# QUALITY ASSURANCE PLAN FOR THE CHESAPEAKE BAY PROGRAM

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## INTRODUCTION

The overall goal of the Chesapeake Bay Program (CBP) is to protect and enhance the quality of the Chesapeake Bay. To this end, the present condition of the Bay must be recorded so that trends can be established. This requires the determination of a variety of parameters such as toxicant and nutrient concentrations in water, sediment and biota. The data resulting from these studies will be used to develop a model to predict the future of the Bay. A control program can then be formulated to optimize the Bay's uses and resources while preserving or improving its quality.

The following three study plans have been proposed for the Chesapeake Bay Program:

"Plan of Action - Toxics Accumulation in Food Chain", prepared by the Toxics Work Group of the CBP;

"A Plan for Ecological Studies of Submerged Aquatic Vegetation and Associated Living Resources of Chesapeake Bay", prepared by the Working Group on Submerged Aquatic Vegetation of the Chesapeake Bay Program; and

"Eutrophication Work Program for Chesapeake Bay", prepared by the CBP Eutrophication Work Group.

The activities proposed in the program plans listed above consist of literature reviews, accumulation and evaluation of available data, extensive sampling in the Bay combined with analyses of the samples, some experimental laboratory work, and management tasks. The sampling and analytical activities described in the various plans are partially overlapping and often not clearly defined. It is desirable to combine sampling events proposed under the various

programs to the highest extent possible. This will not only be more economical and reduce duplication of efforts but concurrent data will be obtained which will greatly facilitate interpretation and modeling (the media to be sampled, as well as the parameters to be determined, are summarized in Appendix A).

Most of the work proposed in the study programs will be performed by as yet unspecified contractors and/or grantees. The data generated will form the input for the model mentioned above, which in turn will be the basis for management decisions on action to be taken. These management decisions can be only as good as the quality of the data they are based upon, and it is imperative that the precision and accuracy of the data be assured. To achieve this, a comprehensive quality assurance (QA) program must be developed covering all work undertaken, and uniform quality control must be imposed on the data collection activities of all contractors and grantees involved.

The purpose of this plan is to outline coordinated and comprehensive QA guidelines for the Chesapeake Bay Program. It should encourage project officers and individual investigators to give adequate thought and sufficient planning to the quality control measures, techniques, and procedures to be used before initiating a project, task, experiment, or contract. It is suggested that this plan, together with copies of the relevant parts of the referenced literature, be issued to all contractors and grantees, to be used as a guideline for the preparation of the QA plans that are required for all experiment, task, project, or contract protocols.

## Quality Assurance in Surveillance Programs

The first step of the CBP, an inventory and baseline determination, consists basically of an extensive surveillance program, which will then be followed by monitoring programs as needed.

The role of such an environmental surveillance program is to provide qualitative and quantitative data on selected environmental parameters. The subjects of such programs are dynamic systems which undergo physical, biological, climatic and man-made changes. The programs usually include the following operational steps:

- Planning
- Sample Collection
- Sample Storage
- Sample Preparation
- Sample Analysis
- Data Manipulation
- Data Interpretation
- Reporting

However, in order to obtain valid data, an overall QA program must apply quality control to all pertinent operational steps. In addition to the usual analytical and equipment QA procedures, a comprehensive QA program should include details on the reliability of the sampling program. Sampling schemes, data analysis strategies, and the objectives of the surveillance program must be well defined in order for a statistician to assist in the development of an efficient collection program. Finally, proper attention must be given to climatic, seasonal, and long-range changes in environmental conditions.

The following items and procedures are obviously important to the success of a comprehensive QA program. They are not intended to be an exhaustive list,

merely representative:

- Satisfactory facilities and equipment
- Adequately trained and experienced personnel
- Use of standard methods
- Routine analysis of control and replicate samples, reagent blanks and standards
- Frequent calibration and servicing of instruments and equipment
- Participation in "round-robin" programs.



An outline which can form the basis for the preparation of QA sections of specific protocols for the CBP plans is presented as Appendix B*. This outline is valid for all media. Special items that go beyond the scope of Appendix B or that supplement the outline are addressed below.

## Evaluation of Literature and Unpublished Data

A substantial part of the data to be used in the CBP have been recorded in the past by different researchers in a variety of studies. While EPA cannot impose any rigid *a priori* criteria for acceptance or rejection of these data, it should be stressed that they must be closely scrutinized. In many instances, data may be of limited value or even useless because precision and accuracy were not reported, or because of inadequate reporting of other parameters (sampling site location, date and time, tide, water temperature, etc.).

## Site Selection

For the northern part of the Chesapeake Bay, 640 sampling sites that have been randomly selected are at present being used to monitor submerged aquatic vegetation (SAV). It has been suggested to extend this random site selection into the southern part of the Bay for SAV research. It should be seriously considered if the same sites (or a certain defined and described selection of

*It is recognized that certain requirements listed in Appendix B might sound superfluous or redundant. However, it should be kept in mind that the manner in which baseline data are determined now might be challenged in court at any time in the future should these baseline data ever be used to demonstrate pollution above the baseline level.

these sites) could be used for all sampling tasks to be carried out. This approach would have obvious logistic advantages because a variety of samples could be taken simultaneously at the same sites. Also, the determination of a wide variety of parameters of the same site would provide a comprehensive site description and thus greatly facilitate the correlation and interpretation of the data. However, the feasibility of using a pattern of randomly selected sites throughout the Bay area must first be evaluated on the basis of anticipated sample numbers and frequency of sampling.

Supplemental Data Acquisition

Whenever samples are collected at field sites, a variety of parameters important for interpretation and correlation of all data must be recorded. These parameters include: sampling site location (coordinates, site number), sampling depth, flow data, date and time of day, tide, meteorological conditions (air temperature, wind speed and direction, percent cloud cover, precipitation, fog), water temperature, water quality parameters (to be determined in the lab or field from collected samples: pH, conductivity, turbidity, color, dissolved oxygen, acidity, alkalinity, BOD, etc.). These data when adequately correlated and evaluated might facilitate trend recognition and might explain patterns that become apparent during the studies.

Interface Measurements

The CBP is basically a multimedia surveillance and monitoring program. One of the proposed study programs includes an investigation of the water/sediment interface (interstitial water, etc.). The water/air interface has not been addressed by any of the proposals. While Bay water contamination resulting from air pollution might indeed pose no problem at present, possible future effects of air pollution on the Bay quality should be evaluated.

Remote Sensing

The use of remote sensing has been considered in one of the program plans to map the general distribution of SAV. More extensive use of remote sensing

by aircraft should be considered. The present state-of-the-art allows remote recording of water temperature, water surface roughness, plumes of a variety of materials, algae blooms and oil films as thin as $10^{-1}$ to $10^{-2}\mu$. EMSL-LV has used remote sensing for water body evaluation for years and has first-hand knowledge of QA requirements in this field.

## Sampling

Sampling should be highly coordinated among the grantees and contractors, to collect a maximum of samples per sampling trip. It is impossible to give directions covering all conditions, and the choice of sampling technique must often be left to the analyst's judgment. However, samples should be truly representative of existing conditions. This can, depending on circumstances, sometimes be achieved by making composites of samples that have been collected over a period of time, or at different sampling points.

In addition to details pertaining to the site and atmospheric conditions, the water current vector should be recorded. Discussions of water movement determinations are presented in limnology textbooks, e.g., "Limnological Methods" by Paul S. Welch, McGraw-Hill Book Co., 1948, p. 141-159; however, no standard method seems to be available. The same book contains a discussion on water sampling methods (p. 199-206) which allow samples to be taken at predetermined depths (sample containers and sample preservation will be addressed later).

All sample containers should be sealed and tagged before they are shipped to the laboratory. Pertinent information should be recorded on a sample tag, e.g., sample number, date and time taken, source of sample, preservative, analyses to be performed, and name of sample collector.

## Water Samples

The "Manual of Methods for Chemical Analysis of Water and Wastes", EPA-625/16-74-003, prepared and published by the Methods Development and Quality

Assurance Research Laboratory, EMSL-Cincinnati, provides standard methods for almost all aspects of water analysis. The following points of importance to the CBP are discussed in detail:

Sample volume, sample preservation, holding containers, holding time, sample analysis (description of analytical procedures including precision and accuracy discussion).

The methods and procedures described also include physical parameters such as temperature, turbidity, pH, conductivity, color, dissolved oxygen, acidity alkalinity, BOD, chemical speciation and oil.

The "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", prepared and published (June 1972) by the Analytical Quality Control Laboratory, EMSL-Cincinnati, addresses in detail the following areas: laboratory facilities, instruments, glassware requirements, and reagents. It further deals with control of analytical performance and data handling and reporting. A separate chapter is devoted to the special requirements for trace organic analysis. Copies of the two handbooks are included as Appendices F and G.

The handbook "Standard Methods for the Examination of Water and Wastewater", 14th edition (1975), published by the American Public Health Association, 1015 Eighteenth Street NW, Washington, DC 20036, describes standard methods for practically all parameters to be determined in Bay water. It provides methods for the determination of color, conductivity, salinity, temperature, turbidity, pH, dissolved oxygen, acidity, alkalinity, and BOD. It covers essentially all elements listed in Appendix A, except Mo and Sn. One chapter is devoted to the determination of organic constituents including oil, pesticides, phenols, surfactants, and tannin and lignin.

Sediment Samples

No standard or tentative EPA-approved or recommended methods are available for sediment sampling or analysis, and the individual investigators must choose their own methods, according to the best of their experience and knowledge. However, it is suggested that individual research groups involved in sediment

sampling and analysis agree in advance on a particular set of methods. Individual skills and the merits of methods could be evaluated in round-robin programs using actual Chesapeake Bay sediment samples and reference material. The National Bureau of Standards is at present certifying a river sediment standard reference material that will become available in late 1978 as SRM 1645.

Every effort must be made to ensure that sediment samples collected are representative. A number of sediment sampling devices and techniques have been described in the literature. The Petersen and the Ponar dredge are preferred for compacted or gravelly sediment, whereas the Ekman dredge is commonly used for soft mud, silt, or finely divided sand bottoms (O. T. Lind, Handbook of Common Methods in Limnology, p. 120. The C. V. Mosby Co., St. Louis, 1974). Bottom samples involving consideration of bottom stratification can be collected with vertical core samplers. An example has been described by P. S. Welch (Limnological Methods, p. 182. McGraw-Hill Book Co., Inc., 1948).

A standard method for extraction of oil and grease from sludge sample and subsequent isolation of the hydrocarbon fraction has been described in "Standard Methods for the Examination of Water and Wastewater", p. 517-21. A manual cold vapor technique for the determination of mercury in sediment has been described in the "Manual of Methods for the Chemical Analysis of Water and Wastes", p. 134-138, and recently the Central Regional Laboratory, Region V, has developed an automated method for the determination of mercury in sediments (Appendix H). EMSL-Cin. has prepared some interim general guidelines as to sediment analysis (Appendix I); however, no tentative method or methods have so far been developed. It should be noted that neither Appendix H nor Appendix I represent EPA-approved methods but are included as examples only.

Aquatic Vegetation, Mollusks, Fish and Birds

The manual "Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents," C. I. Weber, ed., EPA-670/4-73-001, NERC-Cin., is at present available, with issuance of a new edition being planned

for the summer of 1978. While the methods described in this manual are considered to be the best available at this time, the manual does not represent the final position of the EPA in this matter. A comprehensive manual entitled "Quality Assurance Guidelines for Biological Research and Environmental Monitoring" is presently being prepared for publication at EMSL-LV and will probably become available in late 1978. These two manuals complement each other in describing in detail methods and QA procedures covering the following items of importance to the CBP:

Sampling methods for plankton, periphyton, macrophyton, macroinvertebrates, fish, birds, mammals, plants.

Sampling frequency.

Sample preservation and preparation, handling, holding time, container choice and analysis for benthic macroinvertebrates, fish, macrophytes and macroalgae, periphyton, phytoplankton and zooplankton.

Calibration and maintenance of sampling equipment and field instruments.

Culturing, identification, estimation of population size, biomass and productivity.

Field bioassay tests.

A variety of sample forms covering aspects of sampling and analysis are displayed in the appendices of the two manuals.

No compilation of standard methods for the analysis of aquatic vegetation for the pollutants of interest is available. However, scattered throughout the scientific literature, analytical methods have been published for some pollutants of interest; an example is "Environmental Pollutants - Selected Analytical Methods," compiled by W. Gallay, et al., Ann Arbor Science Publishers, Inc., 1975. This book details methods for the determination of Hg in biological media, methylmercury compounds in fish, total Pb and Cd in biological media, Se in biological media, and an estimation of DDT and related compounds together with PCBs in biological media.

Individual investigators will have to select their own methods. Again, it should be attempted to correlate the analytical methodology among the research groups as much as possible.

The Role of EMSL-LV in the QA Section of the CBP

The preceding discussion has demonstrated the importance of a comprehensive and coordinated QA plan for the Chesapeake Bay Program. It is estimated that approximately 10 to 15 percent of the available funds should be spent for QA aspects. The EMSL-LV would act as coordinator and serve as a focal point for all QA activities. In this capacity, the EMSL-LV could assist the CBP management in the following areas:

1. Assist contractors and grantees in identifying and coordinating methods and QA procedures.
2. Evaluate and coordinate the QA parts of all submitted detailed protocols. Define data acceptability for the various tasks and ascertain uniform data reporting (including uncertainties).
3. Provide or arrange for the provision of reference materials as available.
4. Conduct round-robin evaluations as needed to evaluate analyst skills and methods adequacy.

These control and guidance measures would ascertain that all contractors adequately address the use of standardized or comparable procedures in their activities, such as sampling, sample preservation, handling and storage, sample analysis, reference methods, instrument calibration routine, use of reference materials, duplicates, blanks and split samples, chain-of-custody procedures and record-keeping, etc. The EMSL-LV has available the following biological reference materials from various sources that could be distributed to the analytical laboratories involved in the program: fish solubles, oysters, copepod homogenate, animal blood, animal muscle, bovine liver, animal bone, orchard leaves, pine needles, tomato leaves, and spinach. These materials are certified for a variety of stable elements at environmental levels. Reference materials consisting of water hyacinth leaves and a variety of goat tissues, certified for Hg, Pb, As and Cd at environmental and several higher levels are at present being prepared under grants monitored by EMSL and will become available in FY 1979. A round-robin program on water analysis is being conducted by EMSL-Cinc., and all contractors doing this kind of analysis should

be required, at no additional cost to the contractor or the CBP, to participate in this program.

EMSL-LV has years of extensive experience in conducting laboratory intercomparison study programs. Within the framework of the CBP, such a program could be conducted using actual samples collected from the Chesapeake Bay, to demonstrate the comparability and compatibility of analytical data between participating laboratories.

To conduct a QA Coordination program as proposed, EMSL-LV would require the following:

| | FY 1978 | FY 1979 | FY 1980 | FY 1981 |
|---|---|---|---|---|
| Personnel | | | | |
| Permanent | | | | |
| Temporary | | | | |
| Funds | | | | |
| Travel Funds | | | | |
| Material | | | | |

This estimate is based on the following assumptions:

1. The QA plans of a total of 10 contractors will be reviewed and evaluated.
2. Reference materials delivered consist of materials available at EMSL-LV or other government agencies.
3. A total of round-robins involving the 10 contractor laboratories will be conducted during a -year period.
4. The performance and cooperation of the contractor laboratories is generally good.

Not included in the cost estimate are outside expertise used as well as special studies and requests.

APPENDIX A

Major media to be sampled (according to the combined CBP plans)

Bay water (at various depths) and interstitial water, suspended sediment and fluid mud, sediment, benthic vegetation, submerged aquatic vegetation, phytoplankton, mollusks, shellfish, finfish, birds.

Parameters to be determined in the various media (according to the combined CBP plans)

Water:

Temperature, turbidity, water current vectors, pH, conductivity

As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Se, Sn, Zn

Dissolved species of S, C, P, N, Si, Cl

Speciation of As, Hg, Sn

Nutrients (including dissolved organic and inorganic forms of N and P)

Herbicides, insecticides, miticides, bactericides, fungicides, nematocides, wood and metal preservatives, selected persistent organics

Selected compounds from industrial effluents in VA and MD

Low-molecular-weight toxic halogenated hydrocarbons

PCBs

Other toxic substances known to be present in the Bay

Priority toxic organic pollutants

Oil and oil fractions

Previously unidentified organic compounds that show significant changes in concentration or areal distribution during the monitoring period.

Suspended sediment and fluid mud:

Toxicants.

Sediment:

Grain size, bulk water content, fecal pellet analysis, seeds

Heavy metals, bioactive trace metals, heavy minerals, nutrients

Pollen, Pb-210 and C-14 for dating

Toxic substances known to be present in the Bay

Selected persistent organics

Pesticides

PCBs

Oil and oil fractions

Previously unidentified organic compounds that show significant changes in concentration or areal distribution during the monitoring period

Plant pigments

Selected microorganisms.

Submerged aquatic vegetation:

Distribution and abundance, biomass, biogenic structure, growth rate, $CO_2$ fixation, growth rate, respiration

Chlorophyll concentration

Heavy metals

As, Cd, Cu, Hg (inorganic and organic), Ni, Pb, Se, Sn, Zn

Pesticides, herbicides

PCBs

Nutrients

Oil fractions.

Phytoplankton:

Dominant species

Chlorophyll concentrations.

Miscellaneous media:

| | |
|---|---|
| Birds: | determine specimen, their physiological conditions; analyze for contaminants |
| Finfish: | determine number, biomass, length |
| Mollusks: | monitor for toxic substances. |

APPENDIX B

General Outline of the Quality Assurance Section of a Protocol*

The Quality Assurance Section of every protocol should contain, when applicable, a discussion of each of the items addressed below. When published procedures or methods are being utilized without modification, a reference (publication, volume, number, pages, date) to the method may be included.

A. FIELD SAMPLING

1. Describe the criteria used to select the sampling site(s). Explain how the sampling sites are described, identified and recorded.

2. Describe the sampling method(s) used and show why grab or composite sampling is the appropriate method for the particular task. Show that the sampling is representative.

3. Estimate the number of samples to be taken per site and per study; explain the sampling frequency and the rationale upon which it is based.

4. Detail the procedures to be used for the identification and storage of samples (all samples must be identified as to the exact time and date of collection, the exact location, sampling depth, nature and purpose of sample, sample preservation method, and name of sampler).

5. Describe the sample preservation method(s) and the shipment provisions (EPA recommendations for sample containers, preservation techniques and maximum sample holding time must be followed).

6. Define the types of field log books and forms, and the procedures used in recording and maintaining field data (a field sheet or bound notebook must be used for recording all aspects of sample collection, flow measurement, in-situ field testing and other field data collection activities).

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*This outline is largely based on the quality Assurance plan requirements currently in use at EMSL-LV.

7. If there is any probability that a sample or set of samples may be required for any judicial or quasi-judicial proceeding, then formal chain of custody procedures must be followed (an excerpt from the NEIC Compliance Monitoring Procedures Handbook is included as Appendix C).

8. List the procedures to be used for the maintenance and calibration of field instruments, including frequency and record-keeping (sampling equipment, flow measuring devices, and direct-reading field instruments must be calibrated according to proper specifications immediately before and after use in the field. If samples are collected and analyzed over a long period of time, more frequent calibration is necessary. In-situ continuous monitoring devices must be calibrated according to manufacturer's specifications and these calibrations verified by approved manual methodology. Sensor calibration must be verified at least on a daily basis).

9. Define what written instructions and other information will be provided to the sampler(s).

B. STANDARDS, INSTRUMENT CALIBRATION, AND INSTRUMENT MAINTENANCE

1. Identify the standard(s) required for each experiment, task, project, or contract, and the source (or sources) of the required standards; describe the way in which the standards are to be utilized, and their frequency of use.

2. Discuss the known or expected uncertainty of each standard and the effect of this uncertainty on the objectives and the acceptable overall error of the project, experiment or study.

3. Describe or refer to the instrument calibration procedures to be employed and the proposed frequency of calibration (guidance on calibration procedures and suggested frequency for a variety of laboratory instruments is included in Appendix D). Include plans for periodic instrument checks and maintenance.

4. Define the logbooks, forms, records, and/or control charts to be employed in conjunction with the maintenance and calibration of instruments.

C. ANALYTICAL PROCEDURES

1. Outline the responsibility for the custody of samples if applicable (see this Appendix, A-7).

2. Describe formalized handling procedures in the laboratory (the movement of samples through the laboratory should be well organized and controlled, and forms should be prepared for both requests and the reporting of results. Samples which have been held for a period in excess of the maximum recommended holding time should be rejected).

3. Define or reference all analytical procedures (laboratory analyses must be conducted using reference or equivalent methods as described in the Federal Register, or by using EPA-approved alternative test procedures. All reference or equivalent methods must state the precision and accuracy possible with the method. Modifications of referenced methods should be described).

4. Describe the number (or percentage) and the types of samples to be recycled for control purposes. Establish specific control programs for each sampling procedure and analytical test. Provide for record-keeping on all analytical tests such as replicate analyses, parallel testing, and the confirmation or verification of tests (based on sound statistical techniques).

5. Assign responsibility for the preparation of reagents, materials, and pertinent records.

6. Estimate all analytical and instrumental uncertainties, and indicate any expected or real bias. On all new or proposed projects, where preliminary experimental data are unavailable, all factors which may contribute to uncertainties, and the procedures and calculations to be used in estimating error shall be described. The acceptable error, based on the objectives of the experiment or project, should be so indicated.

7. Specify the criteria to be utilized for the acceptance or rejection of anomalous data. Normally, data sets must be within plus or minus 2 standard deviations of the established precision and accuracy (bias).

8. Describe procedures for the analysis and formal reporting of the data.

9. Discuss plans for the use of control charts or other techniques for monitoring daily performance.

10. Describe plans for participating in intercomparisons, cross-checks, performance studies, and interlaboratory calibrations.

11. Identify provisions for recording and storage of actual laboratory and supporting data in bound ledgers and for the maintenance of records of analyses (to be kept for not less than 10 years, as specified by legal mandate).

A check-list, designed to assist investigators and project officers to assess the adequacy of their internal and external controls, is included as Appendix E.

APPENDIX C

ENVIRONMENTAL PROTECTION AGENCY
Office Of Enforcement
NATIONAL ENFORCEMENT INVESTIGATIONS CENTER
Building 53, Box 25227, Denver Federal Center
Denver, Colorado 80225

June 1, 1975

# CHAIN OF CUSTODY PROCEDURES

## General:

The evidence gathering portion of a survey should be characterized by the minimum number of samples required to give a fair representation of the effluent or water body from which taken. To the extent possible, the quantity of samples and sample locations will be determined prior to the survey.

Chain of Custody procedures must be followed to maintain the documentation necessary to trace sample possession from the time taken until the evidence is introduced into court. A sample is in your "custody" if:

1. It is in your actual physical possession, or
2. It is in your view, after being in your physical possession, or
3. It was in your physical possession and then you locked it up in a manner so that no one could tamper with it.

All survey participants will receive a copy of the survey study plan and will be knowledgeable of its contents prior to the survey. A pre-survey briefing will be held to re-appraise all participants of the survey objectives, sample locations and Chain of Custody procedures. After all Chain of Custody samples are collected, a de-briefing will be held in the field to determine adherence to Chain of Custody procedures and whether additional evidence type samples are required.

## Sample Collection:

1. To the maximum extent achievable, as few people as possible should handle the sample.
2. Stream and effluent samples shall be obtained, using standard field sampling techniques.
3. Sample tags (Exhibit I) shall be securely attached to the sample container at the time the complete sample is collected and shall contain, at a minimum, the following information: station number, station location, date taken, time taken, type of sample, sequence number (first sample of the day - sequence No. 1, second sample - sequence No. 2, etc.), analyses required and samplers. The tags must be legibly filled out in ballpoint (waterproof ink).

Sample Collection (Continued)

4. Blank samples shall also be taken with preservatives which will be analyzed by the laboratory to exclude the possibility of container or preservative contamination.

5. A pre-printed, bound Field Data Record logbook shall be maintained to record field measurements and other pertinent information necessary to refresh the sampler's memory in the event he later takes the stand to testify regarding his action's during the evidence gathering activity. A separate set of field notebooks shall be maintained for each survey and stored in a safe place where they could be protected and accounted for at all times. Standard formats (Exhibits II and III) have been established to minimize field entries and include the date, time, survey, type of samples taken, volume of each sample, type of analysis, sample numbers, preservatives, sample location and field measurements such as temperature, conductivity, DO, pH, flow and any other pertinent information or observations. The entries shall be signed by the field sampler. The preparation and conservation of the field logbooks during the survey will be the responsibility of the survey coordinator. Once the survey is complete, field logs will be retained by the survey coordinator, or his designated representative, as a part of the permanent record.

6. The field sampler is responsible for the care and custody of the samples collected until properly dispatched to the receiving laboratory or turned over to an assigned custodian. He must assure that each container is in his physical possession or in his view at all times, or locked in such a place and manner that no one can tamper with it.

7. Colored slides or photographs should be taken which would visually show the outfall sample location and any water pollution to substantiate any conclusions of the investigation. Written documentation on the back of the photo should include the signature of the photographer, time, date and site location. Photographs of this nature, which may be used as evidence, shall also be handled recognizing Chain of Custody procedures to prevent alteration.

Transfer of Custody and Shipment:

1. Samples will be accompanied by a Chain of Custody Record which includes the name of the survey, samplers signatures, station number, station location, date, time, type of sample, sequence number, number of containers and analyses required (Fig. IV). When turning over the possession of samples, the transferor and transferee will sign, date and time the sheet. This record sheet

allows transfer of custody of a group of samples in the field, to the mobile laboratory or when samples are dispatched to the NFIC - Denver laboratory. When transferring a portion of the samples identified on the sheet to the field mobile laboratory, the individual samples must be noted in the column with the signature of the person relinquishing the samples. The field laboratory person receiving the samples will acknowledge receipt by signing in the appropriate column.

2. The field custodian or field sampler, if a custodian has not been assigned, will have the responsibility of properly packaging and dispatching samples to the proper laboratory for analysis. The "Dispatch" portion of the Chain of Custody Record shall be properly filled out, dated, and signed.

3. Samples will be properly packed in shipment containers such as ice chests, to avoid breakage. The shipping containers will be padlocked for shipment to the receiving laboratory.

4. All packages will be accompanied by the Chain of Custody Record showing identification of the contents. The original will accompany the shipment, and a copy will be retained by the survey coordinator.

5. If sent by mail, register the package with return receipt requested. If sent by common carrier, a Government Bill of Lading should be obtained. Receipts from post offices and bills of lading will be retained as part of the permanent Chain of Custody documentation.

6. If samples are delivered to the laboratory when appropriate personnel are not there to receive them, the samples must be locked in a designated area within the laboratory in a manner so that no one can tamper with them. The same person must then return to the laboratory and unlock the samples and deliver custody to the appropriate custodian.

Laboratory Custody Procedures:

1. The laboratory shall designate a "sample custodian." An alternate will be designated in his absence. In addition, the laboratory shall set aside a "sample storage security area." This should be a clean, dry, isolated room which can be securely locked from the outside.

2. All samples should be handled by the minimum possible number of persons.

3. All incoming samples shall be received only by the custodian, who will indicate receipt by signing the Chain of Custody Record Sheet

accompanying the samples and retaining the sheet as permanent records. Couriers picking up samples at the airport, post office, etc. shall sign jointly with the laboratory custodian.

4. Immediately upon receipt, the custodian will place the sample in the sample room, which will be locked at all times except when samples are removed or replaced by the custodian. To the maximum extent possible, only the custodian should be permitted in the sample room.

5. The custodian shall ensure that heat-sensitive or light-sensitive samples, or other sample materials having unusual physical characteristics, or requiring special handling, are properly stored and maintained.

6. Only the custodian will distribute samples to personnel who are to perform tests.

7. The analyst will record in his laboratory notebook or analytical worksheet, identifying information describing the sample, the procedures performed and the results of the testing. The notes shall be dated and indicate who performed the tests. The notes shall be retained as a permanent record in the laboratory and should note any abnormalities which occurred during the testing procedure. In the event that the person who performed the tests is not available as a witness at time of trial, the government may be able to introduce the notes in evidence under the Federal Business Records Act.

8. Standard methods of laboratory analyses shall be used as described in the "Guidelines Establishing Test Procedures for Analysis of Pollutants," 38 F.R. 28758, October 16, 1973. If laboratory personnel deviate from standard procedures, they should be prepared to justify their decision during cross-examination.

9. Laboratory personnel are responsible for the care and custody of the sample once it is handed over to them and should be prepared to testify that the sample was in their possession and view or secured in the laboratory at all times from the moment it was received from the custodian until the tests were run.

10. Once the sample testing is completed, the unused portion of the sample together with all identifying tags and laboratory records, should be returned to the custodian. The returned tagged sample will be retained in the sample room until it is required for trial. Strip charts and other documentation of work will also be turned over to the custodian.

11. Samples, tags and laboratory records of tests may be destroyed only upon the order of the laboratory director, who will first confer with the Chief, Enforcement Specialist Office, to make certain that the information is no longer required or the samples have deteriorated.

EXHIBIT I

☆ GPO 780-003

# CHAIN OF CUSTODY RECORD

## ENVIRONMENTAL PROTECTION AGENCY

National Field Investigations Center—Denver
Denver Federal Center
Denver, Colorado 80225

| SAMPLE NO. | TIME TAKEN (hours) | DATE TAKEN |
|---|---|---|
| | | |

| SOURCE OF SAMPLE | PRESERVATIVE |
|---|---|
| | |

| SAMPLE COLLECTOR | WITNESS(ES) |
|---|---|
| | |

REMARKS: (Analyses Required, Sample Type, etc.)

I hereby certify that I received this sample and disposed of it as noted below:

| RECEIPT OF SAMPLE | RECEIVED FROM | DATE RECEIVED | TIME RECEIVED |
|---|---|---|---|
| | DISPOSITION OF SAMPLE | SIGNATURE | |

I hereby certify that I received this sample and disposed of it as noted below:

| RECEIPT OF SAMPLE | RECEIVED FROM | DATE RECEIVED | TIME RECEIVED |
|---|---|---|---|
| | DISPOSITION OF SAMPLE | SIGNATURE | |

I hereby certify that I obtained this sample and dispatched it as shown below:

| DISPATCH OF SAMPLE | DATE OBTAINED | TIME OBTAINED | SOURCE |
|---|---|---|---|
| | DATE DISPATCHED | TIME DISPATCHED | METHOD OF SHIPMENT |
| | SENT TO | SIGNATURE | |

EXHIBIT IV

ENVIRONMENTAL PROTECTION AGENCY
Office Of Enforcement
NATIONAL ENFORCEMENT INVESTIGATIONS CENTER
Building 53, Box 25227, Denver Federal Center
Denver, Colorado 80225

# CHAIN OF CUSTODY RECORD

| SURVEY | SAMPLERS: *(Signature)* |
|---|---|

| STATION NUMBER | STATION LOCATION | DATE | TIME | SAMPLE TYPE | | | SEQ. NO. | NO. OF CONTAINERS | ANALYSIS REQUIRED |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Water | | Air | | | |
| | | | | Comp. | Grab. | | | | |
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| | | |
|---|---|---|
| Relinquished by: *(Signature)* | Received by: *(Signature)* | Date/Time |
| Relinquished by: *(Signature)* | Received by: *(Signature)* | Date/Time |
| Relinquished by: *(Signature)* | Received by: *(Signature)* | Date/Time |
| Relinquished by: *(Signature)* | Received by Mobile Laboratory for field analysis: *(Signature)* | Date/Time |

| | | | |
|---|---|---|---|
| Dispatched by: *(Signature)* | Date/Time | Received for Laboratory by: | Date/Time |
| Method of Shipment: | | | |

Distribution: Orig.— Accompany Shipment
1 Copy— Survey Coordinator Field Files

APPENDIX D

# INSTRUMENT CALIBRATIONS

| Instrument | Procedure | Frequency |
|---|---|---|
| 1) Analytical Balances | (a) Zero | (a) Before each weighing |
| | (b) Standard weights | (b) Monthly |
| | (c) Full calibration and adjustment | (c) Annually |
| 2) pH Meters | At pH 4,7, and 10 | Daily |
| 3) Conductivity Meters | (a) Obtain cell constant with potassium chloride reference solutions | Daily |
| | (b) Construct temperature curve if measurements are to be made other than at 25 $\pm$ 0.5°C | Monthly |
| 4) Nephelometer/ Turbidimeters | (a) Check instrument scales or develop calibration curve with formazine stds. ($\leq$ 40NTU) | Monthly |
| | (b) If manufacturer's stds. are not formazine, check against formazine stds. ($\leq$ 40NTU) | Annually |
| 5) Colorimeters/Filter Photometers | Curves determined with 5-6 laboratory-prepared std. solutions for each parameter in conc. range of samples | Daily |
| 6) UV/Visible Spectrophotometers | (a) Wavelength calibration with holimum oxide glass or solution, low-pressure mercury arc, benzene vapor (UV), or hydrogen arc (visible) | Quarterly |
| | (b) Absorbance vs. concentration curves with 5-6 std. Solutions for each parameter at analytical wavelength in conc. range of samples | Daily |
| | (c) Full servicing and adjustment | Annually |

INSTRUMENT CALIBRATIONS

| Instrument | Procedure | Frequency |
|---|---|---|
| 7) Infrared Spectrophotometers | (a) Wavelength calibration with polystyrene or indene | Daily |
| | (b) Absorbance vs. concentration curves with 5-6 std. solutions for each parameter at analytical wavelength in conc. range of samples | Daily |
| | (c) Full servicing and adjustment | Semi-annually |
| 8) Atomic Absorption Spectrophotometers | (a) Response vs. concentration curves with 6-8 std. solutions for each metal (std. mixtures are acceptable, but with same acid as samples to be run) in conc. range of samples | Daily |
| | (b) Full servicing and adjustment | Annually |
| 9) Carbon Analyzers | Curves determined with 5-6 std. solutions in conc. range of samples | Daily |
| 10) DO Meters | Calibrated against modified Winkler method on aerated distilled or tap water | Daily |
| 11) Other Selective Ion Electrodes and Electrometers | Curves determined with 5-6 std. solutions in conc. range of samples | Daily |
| 12) Thermometers | Calibrate in constant temperature baths at two temperatures against precision thermometers certified by NBS. | Quarterly |
| 13) Technicon Auto Analyzers | (a) Curves determined with std. solutions for each parameter. | Each set of samples |
| | (b) Full service and adjustment (esp. colorimeter) | Annually |

INSTRUMENT CALIBRATIONS

| Instrument | Procedure | Frequency |
|---|---|---|
| 14) Gas Chromatographs | (a) Retention times and detector response checked with std. solutions | Daily |
| | (b) Response curves for each parameter determined with std. solutions | Monthly |
| 15) Sulfur Dioxide in Air Sampler/Analyzers (Pararosaniline Method) | (a) Calibrate flowmeters and hypodermic needle against a wet test meter | Quarterly |
| | (b) Spectrophotometric calibration curve with 5-6 std. sulfite-TCM solutions at controlled temperature ($\pm 1^{\circ}C$) | Monthly |
| | (c) Sampling calibration curve with 5-6 std. atmospheres from permeation tubes or cylinders | Monthly |
| | (d) Calibrate associated thermometers, barometers, and spectrophotometer (wavelength) | Quarterly |
| 16) Suspended Particulates (High-volume Sampler Method) | (a) Calibrate sampler (curve of true airflow rate vs. rotameter or recorder reading) with orifice calibration unit and differential manometer at 6 air flow rates. | Monthly |
| | (b) Calibrate orifice calibration unit with positive displacement primary standard and differential manometers | Annually |
| | (c) Calibrate relative humidity indicator in the conditioning environment against wet-bulb/dry-bulb psychrometer | Semi-annually |
| | (d) Check elapsed time indicator | Semi-annually |
| | (e) Calibrate associated analytical balances, thermometers, barometers | As needed |

INSTRUMENT CALIBRATIONS

| Instrument | Procedure | Frequency |
|---|---|---|
| 17) Carbon monoxide (Non-dispersive IR) | (a) Determine linearity of detector response (calibration curve) with calibration gases (0, 10, 20, 40, and 80% of full scale, certified to ±2% and checked against auditing gases certified to ±1%) | Monthly |
| | (b) Perform zero and span calibrations | Before and after each sampling period |
| | (c) Calibrate rotameter and sample cell pressure gauge | Semi-annually |
| 18) Photochemical Oxidants (Ozone) | (a) Calibrate standard $KI/I_2$ solutions in terms of calculated $O_3$ equivalents at 352 nm | Weekly |
| | (b) Calibrate instrument response with 6-8 test atmospheres from ozone generator, spanning expected ranged of sample concentrations (usually 0.05-0.5 ppm $O_3$) | Monthly |
| | (c) Calibrate flowmeters, barometer, thermometer | Semi-annually |
| | (d) Calibrate and service spectrophotometer | As specified |
| 19) Hydrocarbons (corrected for Methane) | (a) Determine linearity of detector response with calibration gases (0, 10, 20, 40, and 80% of full scale, certified to ±2%) | Monthly |
| | (b) Perform zero and span calibrations | Before and after each sampling period |
| | (c) Calibrate flowmeters and other associated apparatus | Semi-annually |
| 20) Nitrogen Dioxide (Arsenite 24 hr. Sampling Method) | (a) Calibrate flowmeter with wet test meter | Monthly |
| | (b) Calibrate hypodermic needle (flow restricter) with flowmeter | Each new needle |
| | (c) Obtain colorimetric calibration curves with 5-6 std. nitrite solutions | Weekly |

INSTRUMENT CALIBRATIONS

| Instrument | Procedure | Frequency |
|---|---|---|
| 21) Nitrogen Dioxide (Griess-Saltzman Colorimetric, Continuous) | (a) Dynamic calibration with std. atmospheres (e.g., from permeation tubes) spanning the range of observed concentrations | Monthly |
| | (b) Static colorimetric calibration with 5-6 std. nitrite solutions | Weekly |
| 22) Nitrogen Dioxide (Chemiluminescence, Continuous) | (a) Calibrate std. NO cylinder with ozone generator (pre-calibrated by iodometric procedure) | Each new cylinder |
| | (b) Calibrate NO monitor with std. NO cylinder at several concentrations | Monthly |
| | (c) Calibrate $NO_2$ monitor with std. NO cylinder (diluted NO concentrations determined with NO monitor) and calibrated ozone generator | Monthly |
| | (d) Calibrate associated flow-meters | Semi-annually |
| 23) Autoclaves and Sterilizers | (a) Sterilization effectiveness checked (e.g., B. stearothermophilus, color-indicator tape for ethylene oxide) | Daily |
| | (b) Temperature-recording device calibrated | Semi-annually |

INSTRUMENT CALIBRATION

| Instrument | Procedure | Frequency |
|---|---|---|
| 24) Gamma Spectroscopy | (a) Background | (a) Weekly |
| | (b) Efficiency | (b) Annually, and for every change in library radionuclides or matrix |
| | (c) Time base | (c) Annually |
| | (d) Check source - for energy and efficiency (Bi-207, or Cs-137, Co-60) | (d) Daily |
| 25) Gas Flow Proportional | (a) Plateau | (a) Every change of gas |
| | (b) Background | (b) Daily, when in use |
| | (c) Check source | (c) Daily, when in use |
| | (d) Efficiency vs mass (Am-241, Sr-90) | (d) Semi-annually |
| 26) Radon System | (a) Calibration of cells, photomultiplier, concentrator, and collector | (a) Annually |
| | (b) Background | (b) Daily, when in use |
| | (c) Performance standard | (c) Daily, when in use |
| 27) Liquid Scintillation | (a) Performance standard | (a) Daily, or every 20 samples |
| | (b) Background | (b) Daily, or every 20 samples |
| | (c) Channels ratio for each radionuclide (H-3, Sr-90, Sr-89, Cs-134, I-131, C-14) | (c) Annually |
| 28) Alpha Spectrometer | (a) Performance check | (a) Daily |
| | (b) Background | (b) Weekly |
| | (c) Efficiency (Am-241, Pu-239) | (c) Semi-annually |
| 29) Alpha Scintillation | (a) Performance check | (a) Daily |
| | (b) Background | (b) Daily |
| | (c) Efficiency (Am-241) | (c) Semi-annually |
| 30) Beta Scintillation | (a) Performance check | (a) Daily |
| | (b) Background | (b) Daily |
| | (c) Efficiency (Sr-90) | (c) Semi-annually |

APPENDIX E

INTERNAL AND EXTERNAL CONTROLS

| | Available | |
|---|---|---|
| | Yes | No |
| 1. Control of Analytical Methods and Instruments | | |
| (1) Written Instrument Maintenance and Calibration Procedures and Log Books | | |
| (2) Written Operating Procedures | | |
| 2. Control of Sampling | | |
| (1) Written Sampling Procedures Covering: | | |
| Sampling Plans and Sampling Equipment | | |
| Sample Collection and Preservation | | |
| Identification and Storage of Samples | | |
| Laboratory Handling of Samples (Request for analysis, sample preparation, timely performance, etc.) | | |
| (2) Written Description of the Chain of Custody of Samples | | |
| (3) Written Procedures for Field Measurement (Flow, critical tests: D.O., Residual Cl, etc.) | | |
| (4) Written Procedures for Monitoring (Water supply, effluents, ambient air, stacks, mobile vehicles, pesticides, radiation, etc.) | | |
| 3. Quality Control | | |
| (1) Written Quality Control Program Covering: | | |
| Quality Policy | | |
| Assignment of Responsibility | | |
| Training in Quality Control Methods | | |
| Control of Purchased Chemicals/Reagents | | |
| Internal Field and Laboratory Checks: | | |
| Precision/Accuracy | | |
| Routine Duplicates, Spiked, and Standard Samples | | |
| Statistical Methods, Including Control Charts and/or Computer Methods | | |
| (2) Written Description of Lab Record System (Data handling/calculations, data review, validation and audit) | | |
| (3) Written Description of Lab Report Systems | | |

APPENDIX F

EPA-625-/6-74-003

# METHODS FOR CHEMICAL ANALYSIS OF WATER AND WASTES

METHODS DEVELOPMENT AND QUALITY ASSURANCE
RESEARCH LABORATORY
National Environmental Research Center
Cincinnati, Ohio 45268

U. S. ENVIRONMENTAL PROTECTION AGENCY
Office of Technology Transfer
Washington D.C. 20460

1974

## FOREWORD

The accomplishment of our objectives in protecting the environment requires an awareness of the interdependence of the components we seek to protect - - -air, water, and land. Through individual and joint efforts the National Environmental Research Centers provide this multidisciplinary focus through programs engaged in

studies on the effects of environmental contaminants on man and the biosphere,

the development of efficient means of monitoring these contaminants, and

a search for more effective ways to prevent undesirable modification of the environment and the recycling of valuable resources.

This chemical methods manual was developed by the staff of the Methods Development and Quality Assurance Research Laboratory of the National Environmental Research Center, Cincinnati, to provide methodology for monitoring the quality of our Nation's waters and to determine the impact of waste discharges. The test procedures have been carefully selected to meet the needs of laboratories engaged in protecting the aquatic environment. The contributions and counsel of scientists in other EPA laboratories are gratefully acknowledged.

The manual is published and distributed by the Office of Technology Transfer, as one of a series designed to insure that the latest technologies developed by EPA and private industry are disseminated to states, municipalities and industries who are responsible for environmental pollution control.

The other manuals in this series are:

*Handbook for Monitoring Industrial Wastewater*

*Handbook for Analytical Quality Control in Water and Wastewater Laboratories.*

These are also available through the Office of Technology Transfer, Washington, D.C. 20460.

Robert E. Crowe, Director
Office of Technology Transfer
Washington, D.C.

Andrew W. Breidenbach, Ph.D.
Director, National Envrionmental
Research Center, Cincinnati, Ohio

# INTRODUCTION

This second edition of "Methods for Chemical Analysis of Water and Wastes" contains the chemical analytical procedures used in U.S. Environmental Protection Agency (EPA) laboratories for the examination of ground and surface waters, domestic and industrial waste effluents, and treatment process samples. Except where noted under "Scope and Application," the methods are applicable to both water and wastewaters, and both fresh and saline water samples. The manual provides test procedures for the measurement of physical, inorganic, and selected organic constituents and parameters. Methods for pesticides, industrial organic waste materials, and sludges are given in other publications of the Agency. The methods were chosen through the combined efforts of the EPA Regional Analytical Quality Control Coordinators, the staff of the Physical and Chemical Methods Branch, Methods Development and Quality Assurance Research Laboratory, and other senior chemists in both federal and state laboratories. Method selection was based on the following criteria:

(1) The method should measure the desired property or constituent with precision, accuracy, and specificity sufficient to meet the data needs of EPA, in the presence of the interfering materials encountered in water and waste samples.
(2) The procedure should utilize the equipment and skills available in modern water pollution control laboratories.
(3) The selected method is in use in many laboratories or has been sufficiently tested to establish its validity.
(4) The method should be rapid enough to permit routine use for the examination of a large number of samples.

Instrumental methods have been selected in preference to manual procedures because of the improved speed, accuracy, and precision. In keeping with this policy, procedures for the Technicon AutoAnalyzer have been included for laboratories having this equipment available.

Precision and accuracy statements are provided where such data are available. These statements are derived from interlaboratory studies conducted by the Quality Assurance and Laboratory Evaluation Branch, Methods Development and Quality Assurance Research Laboratory; the American Society for Testing Materials; or the Analytical Reference Service of the US Public Health Service, DHEW.

These methods may be used for measuring both total and dissolved constituents of the sample. *When the dissolved concentration is to be determined, the sample is filtered through a 0.45-micron membrane filter and the filtrate analyzed by the procedure specified.* The sample should be filtered as soon as possible after it is collected, preferably in the field. Where field filtration is not practical, the sample should be filtered as soon as it is received in the laboratory.

Many water and waste samples are unstable. In situations where the interval between sample collection and analysis is long enough to produce changes in either the concentration or the physical state of the constituent to be measured, the preservation practices in Table II are recommended.

This manual is a basic reference for monitoring water and wastes in compliance with the requirements of the Federal Water Pollution Control Act Amendments of 1972. Although other test procedures may be used, as provided in the Federal Register issue of October 16, 1973 (38FR 28758), the methods described in this manual will be used by the Environmental Protection Agency in determining compliance with applicable water and effluent standards established by the Agency.

Although a sincere effort has been made to select methods that are applicable to the widest range of sample types, significant interferences may be encountered in certain isolated samples. In these situations, the analyst will be providing a valuable service to EPA by defining the nature of the interference with the method and bringing this information to the attention of the Director, Methods Development and Quality Assurance Research Laboratory, through the appropriate Regional AQC Coordinator.

## SAMPLE PRESERVATION

Complete and unequivocal preservation of samples, either domestic sewage, industrial wastes, or natural waters, is a practical impossibility. Regardless of the nature of the sample, complete stability for every constituent can never be achieved. At best, preservation techniques can only retard the chemical and biological changes that inevitably continue after the sample is removed from the parent source. The changes that take place in a sample are either chemical or biological. In the former case, certain changes occur in the chemical structure of the constituents that are a function of physical conditions. Metal cations may precipitate as hydroxides or form complexes with other constituents; cations or anions may change valence states under certain reducing or oxidizing conditions; other constituents may dissolve or volatilize with the passage of time. Metal cations may also adsorb onto surfaces (glass, plastic, quartz, etc.), such as, iron and lead. Biological changes taking place in a sample may change the valence of an element or a radical to a different valence. Soluble constituents may be converted to organically bound materials in cell structures, or cell lysis may result in release of cellular material into solution. The well known nitrogen and phosphorus cycles are examples of biological influence on sample composition.

Methods of preservation are relatively limited and are intended generally to (1) retard biological action, (2) retard hydrolysis of chemical compounds and complexes and (3) reduce volatility of constituents.

Preservation methods are generally limited to pH control, chemical addition, refrigeration, and freezing. Table 1 shows the various preservatives that may be used to retard changes in samples.

TABLE 1

| Preservative | Action | Applicable to: |
|---|---|---|
| $HgCl_2$ | Bacterial Inhibitor | Nitrogen forms, Phosphorus forms |
| Acid ($HNO_3$) | Metals solvent, prevents precipitation | Metals |
| Acid ($H_2SO_4$) | Bacterial Inhibitor | Organic samples (COD, oil & grease organic carbon) |
| | Salt formation with organic bases | Ammonia, amines |
| Alkali (NaOH) | Salt formation with volatile compounds | Cyanides, organic acids |
| Refrigeration | Bacterial Inhibitor | Acidity-alkalinity, organic materials, BOD, color, odor, organic P, organic N, carbon, etc., biological organism (coliform, etc.) |

In summary, refrigeration at temperatures near freezing or below is the best preservation technique available, but it is not applicable to all types of samples.

The recommended choice of preservatives for various constituents is given in Table 2. These choices are based on the accompanying references and on information supplied by various Regional Analytical Quality Control Coordinators.

## TABLE 2

## RECOMMENDATION FOR SAMPLING AND PRESERVATION OF SAMPLES ACCORDING TO MEASUREMENT (1)

| Measurement | Vol. Req. (ml) | Container | Preservative | Holding Time(6) |
|---|---|---|---|---|
| Acidity | 100 | P, G[2] | Cool, 4°C | 24 Hrs. |
| Alkalinity | 100 | P, G | Cool, 4°C | 24 Hrs. |
| Arsenic | 100 | P, G | $HNO_3$ to pH <2 | 6 Mos. |
| BOD | 1000 | P, G | Cool, 4°C | 6 Hrs.[3] |
| Bromide | 100 | P, G | Cool, 4°C | 24 Hrs. |
| COD | 50 | P, G | $H_2SO_4$ to pH <2 | 7 Days |
| Chloride | 50 | P, G | None Req. | 7 Days |
| Chlorine Req. | 50 | P, G | Cool, 4°C | 24 Hrs. |
| Color | 50 | P, G | Cool, 4°C | 24 Hrs. |
| Cyanides | 500 | P, G | Cool, 4°C<br>NaOH to pH 12 | 24 Hrs. |
| Dissolved Oxygen | | | | |
| Probe | 300 | G only | Det. on site | No Holding |
| Winkler | 300 | G only | Fix on site | No Holding |

TABLE 2 (Continued)

| Measurement | Vol. Req. (ml) | Container | Preservative | Holding Time(6) |
|---|---|---|---|---|
| Fluoride | 300 | P, G | Cool, 4°C | 7 Days |
| Hardness | 100 | P, G | Cool, 4°C | 7 Days |
| Iodide | 100 | P, G | Cool, 4°C | 24 Hrs. |
| MBAS | 250 | P, G | Cool, 4°C | 24 Hrs. |
| Metals | | | | |
| Dissolved | 200 | P, G | Filter on site $HNO_3$ to pH <2 | 6 Mos. |
| Suspended | | | Filter on site | 6 Mos. |
| Total | 100 | | $HNO_3$ to pH <2 | 6 Mos. |
| Mercury | | | | |
| Dissolved | 100 | P, G | Filter $HNO_3$ to pH <2 | 38 Days (Glass) 13 Days (Hard Plastic) |
| Total | 100 | P, G | $HNO_3$ to pH <2 | 38 Days (Glass) 13 Days (Hard Plastic) |

TABLE 2 (Continued)

| Measurement | Vol. Req. (ml) | Container | Preservative | Holding Time(6) |
|---|---|---|---|---|
| Nitrogen | | | | |
| Ammonia | 400 | P, G | Cool, 4°C<br>$H_2SO_4$ to pH <2 | 24 Hrs.(4) |
| Kjeldahl | 500 | P, G | Cool, 4°C<br>$H_2SO_4$ to pH <2 | 24 Hrs.(4) |
| Nitrate | 100 | P, G | Cool, 4°C<br>$H_2SO_4$ to pH <2 | 24 Hrs.(4) |
| Nitrite | 50 | P, G | Cool, 4°C | 24 Hrs. (4) |
| NTA | 50 | P, G | Cool, 4°C | 24 Hrs. |
| Oil & Grease | 1000 | G only | Cool, 4°C<br>$H_2SO_4$ to pH <2 | 24 Hrs. |
| Organic Carbon | 25 | P, G | Cool, 4°C<br>$H_2SO_4$ to pH <2 | 24 Hrs. |
| pH | 25 | P, G | Cool, 4°C<br>Det. on site | 6 Hrs.(3) |
| Phenolics | 500 | G only | Cool, 4°C<br>$H_3PO_4$ to pH <4<br>1.0 g $CuSO_4$/l | 24 Hrs. |
| Phosphorus<br>Ortho-<br>phosphate,<br>Dissolved | 50 | P, G | Filter on site<br>Cool, 4°C | 24 Hrs.(4) |

TABLE 2 (Continued)

| Measurement | Vol. Req. (ml) | Container | Preservative | Holding Time(6) |
|---|---|---|---|---|
| Hydrolyzable | 50 | P, G | Cool, 4°C $H_2SO_4$ to pH <2 | 24 Hrs.(4) |
| Total | 50 | P, G | Cool, 4°C | 24 Hrs. (4) |
| Total, Dissolved | 50 | P, G | Filter on site Cool, 4°C | 24 Hrs.(4) |
| Residue | | | | |
| Filterable | 100 | P, G | Cool, 4°C | 7 Days |
| Non-Filterable | 100 | P, G | Cool, 4°C | 7 Days |
| Total | 100 | P, G | Cool, 4°C | 7Days |
| Volatile | 100 | P, G | Cool, 4°C | 7 Days |
| Settleable Matter | 1000 | P, G | None Req. | 24 Hrs. |
| Selenium | 50 | P, G | $HNO_3$ to pH <2 | 6 Mos. |
| Silica | 50 | P only | Cool, 4°C | 7 Days |
| Specific Conductance | 100 | P, G | Cool, 4°C | 24 Hrs. (5) |
| Sulfate | 50 | P, G | Cool, 4°C | 7 Days |

TABLE 2 (Continued)

| Measurement | Vol. Req. (ml) | Container | Preservative | Holding Time(6) |
|---|---|---|---|---|
| Sulfide | 50 | P, G | 2 ml zinc acetate | 24 Hrs. |
| Sulfite | 50 | P, G | Cool, 4°C | 24 Hrs. |
| Temperature | 1000 | P, G | Det. on site | No Holding |
| Threshold Odor | 200 | G only | Cool, 4°C | 24 Hrs. |
| Turbidity | 100 | P, G | Cool, 4°C | 7 Days |

1. More specific instructions for preservation and sampling are found with each procedure as detailed in this manual. A general discussion on sampling water and industrial wastewater may be found in ASTM, Part 23, p. 72-91 (1973).

2. Plastic or Glass

3. If samples cannot be returned to the laboratory in less than 6 hours and holding time exceeds this limit, the final reported data should indicate the actual holding time.

4. Mercuric chloride may be used as an alternate preservative at a concentration of 40 mg/1, especially if a longer holding time is required. However, the use of mercuric chloride is discouraged whenever possible.

5. If the sample is stabilized by cooling, it should be warmed to 25°C for reading, or temperature correction made and results reported at 25°C.

6. It has been shown that samples properly preserved may be held for extended periods beyond the recommended holding time.

## ENVIRONMENTAL PROTECTION AGENCY
## REGIONAL ANALYTICAL QUALITY CONTROL COORDINATORS

REGION I

Warren H. Oldaker
New England Basin Office
240 Highland Avenue
Needham Heights, MA 02194
(617-223-7337)

REGION II

Gerard F. McKenna
Edison Environmental Lab.
Edison, NJ 08817
(201-548-3427)

REGION III

Orterio Villa
Annapolis Field Office
Annapolis Science Center
Annapolis, MD 21401
(301-597-3311)
(Ask for: 268-5038)

REGION IV

James H. Finger
Southeast Envr. Res. Lab.
College Station Road
Athens, GA 30601
(404-546-3111)

REGION V

David Payne
Central Regional Lab.
Quality Assurance Officer
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(312-353-8370)

REGION VI

Dr. Timothy Matzke
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Dallas, TX 75201
(214-749-1121)

REGION VII

Dr. Harold G. Brown
25 Funston Road
Kansas City, KS 66115
(816-374-4286)

REGION VIII

John R. Tilstra
Denver Federal Center
P.O. Box 25345
Denver, CO 80225
(303-234-3263)

REGION IX

Dr. Ho L. Young
620 Central Ave., Bldg 1
Alameda, CA 94501
(415-273-7502)

REGION X

Arnold R. Gahler
15345 N.E. 36th Street
Redmond, WA 98052
(206-442-0111)
(Ask for: 883-0833)

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# ACIDITY

STORET NO. 70508

1. Scope and Application
   1.1 This method is applicable to surface waters, sewages and industrial wastes, particularly mine drainage and receiving streams, and other waters containing ferrous iron or other polyvalent cations in a reduced state.
   1.2 The method covers the range from approximately 10 mg/1 acidity to approximately 1000 mg/1 as $CaCO_3$, using a 50 ml sample.
2. Summary of Method
   2.1 The pH of the sample is determined and a measured amount of standard acid is added, as needed, to lower the pH to 4 or less. Hydrogen peroxide is added, the solution boiled for several minutes, cooled, and titrated electrometrically with standard alkali to pH 8.2.
3. Definitions
   3.1 This method measures the mineral acidity of a sample plus the acidity resulting from oxidation and hydrolysis of polyvalent cations, including salts of iron and aluminum.
4. Interferences
   4.1 Suspended matter present in the sample, or precipitates formed during the titration may cause a sluggish electrode response. This may be offset by allowing a 15-20 second pause between additions of titrant or by slow dropwise addition of titrant as the endpoint pH is approached.
5. Apparatus
   5.1 pH meter, suitable for electrometric titrations.
6. Reagents
   6.1 Hydrogen peroxide ($H_2O_2$, 30% solution).
   6.2 Standard sodium hydroxide, 0.02 N.
   6.3 Standard sulfuric acid, 0.02 N.
7. Procedure
   7.1 Pipet 50 ml of the sample into a 250 ml beaker.
   7.2 Measure the pH of the sample. If the pH is above 4.0 add standard sulfuric acid in 5.0 ml increments to lower the pH to 4.0 or less. If the initial pH of the sample is less than 4.0, the incremental addition of sulfuric acid is not required.
   7.3 Add 5 drops of hydrogen peroxide.

7.4 Heat the sample to boiling and continue boiling for 2 to 4 minutes. In some instances, the concentration of ferrous iron in a sample is such that an additional amount of hydrogen peroxide and a slightly longer boiling time may be required.

7.5 Cool the sample to room temperature and titrate electrometrically with standard alkali to pH 8.2.

8. Calculations

8.1 Acidity, as mg/1 $CaCO_3 = \frac{(A \times B) - (C \times D) \times 50{,}000}{\text{ml sample}}$

where:

A = vol. of standard alkali used in titration

B = normality of standard alkali

C = volume of standard acid used to reduce pH to 4 or less

D = normality of standard acid

8.2 If it is desired to report acidity in millequivalents per liter, the reported values as $CaCO_3$ are divided by 50, as follows:

$$\text{Acidity as meq/1} = \frac{\text{mg/1 } CaCO_3}{50}$$

9. Precision

9.1 On a round robin conducted by ASTM on 4 acid mine waters, including concentrations up to 2000 mg/1, the precision was found to be ± 10 mg/l.

10. References

10.1 The procedure to be used for this determination can be found in:

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 124, D-1067, Method E (1973).

Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 370, Method 201 (Acidity and Alkalinity) (1971).

# ALKALINITY (pH 4.5)

STORET NO. 00410

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The method is suitable for all concentration ranges of alkalinity; however, appropriate aliquots should be used to avoid a titration volume greater than 50 ml.
   1.3 Automated titrimetric analysis is equivalent.
2. Summary of Method
   2.1 An unaltered sample is titrated to an electrometrically determined end point of pH 4.5. The sample must not be filtered, diluted, concentrated, or altered in any way.
3. Comments
   3.1 The sample must be analyzed as soon as practical; preferably, within a few hours. Do not open sample bottle before analyses.
   3.2 Substances, such as salts of weak organic and inorganic acids present in large amounts, may cause interference in the electrometric pH measurements.
   3.3 Oil and grease, by coating the pH electrode, may also interfere, causing sluggish response.
4. Precision and Accuracy
   4.1 Forty analysts in seventeen laboratories analyzed synthetic water samples containing increments of bicarbonate, with the following results:

| Increment as Alkalinity mg/liter, $CaCO_3$ | Precision as Standard Deviation mg/liter, $CaCO_3$ | Accuracy as Bias, % | Accuracy as Bias, mg/l, $CaCO_3$ |
|---|---|---|---|
| 8 | 1.27 | +10.61 | +0.85 |
| 9 | 1.14 | +22.29 | +2.0 |
| 113 | 5.28 | - 8.19 | - 9.3 |
| 119 | 5.36 | - 7.42 | - 8.8 |

(FWPCA Method Study 1, Mineral and Physical Analyses)

4.2 In a single laboratory (MDQARL), using surface water samples at an average concentration of 122 mg $CaCO_3$/l, the standard deviation was ±3.

5. References

5.1 The procedure to be used for this determination is found in:

Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 52, Method 102, (1971).

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 119, D-1067, Method B, (1973).

5.2 For samples having high concentrations of mineral acids, such as mine wastes and associated receiving waters, titrate to an electrometric endpoint of pH 3.9, using the procedure in:

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 123, D-1067, Method D, (1973).

# ALKALINITY

(Automated, Methyl Orange)

STORET NO. 00410

1. Scope and Application
   1.1 This automated method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The applicable range is 10 to 200 mg/l as $CaCO_3$.
   1.2 This method is not applicable to samples with pH lower than 3.1.
2. Summary of Method
   2.1 Methyl orange is used as the indicator in this method because its pH range is in the same range as the equivalence point for total alkalinity, and it has a distinct color change that can be easily measured. The methyl orange is dissolved in a weak buffer at a pH of 3.1, just below the equivalence point, so that any addition of alkalinity causes a loss of color directly proportional to the amount of alkalinity.
3. Sample Handling and Preservation
   3.1 Sample should be refrigerated at 4°C and run as soon as practical.
4. Interferences
   4.1 Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used will also interfere.
5. Apparatus
   5.1 Technicon AutoAnalyzer consisting of:
      5.1.1 Sampler I.
      5.1.2 Manifold.
      5.1.3 Proportioning pump.
      5.1.4 Colorimeter equipped with 15 mm tubular flow cell and 550 nm filters.
      5.1.5 Recorder equipped with range expander.
6. Reagents
   6.1 Methyl Orange: Dissolve 0.125 g of methyl orange in 1 liter of distilled water.
   6.2 pH 3.1 Buffer: Dissolve 5.1047 g of potassium acid phthalate in distilled water and add 87.6 ml 0.1 N HCl and dilute to 1 liter. Stable for one week.
   6.3 Methyl Orange-Buffered Indicator: Add 1 liter of pH 3.1 buffer to 200 ml methyl orange solution and mix well. Stable for 24 hours.

6.4 Stock Solution: Dissolve 1.060 g of anhydrous sodium carbonate (oven-dried at 140°C for 1 hour) in distilled water and dilute to 1000 ml. 1.0 ml = 1.00 mg $CaCO_3$.

6.4.1 Prepare a series of standards by diluting suitable volumes of stock solution to 100.0 ml with distilled water. The following dilutions are suggested:

| ml of Stock Solution | Conc., mg/l as $CaCO_3$ |
|---|---|
| 1.0 | 10 |
| 2.0 | 20 |
| 4.0 | 40 |
| 6.0 | 60 |
| 8.0 | 80 |
| 10.0 | 100 |
| 18.0 | 180 |
| 20.0 | 200 |

7. Procedure

7.1 No advance sample preparation is required. Set up manifold as shown in Figure 1.

7.2 Allow both colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on colorimeter to obtain stable baseline.

7.3 Place distilled water wash tubes in alternate openings on sampler and set sample timing at 2.0 minutes.

7.4 Place working standards in sampler in order of decreasing concentration. Complete filling of sampler tray with unknown samples.

7.5 Switch sample line from distilled water to sampler and begin analysis.

8. Calculation

8.1 Prepare standard curve by plotting peak heights of processed standards against known concentrations. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (MDQARL), using surface water samples at concentrations of 15, 57, 154, and 193 mg/l as $CaCO_3$ the standard deviation was ±0.5.

9.2 In a single laboratory (MDQARL), using surface water samples at concentrations of 31 and 149 mg/l as $CaCO_3$ recoveries were 100% and 99%, respectively.

## Bibliography

1. Technicon AutoAnalyzer Methodology, Bulletin 1261, Technicon Controls, Inc., Chauncey, N.Y. (1961).
2. Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 52, Method 102 (1971).

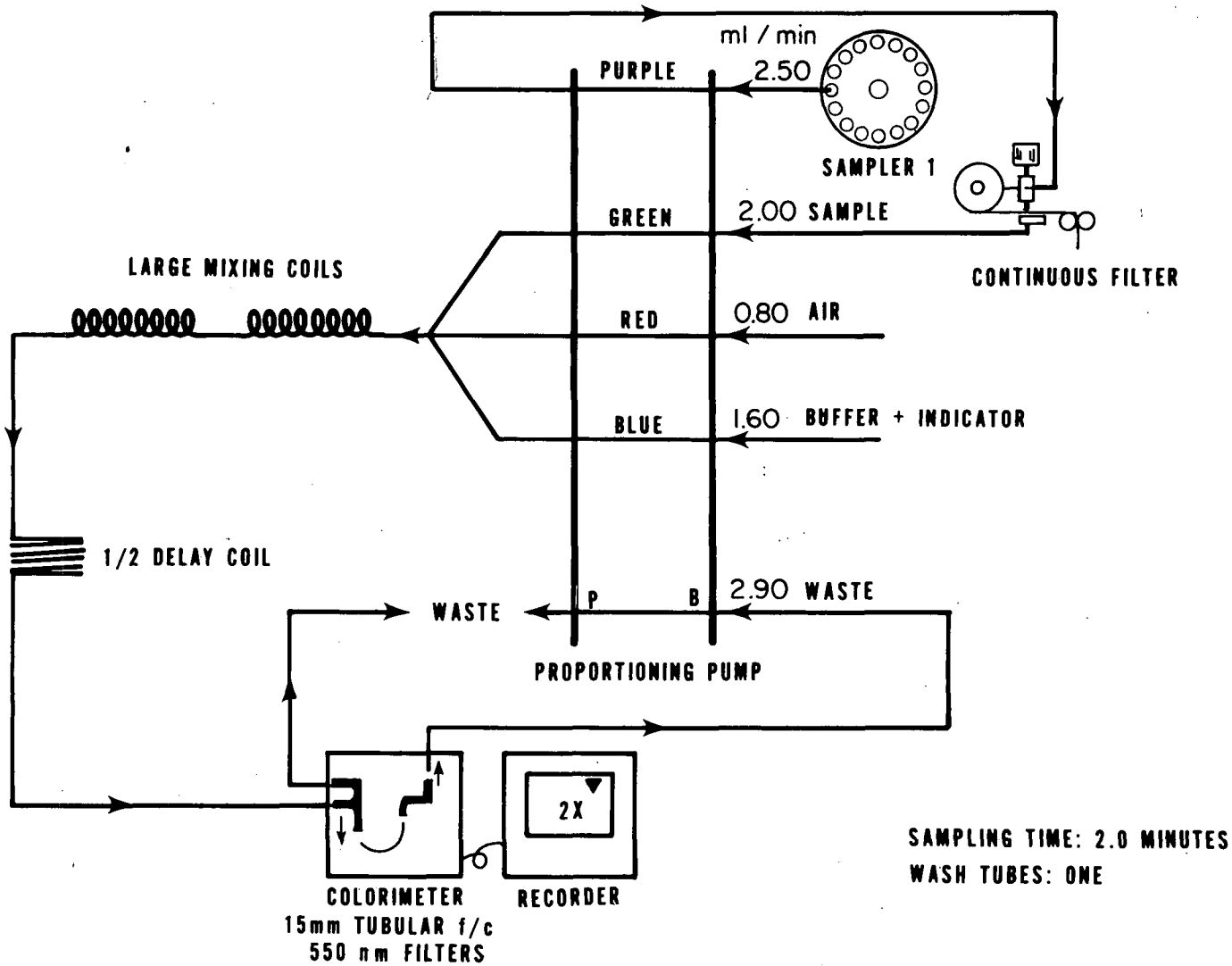



**FIGURE 1. ALKALINITY MANIFOLD AA-I**

# ARSENIC

STORET NO. Total 01002
Inorganic, Dissolved 00995
Inorganic, Total 00997

1. Scope and Application

1.1 The silver diethyldithiocarbamate method determines inorganic arsenic when present in concentrations at or above 10 μg/l. The method is applicable to most fresh and saline waters in the absence of high concentrations of chromium, cobalt, copper, mercury, molybdenum, nickel, and silver. Domestic and industrial wastes may also be analyzed after digestion (See 3.3).

1.2 Difficulties may be encountered with certain industrial waste materials containing volatile substances. High sulfur content of wastes may exceed removal capacity of the lead acetate scrubber.

2. Summary of Method

2.1 Arsenic in the sample is reduced to arsine, $AsH_3$, in acid solution in a hydrogen generator. The arsine is passed through a scrubber to remove sulfide and is absorbed in a solution of silver diethyldithiocarbamate dissolved in pyridine. The red complex thus formed is measured in a spectrophotometer at 535 nm.

3. Comments

3.1 In analyzing most surface and ground waters, interferences are rarely encountered. Industrial waste samples should be spiked with a known amount of arsenic to establish adequate recovery.

3.2 It is essential that the system be airtight during evolution of the arsine, to avoid losses.

3.3 If concentration of the sample and/or oxidation of any organic matter is required, refer to Standard Methods, 13th Edition, Method 104B, p 65, Procedure 4.a (1971).

3.3.1 Since nitric acid gives a negative interference in this colorimetric test, use sulfuric acid as a preservative if only inorganic arsenic is being measured.

3.4 1-Ephedrine in chloroform has been found to be a suitable solvent for silver diethyldithiocarbamate if the analyst finds the odor of pyridine objectionable [Anal. Chem. *45*, 1786 (1973)].

4. Precision and Accuracy

4.1 A synthetic unknown sample containing 40 μg/1, as As, with other metals was analyzed in 46 laboratories. Relative standard deviation was ±13.8% and relative error was 0%.

5. Reference

5.1 The procedure to be used for this determination is found in:
Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 62, Method 104A (1971).

# BIOCHEMICAL OXYGEN DEMAND
## (5 Days, 20°C)

STORET NO. 00310

1. Scope and Application
   1.1 The biochemical oxygen demand test (BOD) is used for determining the relative oxygen requirements of municipal and industrial wastewaters. Application of the test to organic waste discharges allows calculation of the effect of the discharges on the oxygen resources of the receiving water. Data from BOD tests are used for the development of engineering criteria for the design of wastewater treatment plants.
   1.2 The BOD test is an empirical bioassay-type procedure which measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present. The standard test conditions include dark incubation at 20°C for a specified time period (often 5 days). The actual environmental conditions of temperature, biological population, water movement, sunlight, and oxygen concentration cannot be accurately reproduced in the laboratory. Results obtained must take into account the above factors when relating BOD results to stream oxygen demands.
2. Summary of Method
   2.1 The sample of waste, or an appropriate dilution, is incubated for 5 days at 20°C in the dark. The reduction in dissolved oxygen concentration during the incubation period yields a measure of the biochemical oxygen demand.
3. Comments
   3.1 Determination of dissolved oxygen in the BOD test may be made by use of either the Modified Winkler with Full-Bottle Technique or the Probe Method in this manual.
   3.2 Additional information relating to oxygen demanding characteristics of wastewaters can be gained by applying the Total Organic Carbon and Chemical Oxygen Demand tests (also found in this manual).
4. Precision and Accuracy
   4.1 Eighty-six analysts in fifty-eight laboratories analyzed natural water samples plus an exact increment of biodegradable organic compounds. At a mean value of 2.1 and 175 mg/l BOD, the standard deviation was ±0.7 and ±26 mg/l, respectively. (EPA Method Research Study 3).
   4.2 There is no acceptable procedure for determining the accuracy of the BOD test.

5. References

5.1 The procedure to be used for this determination is found in:
Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 489, Method 219 (1971).

# BORON

## (Curcumin Method)

STORET NO. 01022

1. Scope and Application
   1.1 This colorimetric method finds maximum utility for waters whose boron content is below 1 mg/1.
   1.2 The optimum range of the method on undiluted or unconcentrated samples is 0.1-1.0 mg/1 of boron.
2. Summary of Method
   2.1 When a sample of water containing boron is acidified and evaporated in the presence of curcumin, a red-colored product called rosocyanine is formed. The rosocyanine is taken up in a suitable solvent, and the red color is compared with standards either visually or photometrically.
3. Comments
   3.1 Nitrate nitrogen concentrations above 20 mg/1 interfere.
   3.2 Significantly high results are possible when the total of calcium and magnesium hardness exceeds 100 mg/1 as $CaCO_3$. Passing the sample through a cation exchange resin eliminates this problem.
   3.3 Close control of such variables as volumes and concentrations of reagents, as well as time and temperature of drying, must be exercised for maximum accuracy.
   3.4 Data to be entered into STORET must be reported as µg/1.
4. Precision and Accuracy
   4.1 A synthetic sample prepared by the Analytical Reference Service, PHS, containing 240 µg/1 B, 40 µg/1 As, 250 µg/1 Be, 20 µg/1 Se, and 6 µg/1 V in distilled water, was analyzed by the curcumin method with a relative standard deviation of 22.8% and a relative error of 0% in 30 laboratories.
5. Reference
   5.1 The procedure to be used for this determination is found in:
   Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 69, Method 107A (1971).

# BROMIDE

(Titrimetric)

STORET NO. 71870

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial waste effluents.
   1.2 The concentration range for this method is 2-20 mg bromide/l.
2. Summary of Method
   2.1 After pretreatment to remove interferences, the sample is divided into two aliquots. One aliquot is analyzed for iodide by converting the iodide to iodate with bromine water and titrating iodometrically with phenylarsine oxide (PAO) or sodium thiosulfate. The other aliquot is analyzed for iodide plus bromide by converting these halides to iodate and bromate with calcium hypochlorite and titrating iodometrically with PAO or sodium thiosulfate. Bromide is then calculated by difference.
3. Sample Handling and Preservation
   3.1 Store at 4°C and analyze as soon as possible.
4. Interferences
   4.1 Iron, manganese and organic matter can interfere; however, the calcium oxide pretreatment removes or reduces these to insignificant concentrations.
   4.2 Color interferes with the observation of indicator and bromine-water color changes. This interference is eliminated by the use of a pH meter instead of a pH indicator and the use of standardized amounts of oxidant and oxidant-quencher.
5. Reagents
   5.1 Acetic Acid Solution (1:8): Mix 100 ml of glacial acetic acid with 800 ml of distilled water.
   5.2 Bromine Water: In a fume hood, add 0.2 ml bromine to 500 ml distilled water. Stir with a magnetic stirrer and a Teflon-coated stirring bar for several hours or until the bromine dissolves. Store in a glass-stoppered colored bottle.
   5.3 Calcium Carbonate ($CaCO_3$): Powdered
   5.4 Calcium Hypochlorite Solution ($Ca(OCl)_2$): Add 35 g of $Ca(OCl)_2$ to approximately 800 ml of distilled water in a 1 liter volumetric flask. Stir on a magnetic stirrer for approximately 30 minutes. Dilute to 1 liter and filter. Store in a glass-stoppered, colored flask.

5.5 Calcium Oxide (CaO): Anhydrous, powdered.

5.6 Hydrochloric Acid Solution (1:4): Mix 100 ml of HCl (sp. gr. 1.19) with 400 ml of distilled water.

5.7 Potassium Iodide (KI): Crystals, ACS Reagent Grade

5.8 Sodium Acetate Solution (275 g/l): Dissolve 275 g sodium acetate trihydrate ($NaC_2H_3O_2 \cdot 3H_2O$) in distilled water. Dilute to 1 liter and filter.

5.9 Sodium Chloride (NaCl): Crystals, ACS Reagent Grade

5.10 Sodium Formate Solution (500 g/l): Dissolve 50 g sodium formate ($NaCHO_2$) in hot distilled water and dilute to 100 ml.

5.11 Sodium Molybdate Solution (10 g/l): Dissolve 1 g sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in distilled water and dilute to 100 ml.

5.12 Sulfuric Acid Solution (1:4): Slowly add 200 ml $H_2SO_4$ (sp. gr. 1.84) to 800 ml of distilled water.

5.13 Phenylarsine Oxide (0.0375N): Hach Chemical Co., or equivalent. Standardize with 0.0375 N potassium biiodate (5.19, 5.23).

5.14 Phenylarsine Oxide Working Standard (0.0075 N): Transfer 100 ml of commercially available 0.0375 N phenylarsine oxide (5.13) to a 500 ml volumetric flask and dilute to the mark with distilled water. This solution should be prepared fresh daily.

5.15 Amylose Indicator: Mallinckrodt Chemical Works or equivalent.

5.16 Sodium Thiosulfate, Stock Solution, 0.75 N: Dissolve 186.5 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 ml chloroform.

5.17 Sodium Thiosulfate Standard Titrant, 0.0375 N: Prepare by diluting 50.0 ml of stock solution (5.16) to 1.0 liter. Preserve by adding 5 ml of chloroform. Standardize with 0.0375 N potassium biiodate (5.19, or 5.23).

5.18 Sodium Thiosulfate Working Standard (0.0075 N): Transfer 100 ml of sodium thiosulfate standard titrant (5.17) to a 500 ml volumetric flask and dilute to the mark with distilled water. This solution should be prepared fresh daily.

5.19 Potassium Biiodate Standard, 0.0375 N: Dissolve 4.387 g potassium biiodate, previously dried 2 hours at 103°C, in distilled water and dilute to 1.0 liter. Dilute 250 ml to 1.0 liter for 0.0375 N biiodate solution.

5.20 Starch Solution: Prepare an emulsion of 10 g of soluble starch in a mortar or beaker with a small quantity of distilled water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 ml per liter of

chloroform and storage in a 10°C refrigerator. Commercially available dry, powdered starch indicators may be used in place of starch solution.

5.21 Nitrogen Gas: Cylinder

5.22 Potassium Fluoride ($KF \cdot 2H_2O$): ACS Reagent Grade

5.23 Standardization of 0.0375 N Phenylarsine Oxide and 0.0375 N Sodium Thiosulfate: Dissolve approximately 2 g (±1.0 g) KI (5.7) in 100 to 150 ml distilled water; add 10 ml $H_2SO_4$ solution (5.12) followed by 20 ml standard potassium biiodate solution (5.19). Place in dark for 5 minutes, dilute to 300 ml and titrate with the phenylarsine oxide (5.13) or sodium thiosulfate (5.17) to a pale straw color. Add a small scoop of indicator (5.15). Wait until homogeneous blue color develops and continue the titration drop by drop until the color disappears. Run in duplicate. Duplicate determinations should agree within ±0.05 ml.

6. Procedure

6.1 Pretreatment

6.1.1 Add a visible excess of CaO (5.5) to 400 ml of sample. Stir or shake vigorously for approximately 5 minutes. Filter through a dry, moderately retentive filter paper, discarding the first 75 ml.

6.2 Iodide Determination

6.2.1 Place 100 ml of pretreated sample (6.1) or a fraction thereof diluted to that volume, into a 150 ml beaker. Add a Teflon-coated stirring bar and place on a magnetic stirrer. Insert a pH electrode and adjust the pH to approximately 7 or slightly less by the dropwise addition of $H_2SO_4$ solution (5.12).

6.2.2 Transfer the sample to a 250 ml widemouthed conical flask. Wash beaker with small amounts of distilled water and add washings to the flask. A 250 ml iodine flask would increase accuracy and precision by preventing possible loss of the iodine generated upon addition of potassium iodide and sulfuric acid (6.4.1).

6.2.3 Add 15 ml sodium acetate solution (5.8) and 5 ml acetic acid solution (5.1). Mix well. Add 40 ml bromine water solution (5.2); mix well. Wait 5 minutes.

6.2.4 Add 2 ml sodium formate solution (5.10); mix well. Wait 5 minutes.

6.2.5 Purge space above sample with gentle stream of nitrogen (5.21) for approximately 30 seconds to remove bromine fumes.

6.2.6 If a precipitate forms (iron), add 0.5 g $KF \cdot 2H_2O$ (5.22).

6.2.7 A distilled water blank must be run with each set of samples because of iodide in reagents. If the blank is consistently shown to be zero for a particular "lot" of chemicals, it can be ignored.

6.2.8 Proceed to step (6.4).

6.3 Bromide Plus Iodide Determination

6.3.1 Place 100 ml of pretreated sample (6.1) or a fraction thereof diluted to that volume, in a 150 ml beaker. Add 5 g NaCl and stir to dissolve. Neutralize by dropwise addition of HCl solution (5.6) as in (6.2.1). Transfer as in (6.2.2).

6.3.2 Add 20 ml of calcium hypochlorite solution (5.4). Add 1 ml of HCl solution (5.6) and add approximately 0.2 g calcium carbonate (5.3).

6.3.3 Heat to boiling on a hot plate; maintain boiling for 8 minutes.

6.3.4 Remove from hot plate and carefully add 4 ml sodium formate solution (5.10). *Caution: TOO RAPID ADDITION MAY CAUSE FOAMING.* Wash down sides with distilled water.

6.3.5 Return to hot plate and maintain boiling conditions for an additional 8 minutes. Occasionally wash down sides with distilled water if residue is deposited from boiling action.

6.3.6 Remove from hot plate. Wash down sides and allow to cool.

6.3.7 If a precipitate forms (iron), add 0.5 g $KF \cdot 2H_2O$ (5.22).

6.3.8 Add 3 drops sodium molybdate solution (5.11).

6.3.9 A distilled water blank must be run with each set of samples because of iodide, iodate, bromide, and/or bromate in reagents.

6.3.10 Proceed to step (6.4).

6.4 Titration

6.4.1 Dissolve approximately 1 g potassium iodide (5.7) in sample from (6.2.8 or 6.3.10). Add 10 ml of $H_2SO_4$ solution (5.12) and place in dark for 5 minutes.

6.4.2 Titrate with standardized phenylarsine oxide working standard (5.14) or sodium thiosulfate working standard (5.18), adding indicator (5.15, or 5.20) as end point is approached (light straw color). Titrate to colorless solution. Disregard returning blue color.

7. Calculations

7.1 Principle: Iodide is determined by the titration of the sample as oxidized in (6.2): bromide plus iodide is determined by the titration of the sample as oxidized in (6.3). The amount of bromide is then determined by difference. The number of

equivalents of iodine produced a constant of 13,320 as shown in the equation in (7.2). Experimental data is entered in the appropriate place and the equation is solved for mg/l bromide.

7.2 Equation

$$Br(mg/1) = 13{,}320 \left[\left(\frac{A \times B}{C}\right) - \left(\frac{D \times E}{F}\right)\right]$$

where

A = the number of ml of PAO needed to titrate the sample for bromide plus iodide (with the number of ml of PAO needed to titrate the blank subtracted).

B = the normality of the PAO needed to titrate the sample for bromide plus iodide

C = the volume of sample taken (100 ml or a fraction thereof) to be titrated for bromide plus iodide.

D = the number of ml of PAO needed to titrate the sample for iodide (with the number of ml of PAO needed to titrate the blank subtracted). The blank for the iodide titration is often zero.

E = the normality of the PAO used to titrate the sample for iodide.

F = the volume of sample taken (100 ml or a fraction thereof) to be titrated for iodide.

8. Precision and Accuracy

8.1 In a single laboratory (MDQARL), using a mixed domestic and industrial waste effluent, at concentrations of 0.3, 2.8, 5.3, 10.3 and 20.3 mg/l of bromide, the standard deviations were ±0.13, ±0.37, ±0.38, ±0.44 and ±0.42 mg/l, respectively.

8.2 In a single laboratory (MDQARL), using a mixed domestic and industrial waste effluent, at concentrations of 2.8, 5.3, 10.3 and 20.3 mg/l of bromide, recoveries were 96, 83, 97 and 99%, respectively.

## Bibliography

1. ASTM Standards, Part 23, Water; Atmospheric Analysis, p 331-333, Method D1246-C (1973).

# CALCIUM

STORET NO. Calcium (mg/l $CaCO_3$) 00910
Calcium, Total (mg/l Ca) 00916

1. Scope and Application
   1.1 This method is applicable to drinking and surface waters, domestic and industrial wastes.
   1.2 The lower detection limit of this method is approximately 0.5 mg/l as $CaCO_3$; the upper limit can be extended to all concentrations by sample dilution. It is recommended that a sample aliquot containing not more than 25 mg $CaCO_3$ be used.
2. Summary of Method
   2.1 Calcium ion is sequestered upon the addition of disodium dihydrogen ethylenediamine tetraacetate (EDTA). The titration end point is detected by means of an indicator which combines with calcium only.
3. Interferences
   3.1 Strontium and barium interfere and alkalinity in excess of 30 mg/l may cause an indistinct end point. Magnesium interference is reduced or eliminated by raising the pH between 12-13 to precipitate magnesium hydroxide.
4. Precision and Accuracy
   4.1 A synthetic unknown sample containing 108 mg/l Ca, 82 mg/l Mg, 3.1 mg/l K, 19.9 mg/l Na, 241 mg/l chloride, 1.1 mg/l nitrate N, 250 µg/l nitrite N, 259 mg/l sulfate, and 42.5 mg/l total alkalinity in distilled water was determined by this method with a relative standard deviation of 9.2% and a relative error of 1.9% in 44 laboratories.
5. Reference
   5.1 The procedure to be used for this determination is found in: Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 84, Method 110C (1971).

# CHEMICAL OXYGEN DEMAND

STORET NO. 00340

1. Scope and Application

   1.1 The Chemical Oxygen Demand (COD) method determines the quantity of oxygen required to oxidize the organic matter in a waste sample, under specific conditions of oxidizing agent, temperature, and time.

   1.2 Since the test utilizes a rigorous chemical oxidation rather than a biological process, the result has no defineable relationship to the Biochemical Oxygen Demand (BOD) of the waste. The test result should be considered as an independent measurement of organic matter in the sample, rather than as a substitute for the BOD test.

   1.3 The method can be applied to domestic and industrial waste samples having an organic carbon concentration greater than 15 mg/1. For lower concentrations of carbon such as in surface water samples, the Low Level Modification should be used. When the chloride concentration of the sample exceeds 2000 mg/1, the modification for saline waters is required.

2. Summary of Method

   2.1 Organic substances in the sample are oxidized by potassium dichromate in 50% sulfuric acid solution at reflux temperature. Silver sulfate is used as a catalyst and mercuric sulfate is added to remove chloride interference. The excess dichromate is titrated with standard ferrous ammonium sulfate, using orthophenanthroline ferrous complex as an indicator.

3. Comments

   3.1 To reduce loss of volatile organics, the flask should be cooled during addition of the sulfuric acid solution.

4. Precision and Accuracy

   4.1 Eighty-six analysts in fifty-eight laboratories analyzed a distilled water solution containing oxidizable organic material equivalent to 270 mg/1 COD. The standard deviation was ±17.76 mg/l COD with an accuracy as percent relative error (bias) of -4.7%. (EPA Method Research Study 3).

5. References

   5.1 The procedure to be used for this determination is found in:

   Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 495, Method 220 (1971).

   ASTM Standards, Part 23, Water; Atmospheric Analysis, p 470, Method D 1252-67 (1973).

## CHEMICAL OXYGEN DEMAND
(Low Level)

STORET NO. 00335

1. Scope and Application
   1.1 The scope of this modification of the Chemical Oxygen Demand (COD) test is the same as for the high level test. It is applicable to the analysis of surface waters, domestic and industrial wastes with low demand characteristics.
   1.2 This method (low level) is applicable for samples having a COD in the range of 5-50 mg/l COD.
2. Summary of Method
   2.1 Organic and oxidizable inorganic substances in an aqueous sample are oxidized by potassium dichromate solution in 50 percent (by volume) sulfuric acid in solution. The excess dichromate is titrated with standard ferrous ammonium sulfate using orthophenanthroline ferrous complex (ferroin) as an indicator.
3. Sampling and Preservation
   3.1 Collect the samples in glass bottles, if possible. Use of plastic containers is permissible if it is known that no organic contaminants are present in the containers.
   3.2 Biologically active samples should be tested as soon as possible. Samples containing settleable material should be well mixed, preferably homogenized, to permit removal of representative aliquots.
   3.3 Samples may be preserved with sulfuric acid at a rate of 2 ml of conc. $H_2SO_4$ per liter of sample.
4. Interferences
   4.1 Traces of organic material either from the glassware or atmosphere may cause a gross, positive error.
      4.1.1 Extreme care should be exercised to avoid inclusion of organic materials in the distilled water used for reagent preparation or sample dilution.
      4.1.2 Glassware used in the test should be conditioned by running blank procedures to eliminate traces of organic material.
   4.2 Volatile materials may be lost when the sample temperature rises during the sulfuric acid addition step.
   4.3 Chlorides are quantitatively oxidized by dichromate and represent a positive interference. Mercuric sulfate is added to the digestion flask to complex the

chlorides, thereby effectively eliminating the interference on all but brine and estuarine samples.

5. Apparatus

5.1 Reflux apparatus: Glassware should consist of a 500 ml Erlenmeyer flask or a 300 ml round bottom flask made of heat-resistant glass connected to a 12 inch Allihn condenser by means of a ground glass joint. Any equivalent reflux apparatus may be substituted provided that a ground-glass connection is used between the flask and the condenser.

6.1 Distilled water: Special precautions should be taken to insure that distilled water used in this test be low in organic matter.

6.2 Standard potassium dichromate solution (0.025 N): Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 103°C for two hours, in distilled water and dilute to 1000 ml. Mix this solution thoroughly then dilute 100.0 ml to 1000 ml with distilled water.

6.3 Sulfuric acid reagent: Conc. $H_2SO_4$ containing 23.5 g silver sulfate, $Ag_2SO_4$, per 9 lb. bottle (one to two days required for dissolution).

6.4 Standard ferrous ammonium sulfate (0.025 N): Dissolve 98 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 ml of conc. $H_2SO_4$ (6.8), cool and dilute to 1 liter. Dilute 100 ml of this solution to 1 liter with distilled water. This solution must be standardized daily against $K_2Cr_2O_7$ solution.

6.4.1 Standardization: To 15 ml of distilled water add 10.0 ml of 0.025 N $K_2Cr_2O_7$ (6.2) solution. Add 20 ml of $H_2SO_4$ (6.8) and cool. Titrate with ferrous ammonium sulfate (6.4) using 1 drop of ferroin indicator (6.6). The color change is sharp, going from blue-green to reddish-brown.

$$\text{Normality} = \frac{(\text{ml } K_2Cr_2O_7)\,(0.025)}{\text{ml Fe } (NH_4)_2\,(SO_4)_2}$$

6.5 Mercuric sulfate : Powdered $HgSO_4$.

6.6 Phenanthroline ferrous sulfate (ferroin) indicator solution: Dissolve 1.48 g of 1-10 (ortho)phenanthroline monohydrate, together with 0.70 g of $FeSO_4 \cdot 7H_2O$ in 100 ml of water. This indicator may be purchased already prepared.

6.7 Silver sulfate : Powdered $Ag_2SO_4$.

6.8 Sulfuric acid (sp. gr. 1.84) : Concentrated $H_2SO_4$.

7. Procedure

7.1 Place several boiling stones in the reflux flask, followed by 1 g of $HgSO_4$ (6.5). Add 5.0 ml conc. $H_2SO_4$ (6.8); swirl until mercuric sulfate has dissolved. Place

reflux flask in an ice bath and slowly add, with swirling, 25.0 ml of 0.025 N $K_2Cr_2O_7$ (6.2). Now add 70 ml of sulfuric acid-silver sulfate solution (6.3) to the cooled reflux flask, again using slow addition with swirling motion.

7.2 With the reflux flask still in the ice bath, place 50.0 ml of sample or an aliquot diluted to 50.0 ml into the reflux flask.

*Caution:* Care must be taken to assure that the contents of the flask are well mixed. If not, superheating may result, and the mixture may be blown out of the open end of the condenser. Attach the flask to the condenser and start the cooling water.

7.3 Apply heat to the flask and reflux for 2 hours. For some waste waters, the 2-hour reflux period is not necessary. The time required to give the maximum oxidation for a wastewater of constant or known composition may be determined and a shorter period of refluxing may be permissible.

7.4 Allow the flask to cool and wash down the condenser with about 25 ml of distilled water. If a round bottom flask has been used, transfer the mixture to a 500 ml Erlenmeyer flask, washing out the reflux flask 3 or 4 times with distilled water. Dilute the acid solution to about 300 ml with distilled water and allow the solution to cool to about room temperature. Add 8 to 10 drops of ferroin indicator (6.6) to the solution and titrate the excess dichromate with 0.025 N ferrous ammonium sulfate (6.4) solution to the end point. The color change will be sharp, changing from a blue-green to a reddish hue.

7.5 Blank – Simultaneously run a blank determination following the details given in (7.1) and (7.2), but using low COD water in place of sample.

8. Calculation

8.1 Calculate the COD in the sample in mg/l as follows:

$$\text{COD, mg/liter} = \frac{(A-B)N \times 8000}{S}$$

where

A = milliliters of $Fe(NH_4)_2(SO_4)_2$ solution required for titration of the blank,

B = milliliters of $Fe(NH_4)_2(SO_4)_2$ solution required for titration of the sample,

N = normality of the $Fe(NH_4)_2(SO_4)_2$ solution, and

S = milliliters of sample used for the test.

9. Precision and Accuracy

9.1 Eighty-six analysts in fifty-eight laboratories analyzed a distilled water solution containing oxidizable organic material equivalent to 12.3 mg/l COD. The standard deviation was ±4.15 mg/l COD with an accuracy as percent relative error (bias) of 0.3%. (EPA Method Research Study 3.)

## CHEMICAL OXYGEN DEMAND
(High Level for Saline Waters)

STORET NO. 00340

1. Scope and Application

   1.1 When the chloride level exceeds 1000 mg/1 the minimum accepted value for the COD will be 250 mg/1. COD levels which fall below this value are highly questionable because of the high chloride correction which must be made.

2. Summary of Method

   2.1 Organic and oxidizable inorganic substances in an aqueous sample are oxidized by potassium dichromate solution in 50 percent (by volume) sulfuric acid solution. The excess dichromate is titrated with standard ferrous ammonium sulfate using orthophenanthroline ferrous complex (ferroin) as an indicator.

3. Sample Handling and Preservation

   3.1 Collect the samples in glass bottles, if possible. Use of plastic containers is permissible if it is known that no organic contaminants are present in the containers.

   3.2 Biologically active samples should be tested as soon as possible. Samples containing settleable material should be well mixed, preferably homogenized, to permit removal of representative aliquots.

   3.3 Samples are preserved by the addition of 2 ml of conc. $H_2SO_4$ per liter of sample.

4. Interferences

   4.1 Traces of organic material either from the glassware or atmosphere may cause a gross, positive error.

      4.1.1 Extreme care should be exercised to avoid inclusion of organic materials in the distilled water used for reagent preparation or sample dilution.

      4.1.2 Glassware used in the test should be conditioned by running blank procedures to eliminate traces of organic material.

   4.2 Volatile materials may be lost when the sample temperature rises during the sulfuric acid addition step.

   4.3 Chlorides are quantitatively oxidized by dichromate and represent a positive interference. Mercuric sulfate is added to the digestion flask to complex the chlorides, thereby effectively eliminating the interference on all but brine samples.

5. Apparatus

5.1 Reflux apparatus: Glassware should consist of a 500 ml Erlenmeyer flask or a 300 ml round bottom flask made of heat-resistant glass connected to a 12 inch Allihn condenser by means of a ground glass joint. Any equivalent reflux apparatus may be substituted provided that a ground-glass connection is used between the flask and the condenser.

6. Reagents

6.1 Standard potassium dichromate solution, (0.25 N): Dissolve 12.2588 g of $K_2Cr_2O_7$, primary standard grade, previously dried for 2 hours at 103°C in water and dilute to 1000 ml.

6.2 Sulfuric acid reagent: Conc. $H_2SO_4$ containing 23.5 g silver sulfate, $Ag_2SO_4$, per 9 lb. bottle (1 to 2 days required for dissolution).

6.3 Standard ferrous ammonium sulfate, 0.250 N; Dissolve 98 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 ml of conc. $H_2SO_4$, (6.7), cool and dilute to 1 liter. This solution must be standardized against the standard potassium dichromate solution (6.1) daily.

6.3.1 Standardization: Dilute 25.0 ml of standard dichromate solution (6.1) to about 250 ml with distilled water. Add 75 ml conc. sulfuric acid (6.7). Cool, then titrate with ferrous ammonium sulfate titrant (6.3), using 10 drops of ferroin indicator (6.5).

$$\text{Normality} = \frac{(\text{ml } K_2Cr_2O_7)\,(0.25)}{\text{ml Fe } (NH_4)_2\,(SO_4)_2}$$

6.4 Mercuric sulfate : Powdered $HgSO_4$.

6.5 Phenanthroline ferrous sulfate (ferroin) indicator solution: Dissolve 1.48 g of 1-10-(ortho)-phenanthroline monohydrate, together with 0.70 g of $FeSO_4 \cdot 7H_2O$ in 100 ml of water. This indicator may be purchased already prepared.

6.6 Silver sulfate : Powdered $Ag_2SO_4$.

6.7 Sulfuric acid (sp. gr. 1.84) : Concentrated $H_2SO_4$.

7. Procedure

7.1 Pipet a 50.0 ml aliquot of sample not to exceed 800 mg/l of COD into a 500 ml, flat bottom, Erlenmeyer flask. Add 25.0 ml of 0.25 N $K_2Cr_2O_7$ (6.1), then 5 ml of conc. $H_2SO_4$ (6.7). Add $HgSO_4$ (6.4) in the ratio of 10 mg to 1 mg chloride, based upon the mg of chloride in the sample aliquot. Swirl until all the mercuric sulfate has dissolved. Carefully add 70 ml of sulfuric acid-silver sulfate solution

(6.2) and gently swirl until the solution is thoroughly mixed. Glass beads should be added to the reflux mixture to prevent bumping, which can be severe and dangerous.

*Caution:* The reflux mixture must be thoroughly mixed before heat is applied. If this is not done, local heating occurs in the bottom of the flask, and the mixture may be blown out of the condenser.

7.1.1 If volatile organics are present in the sample, use an Allihn condenser and add the sulfuric acid-silver sulfate solution through the condenser, while cooling the flask, to reduce loss by volatilization.

7.2 Attach the flask to the condenser and reflux the mixture for two hours.

7.3 Cool, and wash down the interior of the condenser with 25 ml of distilled water. Disconnect the condenser and wash the flask and condenser joint with 25 ml of distilled water so that the total volume is 350 ml. Cool to room temperature.

7.4 Titrate with standard ferrous ammonium sulfate (6.3) using 10 drops of ferroin (6.5) indicator. (This amount must not vary from blank, sample and standardization). The color change is sharp, going from blue-green to reddish-brown and should be taken as the end point although the blue-green color may reappear within minutes.

7.5 Run a blank, using 50 ml of distilled water in place of the sample together with all reagents and subsequent treatment.

7.6 For COD values greater than 800 mg/l, a smaller aliquot of sample should be taken; however, the volume should be readjusted to 50 ml with distilled water having a chloride concentration equal to the sample.

7.7 Chloride correction [1]: Prepare a standard curve of COD versus mg/l of chloride, using sodium chloride solutions of varying concentrations following exactly the procedure outlined. The chloride interval, as a minimum should be 4000 mg/l up to 20,000 mg/l chloride. Lesser intervals of greater concentrations must be run as per the requirements of the data, but in no case must extrapolation be used.

8. Calculation

8.1 $$\text{mg/l COD} = \frac{[(A-B)\,C \times 8{,}000] - 50D}{\text{ml sample}} \times 1.2$$

Where:

COD = chemical oxygen demand from dichromate

A = ml Fe $(NH_4)_2$ $(SO_4)_2$ for blank;

B = ml Fe $(NH_4)_2$ $(SO_4)_2$ for sample;

C = normality of Fe $(NH_4)_2$ $(SO_4)_2$;

D = chloride correction from curve (step 7.7)

1.2 = compensation factor to account for the extent of chloride oxidation which is dissimilar in systems containing organic and non-organic material.

9. Precision and Accuracy.

9.1 Precision and accuracy data are not available at this time.

## Bibliography

1. Burns, E. R., Marshall, C., Journal WPCF, Vol. 37, p 1716-1721 (1965).

# CHLORIDE

STORET NO. 00940

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The method is suitable for all concentration ranges of chloride content; however, in order to avoid large titration volumes, use a sample aliquot containing not more than 10 to 20 mg Cl per 50 ml.
   1.3 Automated titration may be used.
2. Summary of Method
   2.1 Dilute mercuric nitrate solution is added to an acidified sample in the presence of mixed diphenylcarbazone-bromophenol blue indicator. The end point of the titration is the formation of the blue-violet mercury diphenylcarbazone complex.
3. Comments
   3.1 Anions and cations at concentrations normally found in surface waters do not interfere.
   3.2 Sulfites interfere. If presence is suspected, oxidize by treating 50 ml of sample with 0.5 to 1 ml of $H_2O_2$.
4. Precision and Accuracy
   4.1 Forty-two analysts in eighteen laboratories analyzed synthetic water samples containing exact increments of chloride, with the following results:

| Increment as Chloride mg/liter | Precision as Standard Deviation mg/liter | Accuracy as Bias, % | Accuracy as Bias, mg/liter |
|---|---|---|---|
| 17 | 1.54 | +2.16 | +0.4 |
| 18 | 1.32 | +3.50 | +0.6 |
| 91 | 2.92 | +0.11 | +0.1 |
| 97 | 3.16 | - 0.51 | - 0.5 |
| 382 | 11.70 | - 0.61 | - 2.3 |
| 398 | 11.80 | - 1.19 | - 4.7 |

(FWPCA Method Study 1, Mineral and Physical Analyses)

4.2 In a single laboratory (MDQARL), using surface water samples at an average concentration of 34 mg Cl/l, the standard deviation was ±1.0.

5. Reference

5.1 The procedure to be used for this determination is found in:
ASTM Standards, Part 23, Water; Atmospheric Analysis, p 273, Method 512-67, Referee Method A (1973).

# CHLORIDE
## (Automated)

STORET NO. 00940

1. Scope and Application
   1.1 This automated method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The applicable range is 1 to 250 mg Cl/1. Approximately 15 samples per hour can be analyzed.
2. Summary of Method
   2.1 Thiocyanate ion (SCN) is liberated from mercuric thiocyanate, through sequestration of mercury by chloride ion to form un-ionized mercuric chloride. In the presence of ferric ion, the liberated SCN forms highly colored ferric thiocyanate, in concentration proportional to the original chloride concentration.
3. Sample Handling and Preservation
   3.1 No special requirements.
4. Interferences
   4.1 No significant interferences.
5. Apparatus
   5.1 Technicon AutoAnalyzer consisting of:
      5.1.1 Sampler I.
      5.1.2 Continuous filter.
      5.1.3 Manifold.
      5.1.4 Proportioning pump.
      5.1.5 Colorimeter equipped with 15 mm tubular flow cell and 480 nm filters.
      5.1.6 Recorder.
6. Reagents
   6.1 Ferric Ammonium Sulfate: Dissolve 60 g of $FeNH_4(SO_4)_2 \cdot 12H_2O$ in approximately 500 ml distilled water. Add 355 ml of conc. $HNO_3$ and dilute to 1 liter with distilled water. Filter.
   6.2 Saturated Mercuric Thiocyanate: Dissolve 5 g of $Hg(SCN)_2$ in 1 liter of distilled water. Decant and filter a portion of the saturated supernatant liquid to use as the reagent and refill the bottle with distilled water.
   6.3 Stock Solution (0.0141 N NaCl): Dissolve 0.8241 g of pre-dried (140°C) NaCl in distilled water. Dilute to 1 liter in a volumetric flask. 1 ml = 0.5 mg Cl.

6.3.1 Prepare a series of standards by diluting suitable volumes of stock solution to 100.0 ml with distilled water. The following dilutions are suggested:

| ml of Stock Solution | Conc., mg/l |
|---|---|
| 1.0 | 5.0 |
| 2.0 | 10.0 |
| 4.0 | 20.0 |
| 8.0 | 40.0 |
| 15.0 | 75.0 |
| 20.0 | 100.0 |
| 30.0 | 150.0 |
| 40.0 | 200.0 |
| 50.0 | 250.0 |

7. Procedure

7.1 No advance sample preparation is required. Set up manifold as shown in Figure 1. For water samples known to be consistently low in chloride content, it is advisable to use only one distilled water intake line.

7.2 Allow both colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on colorimeter to obtain stable baseline.

7.3 Place distilled water wash tubes in alternate openings in sampler and set sample timing at 2.0 minutes.

7.4 Place working standards in sampler in order of decreasing concentrations. Complete filling of sampler tray with unknown samples.

7.5 Switch sample line from distilled water to sampler and begin analysis.

8. Calculation

8.1 Prepare standard curve by plotting peak heights of processed standards against known concentrations. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (MDQARL), using surface water samples at concentrations of 1, 100, and 250 mg Cl/1, the standard deviation was ±0.3.

9.2 In a single laboratory (MDQARL), using surface water samples at concentrations of 10 and 100 mg Cl/1, recoveries were 97% and 104%, respectively.

## Bibliography

1. J. E. O'Brien, "Automatic Analysis of Chlorides in Sewage," Waste Engr., 33, 670-672 (Dec. 1962).

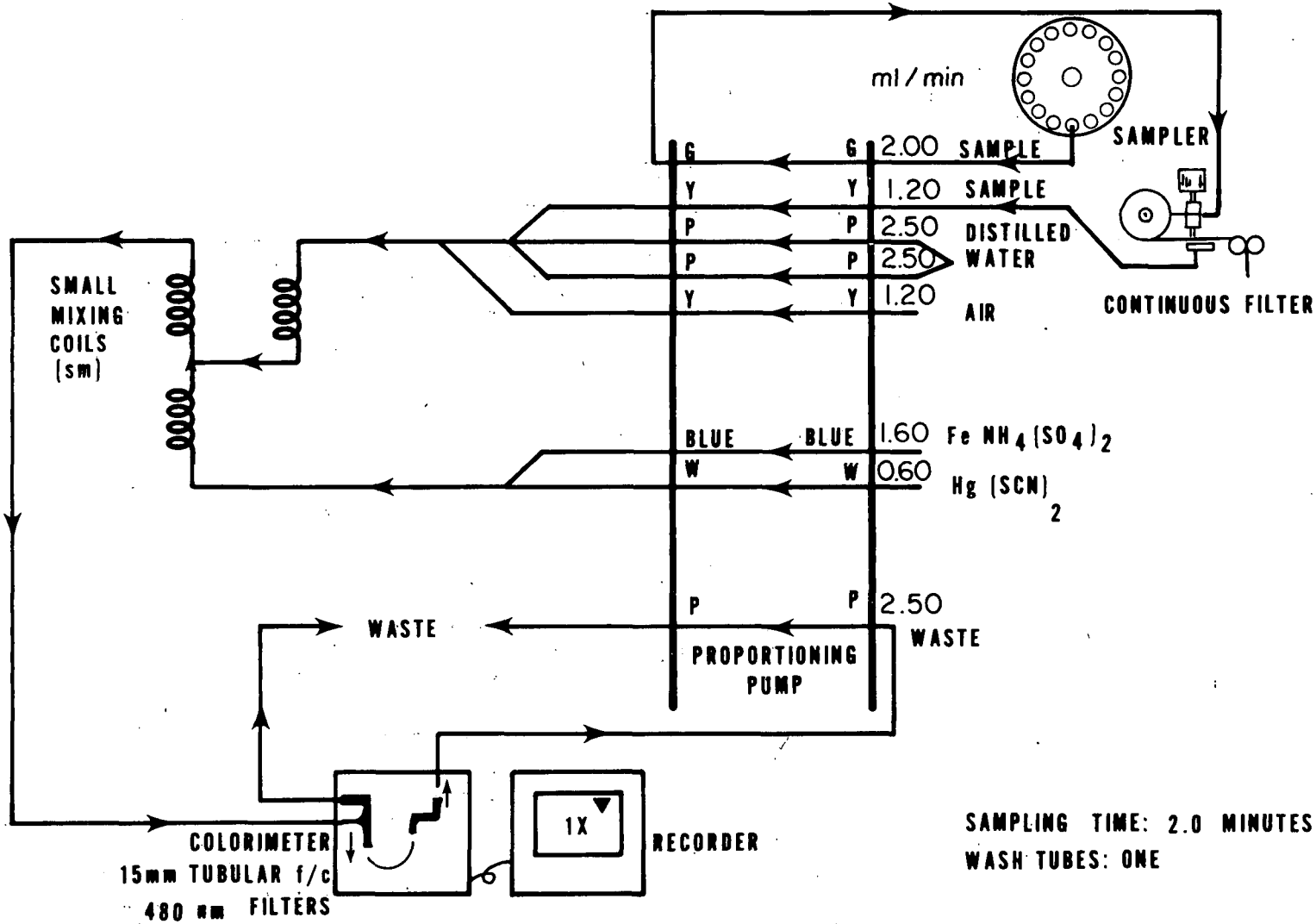



FIGURE 1. CHLORIDE MANIFOLD AA-I

## CHLORINE, Total Residual

STORET NO. 50060

1. Scope and Application
   1.1 The Amperometric Titration method is applicable to all types of waters and wastes that do not contain a substantial amount of organic matter. This method cannot be used for samples containing above 5 mg/1 total residual chlorine.
2. Summary of Method
   2.1 Phenylarsine oxide is titrated into a buffered sample contained in an amperometric titration cell until the generation of current ceases. Potassium iodide is added when chlorine is present as a chloramine.
   2.2 In the iodometric titration, chlorine liberates free iodine from potassium iodide solutions when its pH is 8 or less. The liberated iodine is titrated with a standard solution of sodium thiosulfate or phenylarsine oxide with starch as an indicator.
3. Interferences
   3.1 Samples containing significant amounts of organic matter interfere with the amperometric titration and the iodometric method must be used.
   3.2 The amperometric titration is not subject to interference from color, turbidity, iron, manganese, or nitrite nitrogen.
4. Sample Handling and Preservation
   4.1 Chlorine determinations must be started immediately after sampling, avoiding excessive light and agitation. Samples to be analyzed for chlorine cannot be stored.
5. Reference
   5.1 The procedure to be used for this determination is found in:
   Standard Methods for the Examination of Water and wastewater, 13th Edition, p 382, Method 204A (1971).
   ASTM Standards, Part 23, Water; Atmospheric Analysis, p 280, Method D1253-68 (1973).

# COLOR

## (Platinum-Cobalt)

STORET NO. 00080

1. Scope and Application

   1.1 The Platinum-Cobalt method is useful for measuring color of water derived from naturally occurring materials, i.e., vegetable residues such as leaves, barks, roots, humus and peat materials. *The method is not applicable to color measurement on waters containing highly colored industrial wastes.*

   NOTE 1: The Spectrophotometric and Tristimulus methods are useful for detecting specific color problems. The use of these methods, however, is laborious and unless determination of the hue, purity, and luminance is desired, they are of limited value.

2. Summary of Method

   2.1 Color is measured by visual comparison of the sample with platinum-cobalt standards. One unit of color is that produced by 1 mg/l platinum in the form of the chloroplatinate ion.

3. Interferences

   3.1 Since very slight amounts of turbidity interfere with the determination, samples showing visible turbidity should be clarified by centrifugation.

   3.2 Method is pH dependent.

4. Sample Handling and Preservation

   4.1 Representative samples shall be taken in scrupulously clean glassware.

   4.2 Since biological activity may change the color characteristics of a sample, the determination should be made as soon as possible. Refrigeration at 4°C is recommended.

5. Apparatus

   5.1 Nessler tubes : Matched, tall form, 50 ml capacity.

6. Reagents

   6.1 Standard chloroplatinate solution: Dissolve 1.246 g potassium chlorplatinate, $K_2PtCl_6$, (equivalent to 0.500 g metallic Pt) and 1 g crystalline cobaltous chloride, $CoCl_2 \cdot 6H_2O$, in distilled water containing 100 ml of conc. HCl. Dilute to 1000 ml with distilled water. This standard solution is equivalent to 500 color units.

7. Preparation of Standards

7.1 Prepare standards in increments from 5 to 70 units. The following series is suggested:

| ml of Standard Solution Diluted to 50.0 ml with Distilled Water | Color in Chloroplatinate Units |
|---|---|
| 0.0 | 0 |
| 0.5 | 5 |
| 1.0 | 10 |
| 1.5 | 15 |
| 2.0 | 20 |
| 2.5 | 25 |
| 3.0 | 30 |
| 3.5 | 35 |
| 4.0 | 40 |
| 4.5 | 45 |
| 5.0 | 50 |
| 6.0 | 60 |
| 7.0 | 70 |

7.2 Protect these standards against evaporation and contamination by use of clean, inert stoppers.

NOTE 2: The standards also must be protected against the absorption of ammonia since an increase in color will result.

8. Procedure

8.1 Apparent color: Observe the color of the sample by filling a matched Nessler tube to the 50 ml mark with the water and compare with standards. This comparison is made by looking vertically downward through the tubes toward a white or specular surface placed at such an angle that light is reflected upward through the columns of liquid. If turbidity has not been removed by the procedure given in (8.2), report the color as "apparent color". If the color exceeds 70 units, dilute the sample with distilled water in known proportions until the color is within the range of the standards.

8.2 True color: Remove turbidity by centrifuging the sample until the supernatant is clear. The time required will depend upon the nature of the sample, the speed of the motor, and the radius of the centrifuge, but rarely will more than one hour be necessary. Compare the centrifuged sample with distilled water to insure that turbidity has been removed. If the sample is clear, then compare with standards as given in (8.1).

9. Calculation

9.1 Calculate the color units by means of the following equation:

$$\text{Color units} = \frac{A \times 50}{V}$$

where:

A = estimated color of diluted sample.

V = ml sample taken for dilution.

9.2 Report the results in whole numbers as follows:

| Color Units | Record to Nearest |
|---|---|
| 1-50 | 1 |
| 51-100 | 5 |
| 101-250 | 10 |
| 251-500 | 20 |

10. Precision and Accuracy

10.1 Precision and accuracy data are not available at this time.

11. Reference

11.1 The procedure to be used for this determination is found in:

Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 160, Method 118 (1971).

# COLOR

(Spectrophotometric)

STORET NO. 00080

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. It must be used for industrial wastes that cannot be determined by the Platinum-Cobalt method.
2. Summary of Method
   2.1 Color characteristics are measured at pH 7.6 and at the original pH by obtaining the visible absorption spectrum of the sample on a spectrophotometer. The percent transmission at certain selected wavelengths is used to calculate the results.
   2.2 The results are expressed in terms of dominant wavelength, hue, luminance, and purity.
3. Interferences
   3.1 Since very slight amounts of turbidity interfere with the determination, samples must be filtered before analysis.
4. Sample Handling and Preservation
   4.1 Since biological activity may change the color characteristics of a sample, the determination should be made as soon as possible. Refrigeration at 4°C is recommended.
5. Reference
   5.1 The procedure to be used for this determinationis found in:
   Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 391, Method 206A (1971).

CYANIDE, Total

STORET NO. 00720

1. Scope and Application
   1.1 This method is applicable to the determination of cyanide in drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The titration procedure using silver nitrate with p-dimethylamino-benzal-rhodanine indicator is used for measuring concentrations of cyanide exceeding 1 mg/1 (0.2 mg/200 ml of absorbing liquid).
   1.3 The colorimetric procedure is used for concentrations below 1 mg/1 of cyanide and is sensitive to about 0.02 mg/1.
2. Summary of Method
   2.1 The cyanide as hydrocyanic acid (HCN) is released from cyanide complexes by means of a reflux-distillation operation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined by volumetric titration or colorimetrically.
   2.2 In the colorimetric measurement the cyanide is converted to cyanogen chloride, CNCl, by reaction with chloramine-T at a pH less than 8 without hydrolyzing to the cyanate. After the reaction is complete, color is formed on the addition of pyridine-pyrazolone or pyridine-barbituric acid reagent. The absorbance is read at 620 nm when using pyridine-pyrazolone or 578 nm for pyridine-barbituric acid. To obtain colors of comparable intensity, it is essential to have the same salt content in both the sample and the standards.
   2.3 The titrimetric measurement uses a standard solution of silver nitrate to titrate cyanide in the presence of a silver sensitive indicator.
3. Definitions
   3.1 Cyanide is defined as cyanide ion and complex cyanides converted to hydrocyanic acid (HCN) by reaction in a reflux system of a mineral acid in the presence of cuprous ion.
4. Sample Handling and Preservation
   4.1 The sample should be collected in plastic bottles of 1 liter or larger size. All bottles must be thoroughly cleansed and thoroughly rinsed to remove soluble material from containers.
   4.2 Samples must be preserved with 2 ml of 10 N sodium hydroxide per liter of sample ($pH \geq 12$) at the time of collection.

4.3 Samples should be analyzed as rapidly as possible after collection. If storage is required, the samples should be stored in a refrigerator or in an ice chest filled with water and ice to maintain temperature at 4°C.

4.4 Oxidizing agents such as chlorine decompose most of the cyanides. Test a drop of the sample with potassium iodide-starch test paper (KI-starch paper); a blue color indicates the need for treatment. Add ascorbic acid, a few crystals at a time, until a drop of sample produces no color on the indicator paper. Then add an additional 0.6 g of ascorbic acid for each liter of sample volume.

5. Interferences

5.1 Interferences are eliminated or reduced by using the distillation procedure described in Procedure (8.1 through 8.5).

5.2 Sulfides adversely affect the colorimetric and titration procedures. If a drop of the sample on lead acetate test paper indicates the presence of sulfides, treat 25 ml more of the stabilized sample (pH≥12) than that required for the cyanide determination with powdered cadmium carbonate. Yellow cadmium sulfide precipitates if the sample contains sulfide. Repeat this operation until a drop of the treated sample solution does not darken the lead acetate test paper. Filter the solution through a dry filter paper into a dry beaker, and from the filtrate, measure the sample to be used for analysis. Avoid a large excess of cadmium and a long contact time in order to minimize a loss by complexation or occlusion of cyanide on the precipitated material.

5.3 Fatty acids will distill and form soaps under the alkaline titration conditions, making the end point almost impossible to detect.

5.3.1 Acidify the sample with acetic acid (1+9) to pH 6.0 to 7.0.

*Caution:* This operation must be performed in the hood and the sample left there until it can be made alkaline again after the extraction has been performed.

5.3.2 Extract with iso-octane, hexane, or chloroform (preference in order named) with a solvent volume equal to 20% of the sample volume. One extraction is usually adequate to reduce the fatty acids below the interference level. Avoid multiple extractions or a long contact time at low pH in order to keep the loss of HCN at a minimum. When the extraction is completed, immediately raise the pH of the sample to above 12 with NaOH solution.

6. Apparatus

6.1 Reflux distillation apparatus such as shown in Figure 1 or Figure 2. The boiling

flask should be of 1 liter size with inlet tube and provision for condenser. The gas absorber may be a Fisher-Milligan scrubber.

6.2 Microburet, 5.0 ml (for titration).

6.3 Spectrophotometer suitable for measurements at 578 nm or 620 nm with a 1.0 cm cell or larger.

7. Reagents

7.1 Sodium hydroxide solution: Dissolve 50 g of NaOH in distilled water, and dilute to 1 liter with distilled water.

7.2 Cadmium carbonate: powdered.

7.3 Ascorbic acid: crystals.

7.4 Cuprous Chloride Reagent: Weigh 20 g of finely powdered $Cu_2Cl_2$ into an 800 ml beaker. Wash twice, by decantation, with 250 ml portions of dilute sulfuric acid ($H_2SO_4$, 1 + 49) and then twice with water. Add about 250 ml of water and then hydrochloric acid (HCl, sp gr 1.19) in 1/2 ml portions until the salt dissolves (See Note 1). Dilute to 1 liter with distilled water and store in a tightly stoppered bottle containing a few lengths of pure copper wire or rod extending from the bottom to the mouth of the bottle (See Note 2).

Note 1: The reagent should be clear; dark discoloration indicates the presence of cupric salts.

Note 2: If it is desired to use a reagent bottle of smaller volume, it should be kept completely filled and tightly stoppered. Refill it from the stock solution after each use.

7.5 Sulfuric acid: concentrated.

7.6 Sodium dihydrogenphosphate, 1 M: Dissolve 138 g of $NaH_2PO_4 \cdot H_2O$ in 1 liter of distilled water. Refrigerate this solution.

7.7 Stock cyanide solution: Dissolve 2.51 g of KCN and 2 g KOH in 1 liter of distilled water. Standardize with 0.0192 N $AgNO_3$. Dilute to appropriate concentration so that 1 ml = 1 mg CN.

7.8 Standard cyanide solution, intermediate: Dilute 10.0 ml of stock (1 ml = 1 mg CN) to 1000 ml with distilled water (1 ml = 10$\mu$g).

7.9 Standard cyanide solution: Prepare fresh daily by diluting 100.0 ml of intermediate cyanide solution to 1000 ml with distilled water and store in a glass stoppered bottle. 1 ml = 1.0$\mu$g CN (1.0 mg/1).

7.10 Standard silver nitrate solution, 0.0192 N: Prepare by crushing approximately 5 g $AgNO_3$ crystals and drying to constant weight at 40°C. Weigh out 3.2647 g of dried $AgNO_3$, dissolve in distilled water, and dilute to 1000 ml (1 ml = mg CN).

7.11 Rhodanine indicator: Dissolve 20 mg of p-dimethyl-amino-benzalrhodanine in 100 ml of acetone.

7.12 Chloramine T solution: Dissolve 1.0 g of white, water soluble Chloramine T in 100 ml of distilled water and refrigerate until ready to use. Prepare fresh weekly.

7.13 Color Reagent – One of the following may be used:

7.13.1 Pyridine-Barbituric Acid Reagent: Place 15 g of barbituric acid in a 250 ml volumetric flask and add just enough distilled water to wash the sides of the flask and wet the barbituric acid. Add 75 ml of pyridine and mix. Add 15 ml of HCl (sp gr 1.19), mix, and cool to room temperature. Dilute to 250 ml with distilled water and mix. This reagent is stable for approximately six months if stored in a cool, dark plate.

7.13.2 Pyridine-pyrazolone solution:

7.13.2.1 3-Methyl-1-phenyl-2-pyrazolin-5-one reagent, saturated solution. Add 0.25 g of 3-methyl-1-phenyl-2-pyrazolin-5-one to 50 ml of distilled water, heat to 60°C with stirring. Cool to room temperature.

7.13.2.2 3,3′Dimethyl-1,1′-diphenyl-[4,4′-bi-2 pyrazoline]-5,5′dione (bispyrazolone). Dissolve 0.01 g of bispyrazolone in 10 ml of pyridine.

7.13.2.3 Pour solution (7.13.2.1) through nonacid-washed filter paper. Collect the filtrate. Through the same filter paper pour solution (7.13.2.2) collecting the filtrate in the same container as filtrate from (7.13.2.1). Mix until the filtrates are homogeneous. The mixed reagent develops a pink color but this does not affect the color production with cyanide if used within 24 hours of preparation.

8. Procedure

8.1 Place 500 ml of sample, or an aliquot diluted to 500 ml in the 1 liter boiling flask. Add 50 ml of sodium hydroxide (7.1) to the absorbing tube and dilute if necessary with distilled water to obtain an adequate depth of liquid in the absorber. Connect the boiling flask, condenser, absorber and trap in the train.

8.2 Start a slow stream of air entering the boiling flask by adjusting the vacuum source. Adjust the vacuum so that approximately one bubble of air per second enters the boiling flask through the air inlet tube.

*Caution:* The bubble rate will not remain constant after the reagents have been added and while heat is being applied to the flask. It will be necessary to readjust the air rate occasionally to prevent the solution in the boiling flask from backing up into the air inlet tube.

8.3 Slowly add 25 ml conc. sulfuric acid (7.5) through the air inlet tube. Rinse the tube with distilled water and allow the airflow to mix the flask contents for 3 min. Pour 10 ml of $Cu_2Cl_2$ reagent (7.4) into the air inlet and wash down with a stream of water.

8.4 Heat the solution to boiling, taking care to prevent the solution from backing up into and overflowing from the air inlet tube. Reflux for one hour. Turn off heat and continue the airflow for at least 15 minutes. After cooling the boiling flask, disconnect absorber and close off the vacuum source.

8.5 Drain the solution from the absorber into a volumetric flask and bring up to volume with distilled water washings from the absorber tube.

8.6 Withdraw 50 ml of the solution from the volumetric flask and transfer to a 100 ml volumetric flask. Add 15 ml of sodium phosphate solution (7.6) and 2.0 ml of Chloramine T solution (7.12) and mix. Immediately add 5.0 ml pyridine-barbituric acid solution (7.13.1), or pyridine-pyrazolone solution (7.13.2.3), mix and bring to mark with distilled water and mix again.

8.7 For pyridine-pyrazolone solution allow 40 minutes for color development then read absorbance at 620 nm in a 1 cm cell. When using pyridine-barbituric acid, allow 8 minutes for color development then read absorbance at 578 nm in a 1.0 cm cell within 15 minutes.

8.8 Prepare a series of standards by diluting suitable volumes of standard solution to 500.0 ml with distilled water as follows:

| ml of Standard Solution (1.0 = 1μg CN) | Conc., When Diluted to 500 ml, mg/1 CN |
|---|---|
| 0 (Blank) | 0 |
| 5.0 | 0.01 |
| 10.0 | 0.02 |
| 20.0 | 0.04 |
| 50.0 | 0.10 |
| 100.0 | 0.20 |
| 150.0 | 0.30 |
| 200.0 | 0.40 |

8.8.1 Standards must be treated in the same manner as the samples, as outlined in (8.1) through (8.7) above.

8.8.2 Prepare a standard curve by plotting absorbance of standard vs. cyanide concentrations.

8.8.3 Subsequently, at least two standards (a high and a low) should be treated as in (8.8.1) to verify standard curve. If results are not comparable (±20%), a complete new standard curve must be prepared.

8.8.4 To check the efficiency of the sample distillation, add an increment of cyanide from either the intermediate standard (7.8) or the working standard (7.9) to insure a level of 20μg/1 or a significant increase in absorbance value. Proceed with the analysis as in Procedure (8.8.1) using the same flask and system from which the previous sample was just distilled.

8.9 Alternatively, if the sample contains more than 1 mg of CN transfer the distillate, or a suitable aliquot diluted to 250 ml, to a 500 ml Erlenmeyer flask. Add 10-12 drops of the benzalrhodanine indicator.

8.10 Titrate with standard silver nitrate to the first change in color from yellow to brownish-pink. Titrate a distilled water blank using the same amount of sodium hydroxide and indicator as in the sample.

8.11 The analyst should familiarize himself with the end point of the titration and the amount of indicator to be used before actually titrating the samples. A 5 or 10 ml microburet may be conveniently used to obtain a more precise titration.

9. Calculation

9.1 Using the colorimetric procedure, calculate concentration of CN, mg/l, directly from prepared standard curve compensating for sample dilution if less than 500 ml was used for distillation.

9.2 Using the titrimetric procedure, calculate concentration of CN as follows:

$$\text{CN, mg/l} = \frac{\text{(A-B) 1000}}{\text{ml original sample}} \text{ X } \frac{250}{\text{ml of aliquot titrated}}$$

where:

A = volume of $AgNO_3$ for titration of sample.

B = volume of $AgNO_3$ for titration of blank.

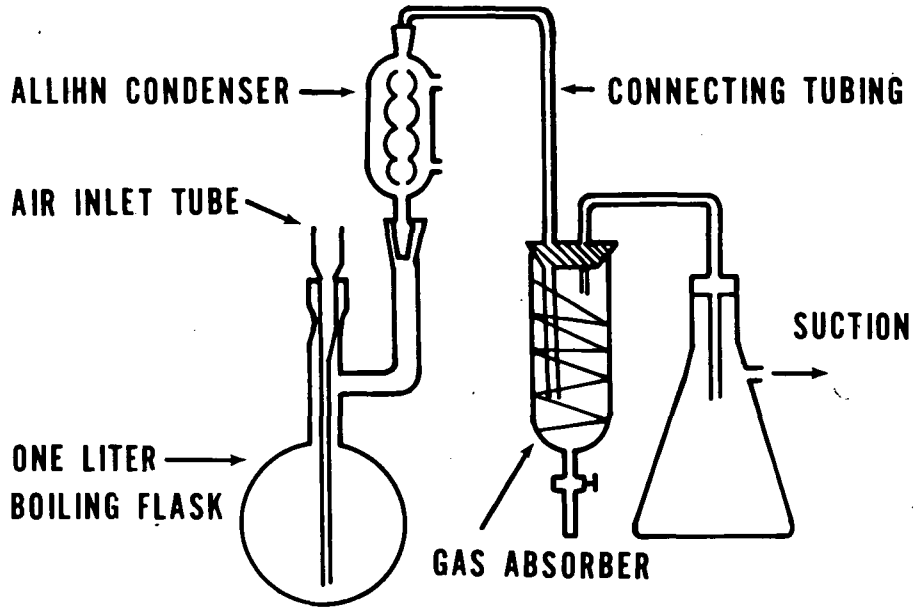
10. Precision and Accuracy

10.1 In a single laboratory (MDQARL), using mixed industrial and domestic waste samples at concentrations of 0.06, 0.13, 0.28 and 0.62 mg/1 CN, the standard deviations were ±0.005, ±0.007, ±0.031, and ±0.094, respectively.

10.2 In a single laboratory (MDQARL), using mixed industrial and domestic waste samples at concentrations of 0.28 and 0.62 mg/l CN, recoveries were 85% and 102%, respectively.

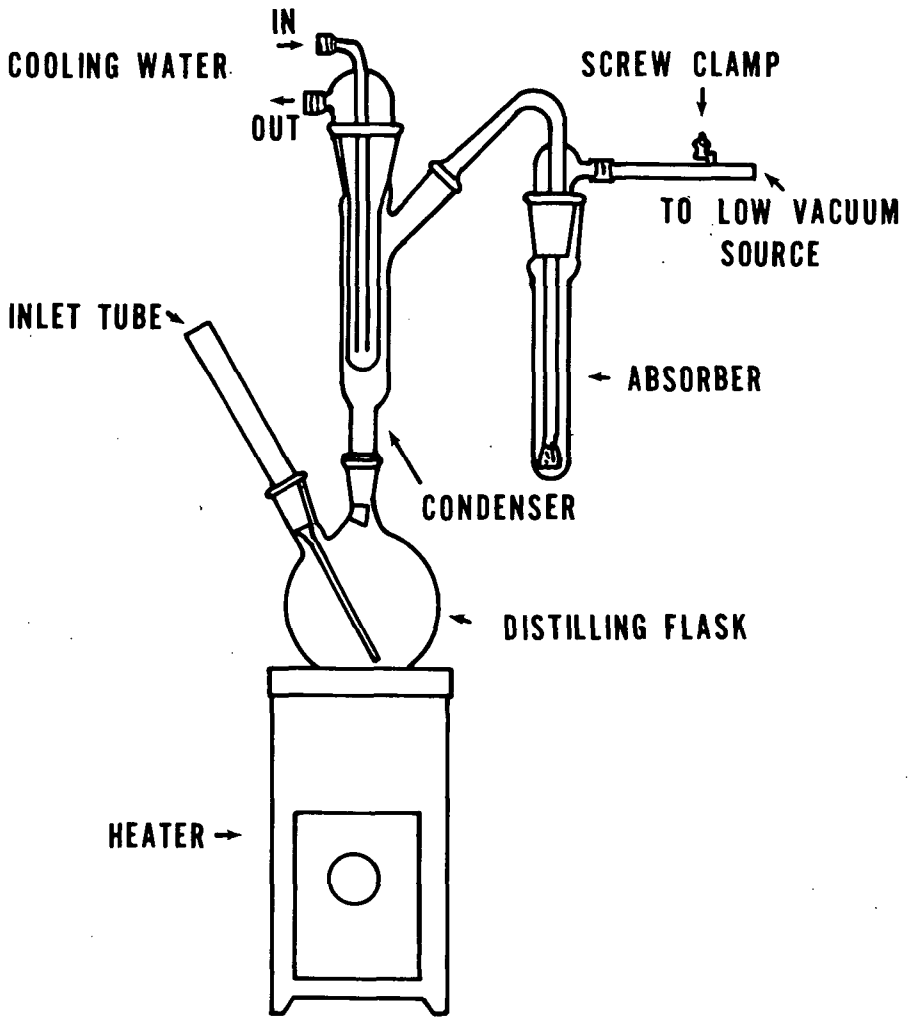
## Bibliography


1. Bark, L. S., and Higson, H. G. "Investigation of Reagents for the Colorimetric Determination of Small Amounts of Cyanide". *Talanta,* 2:471-479 (1964).
2. Elly, C. T. "Recovery of Cyanides by Modified Serfass Distillation". *Journal Water Pollution Control Federation,* 40:848-856 (1968).
3. ASTM Standards, Part 23, Water: Atmospheric Analysis, p 498, Method D2036-72 Referee Method A (1973).




# FIGURE 1

# CYANIDE DISTILLATION APPARATUS




# FIGURE 2
# CYANIDE DISTILLATION APPARATUS

## CYANIDES, Amenable to Chlorination

STORET NO. 00722

1. Scope and Application

   1.1 This method is applicable to the determination of cyanides amenable to chlorination in drinking, surface, and saline waters, and domestic and industrial wastes.

   1.2 The titration procedure is used for measuring concentrations of cyanide exceeding 1 mg/l after removal of the cyanides amenable to chlorination. Below this level the colorimetric determination is used.

2. Summary of Method

   2.1 A portion of the sample is chlorinated at a pH>11 to decompose the cyanide. Cyanide levels in the chlorinated sample are then determined by the method for Cyanide, Total, in this manual. Cyanides amenable to chlorination are then calculated by difference.

3. Reagents

   3.1 Calcium Hypochlorite solution: Dissolve 5 g of calcium hypochlorite ($Ca(OCl)_2$) in 100 ml of distilled water.

   3.2 Sodium Hydroxide solution: Dissolve 50 g of sodium hydroxide (NaOH) in distilled water and dilute to 1 liter.

   3.3 Ascorbic acid: crystals.

   3.4 Potassium Iodide - starch test paper.

4. Procedure

   4.1 Two sample aliquots are required to determine cyanides amenable to chlorination. To one 500 ml aliquot or a volume diluted to 500 ml, add calcium hypochlorite solution (3.1) dropwise while agitating and maintaining the pH between 11 and 12 with sodium hydroxide (3.2).

   *Caution*: The initial reaction product of alkaline chlorination is the very toxic gas cyanogen chloride; therefore, it is recommended that this reaction be performed in a hood. For convenience, the sample may be agitated in a 1 liter beaker by means of a magnetic stirring device.

   4.2 Test for residual chlorine with KI-starch paper (3.4) and maintain this excess for one hour, continuing agitation. A distinct blue color on the test paper indicates a sufficient chlorine level. If necessary, add additional hypochlorite solution.

4.3 After one hour, add 0.5 g portions of ascorbic acid (3.3) until KI-starch paper shows no residual chlorine. Add an additional 0.5 g of ascorbic acid to insure the presence of excess reducing agent.

4.4 Test for total cyanide in both the chlorinated and unchlorinated aliquots as in the method Cyanide, Total, in this manual.

5. Calculation

5.1 Calculate the cyanide amenable to chlorination as follows:

CN, mg/l = A–B

where:

A = mg/l total cyanide in unchlorinated aliquot

B = mg/l total in chlorinated aliquot

## Bibliography

1. ASTM Standards, Part 23, Water; Atmospheric Analysis, p 503, Method B, D2036-72 (1973).

# DISSOLVED OXYGEN

## (Modified Winkler With Full-Bottle Technique)

STORET NO. 00300

1. Scope and Application

   1.1 This method is applicable for use with most wastewaters and streams that contain nitrate nitrogen and not more than 1 mg/l of ferrous iron. Other reducing or oxidizing materials should be absent. If 1 ml of fluoride solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg/l ferric iron.

   1.2 The Dissolved Oxygen (DO) Probe technique gives comparable results on all sample types.

   1.3 The azide modification is not applicable under the following conditions: (a) samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite; (b) samples high in suspended solids; (c) samples containing organic substances which are readily oxidized in a highly alkaline solution. or which are oxidized by free iodine in an acid solution; (d) untreated domestic sewage; (e) biological flocs; and (f) where sample color interferes with endpoint detection. In instances where the azide modification is not applicable, the DO probe should be used.

2. Summary of Method

   2.1 The sample is treated with manganous sulfate, potassium hydroxide, and potassium iodide (the latter two reagents combined in one solution) and finally sulfuric acid. The initial precipitate of manganous hydroxide, $Mn(OH)_2$, combines with the dissolved oxygen in the sample to form a brown precipitate, manganic hydroxide, $MnO(OH)_2$. Upon acidification, the manganic hydroxide forms manganic sulfate which acts as an oxidizing agent to release free iodine from the potassium iodine. The iodine, which is stoichiometrically equivalent to the dissolved oxygen in the sample is then titrated with sodium thiosulfate or phenylarsine oxide (PAO).

3. Interferences

   3.1 There are a number of interferences to the dissolved oxygen test, including oxidizing and reducing agents, nitrate ion, ferrous iron, and organic matter.

   3.2 Various modifications of the original Winkler procedure for dissolved oxygen have been developed to compensate for or eliminate interferences. The Alsterberg

modification is commonly used to successfully eliminate the nitrite interference, the Rideal-Stewart modification is designed to eliminate ferrous iron interference, and the Theriault procedure is used to compensate for high concentration of organic materials.

3.3 Most of the common interferences in the Winkler procedure may be overcome by use of the dissolved oxygen probe.

4. Sample Handling and Preservation

4.1 Where possible, collect the sample in a 300 ml BOD incubation bottle. Special precautions are required to avoid entrainment or solution of atmospheric oxygen or loss of dissolved oxygen.

4.2 Where samples are collected from shallow depths (less than 5 feet), use of an APHA-type sampler is recommended. Use of a Kemmerer type sampler is recommended for samples collected from depths of greater than 5 feet.

4.3 When a Kemmerer sampler is used, the BOD sample bottle should be filled to overflowing. (Overflow for approximately 10 seconds). Outlet tube of Kemmerer should be inserted to bottom of BOD bottle. Care must be taken to prevent turbulence and the formation of bubbles when filling bottle.

4.4 At time of sampling, the sample temperature should be recorded as precisely as required.

4.5 Do not delay the determination of dissolved oxygen in samples having an appreciable iodine demand or containing ferrous iron. If samples must be preserved either method (4.5.1) or (4.5.2) below, may be employed.

4.5.1 Add 2 ml of manganous sulfate solution (6.1) and then 2 ml of alkaline iodide-azide solution (6.2) to the sample contained in the BOD bottle. Both reagents must be added well below the surface of the liquid. Stopper the bottle immediately and mix the contents thoroughly. The sample should be stored at the temperature of the collection water, or water sealed and kept at a temperature of 10 to 20°C, in the dark. Complete the procedure by adding 2 ml $H_2SO_4$ (see 7.1) at time of analysis.

4.5.2 Add 0.7 ml of conc. $H_2SO_4$ (6.3) and 1 ml sodium azide solution (2 g $NaN_3$ in 100 ml distilled water) to the sample in the BOD bottle. Store sample as in (4.5.1). Complete the procedure using 2 ml of manganous sulfate solution (6.1), 3 ml alkaline iodide-azide solution (6.2), and 2 ml of conc. $H_2SO_4$ (6.3) at time of analysis.

4.6 If either preservation technique is employed, complete the analysis within 4-8 hours after sampling.

5. Apparatus

5.1 Sample bottles – 300 ml ±3 ml capacity BOD incubation bottles with tapered ground glass pointed stoppers and flared mouths.

5.2 Pipets – with elongated tips capable of delivering 2.0 ml ±0.10 ml of reagent.

6. Reagents

6.1 Manganous sulfate solution: Dissolve 480 g manganous sulfate ($MnSO_4 \cdot 4H_2O$) in distilled water and dilute to 1 liter.

6.1.1 Alternatively, use 400 g of $MnSO_4 \cdot 2H_2O$ or 364 g of $MnSO_4 \cdot H_2O$ per liter. When uncertainty exists regarding the water of crystallization, a solution of equivalent strength may be obtained by adjusting the specific gravity of the solution to 1.270 at 20°C.

6.2 Alkaline iodide-azide solution: Dissolve 500 g of sodium hydroxide (NaOH) or 700 g of potassium hydroxide (KOH) and 135 g of sodium iodide (NaI) or 150 g of potassium iodide (KI) in distilled water and dilute to 1 liter. To this solution add 10 g of sodium azide ($NaN_3$) dissolved in 40 ml of distilled water.

6.3 Sulfuric acid: concentrated.

6.4 Starch solution: Prepare an emulsion of 10 g soluble starch in a mortar or beaker with a small quantity of distilled water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 ml per liter of chloroform and storage in a 10°C refrigerator.

6.4.1 Dry, powdered starch indicators such as "thyodene" may be used in place of starch solution.

6.5 Potassium fluoride solution: Dissolve 40 g $KF \cdot 2H_2O$ in distilled water and dilute to 100 ml.

6.6 Sodium thiosulfate, stock solution, 0.75 N: Dissolve 186.15 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 ml chloroform.

6.7 Sodium thiosulfate standard titrant, 0.0375 N: Prepare by diluting 50.0 ml of stock solution to 1 liter. Preserve by adding 5 ml of chloroform. Standard sodium thiosulfate, exactly 0.0375 N is equivalent to 0.300 mg of DO per 1.00 ml. Standardize with 0.0375 N potassium biiodate.

6.8 Potassium biiodate standard, 0.0375 N: For stock solution, dissolve 4.873 g of potassium biiodate, previously dried 2 hours at 103°C, in 1000 ml of distilled water. To prepare working standard, dilute 250 ml to 1000 ml for 0.0375 N biiodate solution.

6.9 Standardization of 0.0375 N sodium thiosulfate: Dissolve approximately 2 g (±1.0 g) KI in 100 to 150 ml distilled water; add 10 ml of 10% $H_2SO_4$ followed by 20.0 ml standard potassium biiodate (6.8). Place in dark for 5 minutes, dilute to 300 ml, and titrate with the standard sodium thiosulfate (6.7) to a pale straw color. Add 1-2 ml starch solution and continue the titration drop by drop until the blue color disappears. Run in duplicate. Duplicate determinations should agree within ±0.05 ml.

6.10 As an alternative to the sodium thiosulfate, phenylarsine oxide (PAO) may be used. This is available, already standardized, from commercial sources.

7. Procedure

7.1 To the sample collected in the BOD incubation bottle, add 2 ml of the manganous sulfate solution (6.1) followed by 2 ml of the alkaline iodide-azide solution (6.2), well below the surface of the liquid; stopper with care to exclude air bubbles, and mix well by inverting the bottle several times. When the precipitate settles, leaving a clear supernatant above the manganese hydroxide floc, shake again. When settling has produced at least 200 ml of clear supernant, carefully remove the stopper and immediately add 2 ml of conc. $H_2SO_4$ (6.3)(sulfamic acid packets, 3 g may be substituted for $H_2SO_4$)(1) by allowing the acid to run down the neck of the bottle, re-stopper, and mix by gentle inversion until the iodine is uniformly distributed throughout the bottle. Complete the analysis within 45 minutes.

7.2 Transfer the entire bottle contents by inversion into a 500 ml wide mouth flask and titrate with 0.0375 N thiosulfate solution (6.7) (0.0375 N phenyarsine oxide (PAO) may be substituted as titrant) to a pale straw color. Add 1-2 ml of starch solution (6.4) or 0.1 g of powdered indicator and continue to titrate to the first disappearance of the blue color.

7.3 If ferric iron is present (100 to 200 ppm), add 1.0 ml of KF (6.5) solution before acidification.

7.4 Occasionally, a dark brown or black precipitate persists in the bottle after acidication. This precipitate will dissolve if the solution is kept for a few minutes longer than usual or, if particularly persistent, a few more drops of $H_2SO_4$ will effect dissolution.

8. Calculation

8.1 Each ml of 0.0375 sodium thiosulfate (or PAO) titrant is equivalent to 1 mg DO when the entire bottle contents are titrated.

8.2 If the results are desired in milliliters of oxygen gas per liter at 0°C and 760 mm pressure, multiply mg/1 DO by 0.698.

8.3 To express the results as percent saturation at 760 mm atmospheric pressure, the solubility data in Table 218 (Whipple & Whipple Table, p 480, Standard Methods, 13th Edition) may be used. Equations for correcting the solubilities to barometric pressures other than mean sea level are given below the table.

8.4 The solubility of DO in distilled water at any barometric pressure, p (mm Hg), temperature, T°C, and saturated vapor pressure, $\mu$ (mm Hg), for the given T, may be calculated between the temperature of 0° and 30°C by:

$$\text{ml/l DO} = \frac{(P-\mu) \times 0.678}{35 + T}$$

and between 30° and 50°C by:

$$\text{ml/l DO} = \frac{(P-\mu) \times 0.827}{49 + T}$$

9. Precision and Accuracy

9.1 Exact data are unavailable on the precision and accuracy of this technique; however, reproducibility is approximately 0.2 ppm of DO at the 7.5 ppm level due to equipment tolerances and uncompensated displacement errors.

## Bibliography

1. Kroner, R. C., Longbottom, J. E., Gorman, R. A., "A Comparison of Various Reagents Proposed for Use in the Winkler Procedure for Dissolved Oxygen", PHS Water Pollution Surveillance System Applications and Development, Report #12, Water Quality Section, Basic Data Branch, July 1964.

# DISSOLVED OXYGEN

## (Electrode)

STORET NO. 00299

1. Scope and Application
   1.1 The probe method for dissolved oxygen is recommended for those samples containing materials which interfere with the modified Winkler procedure such as sulfite, thiosulfate, polythionate, mercaptans, free chlorine or hypochlorite, organic substances readily hydrolyzed in alkaline solutions, free iodine, intense color or turbidity, biological flocs, etc.
   1.2 The probe method is recommended as a substitute for the modified Winkler procedure in monitoring of streams, lakes, outfalls, etc., where it is desired to obtain a continuous record of the dissolved oxygen content of the water under observation.
   1.3 The probe method may be used as a substitute for the modified Winkler procedure in BOD determinations where it is desired to perform nondestructive DO measurements on a sample.
   1.4 The probe method may be used under any circumstances as a substitute for the modified Winkler procedure provided that the probe itself is standardized against the Winkler method on samples free of interfering materials.
   1.5 The electronic readout meter for the output from dissolved oxygen probes is normally calibrated in convenient scale (0 to 10, 0 to 15, 0 to 20 mg/l for example) with a sensitivity of approximately 0.05 mg/liter.
2. Summary of Method
   2.1 The most common instrumental probes for determination of dissolved oxygen in water are dependent upon electrochemical reactions. Under steady-state conditions, the current or potential can be correlated with DO concentrations. Interfacial dynamics at the probe-sample interface are a factor in probe response and a significant degree of interfacial turbulence is necessary. For precision performance, turbulence should be constant.
3. Sample Handling and Preservation
   3.1 See 4.1, 4.2, 4.3, 4.4 under Modified Winkler Method.
4. Interferences
   4.1 Dissolved organic materials are not known to interfere in the output from dissolved oxygen probes.

4.2 Dissolved inorganic salts are a factor in the performance of dissolved oxygen probe.

4.2.1 Probes with membranes respond to partial pressure of oxygen which in turn is a function of dissolved inorganic salts. Conversion factors for seawater and brackish waters may be calculated from dissolved oxygen saturation versus salinity data. Conversion factors for specific inorganic salts may be developed experimentally. Broad variations in the kinds and concentrations of salts in samples can make the use of a membrane probe difficult.

4.2.2 The thallium probe requires the presence of salts in concentrations which provide a minimum conductivity of approximately 200 micromhos.

4.3 Reactive compounds can interfere with the output or the performance of dissolved oxygen probes.

4.3.1 Reactive gases which pass through the membrane probes may interfere. For example, chlorine will depolarize the cathode and cause a high probe-output. Long-term exposures to chlorine will coat the anode with the chloride of the anode metal and eventually desensitize the probe. Alkaline samples in which free chlorine does not exist will not interfere. Hydrogen sulfide will interfere with membrane probes if the applied potential is greater than the half-wave potential of the sulfide ion. If the applied potential is less than the half-wave potential, an interfering reaction will not occur, but coating of the anode with the sulfide of the anode metal can take place.

4.3.2 Sulfur compounds (hydrogen sulfide, sulfur dioxide and mercaptans, for example) cause interfering outputs from the thallium probe. Halogens do not interfere with the thallium probe.

4.4 At low dissolved oxygen concentrations, pH variation below pH 5 and above pH 9 interfere with the performance of the thallium probe (approximately ±0.05 mg/l DO per pH unit). The performance of membranes is not affected by pH changes.

4.5 Dissolved oxygen probes are temperature sensitive, and temperature compensation is normally provided by the manufacturer. The thallium probe has a temperature coefficient of 1.0 mv/°C. Membrane probes have a temperature coefficient of 4 to 6 percent/°C dependent upon the membrane employed.

5. Apparatus

5.1 No specific probe or accessory is especially recommended as superior. However, probes which have been evaluated or are in use and found to be reliable are the

Weston & Stack DO Analyzer Model 30, the Yellow Springs Instrument (YSI) Model 54, and the Beckman Fieldlab Oxygen Analyzer.

6. Calibration

   Follow manufacturer instructions.

7. Procedure

   Follow manufacturer instructions.

8. Calculation

   Follow manufacturer instructions.

9. Precision and Accuracy

   Manufacturer's specification claim 0.1 mg/l repeatability with ±1% accuracy.

# FLUORIDE, Total

## (SPADNS Method with Bellack Distillation)

STORET NO. Total 00951
Dissolved 00950

1. Scope and Application
   1.1 This method is applicable to the measurement of fluoride in drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The method covers the range from 0.1 to about 2.5 mg/1 F.

2. Summary of Method
   2.1 Following distillation to remove interferences, the sample is treated with the SPADNS reagent. The loss of color resulting from the reaction of fluoride with the zirconyl-SPADNS dye is a function of the fluoride concentration.

3. Comments
   3.1 The SPADNS reagent is more tolerant of interfering materials than other accepted fluoride reagents. Reference to Table 121(1), p 169, Standard Methods for the Examination of Waters and Wastewaters, 13th Edition, will help the analyst decide if distillation is required. The addition of the highly colored SPADNS reagent must be done with utmost accuracy because the fluoride concentration is measured as a difference of absorbance in the blank and the sample. A small error in reagent addition is the most prominent source of error in this test.
   3.2 Care must be taken to avoid overheating the flask above the level of the solution. This is done by maintaining an even flame entirely under the boiling flask.

4. Precision and Accuracy
   4.1 On a sample containing 0.83 mg/1 F with no interferences, 53 analysts using the Bellack distillation and the SPADNS reagent obtained a mean of 0.81 mg/1 F with a standard deviation of ±0.089 mg/1.
   4.2 On a sample containing 0.57 mg/1 F (with 200 mg/1 $SO_4$ and 10 mg/1 Al as interferences) 53 analysts using the Bellack distillation obtained a mean of 0.60 mg F/1 with a standard deviation of ±0.103 mg/1.
   4.3 On a sample containing 0.68 mg/1 F (with 200 mg/1 $SO_4$ 2 mg/1 Al and 2.5 mg/1 [$Na(PO_3)_6$] as interferences), 53 analysts using the Bellack distillation obtained a mean of 0.72 mg/1 F with a standard deviation of ±0.092 mg/1.
   (Analytical Reference Service, Sample 111-B water, Fluoride, August, 1961.)

5. Reference

5.1 The procedure to be used for this determination is found in:

Standard Methods for the Examination of Water and Wastewaters, p 171-172 (Method No. 121A, Preliminary Distillation Step) and p 174-176 (Method 121 C, SPADNS) 13th Edition, (1971).

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 312, Method D 1179-72, (1973).

# FLUORIDE

## (Automated Complexone Method)

STORET NO. 00950

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The applicable range of the method is 0.05 to 1.5 mg F/1. Twelve samples per hour can be analyzed.
   1.2 For Total or Total Dissolved Fluoride, the Bellack Distillation must be performed on the samples prior to analysis by the complexone method.
2. Summary of Method
   2.1 Fluoride ion reacts with the red cerous chelate of alizarin complexone. It is unlike other fluoride procedures in that a positive color is developed as contrasted to a bleaching action in previous methods.
3. Sample Handling and Preservation
   3.1 No special requirements.
4. Interferences
   4.1 Method is free from most anionic and cationic interferences, except aluminum, which forms an extremely stable fluoro compound, $AlF_6^{-3}$. This is overcome by treatment with 8-hydroxyquinoline to complex the aluminum and by subsequent extraction with chloroform. At aluminum levels below 0.2 mg/1, the extraction procedure is not required.
5. Apparatus
   5.1 Technicon AutoAnalyzer Unit consisting of:
      5.1.1 Sampler I.
      5.1.2 Manifold.
      5.1.3 Proportioning pump.
      5.1.4 Continuous filter.
      5.1.5 Colorimeter equipped with 15 mm tubular flow cell and 650 nm filters.
      5.1.6 Recorder equipped with range expander.
6. Reagents
   6.1 Sodium acetate solution: Dissolve 272 g (2 moles) of sodium acetate in distilled water and dilute to 1 liter.
   6.2 Acetic acid-8-hydroxyquinoline solution: Dissolve 6 g of 8-hydroxyquinoline in 34 ml of conc. acetic acid, and dilute to 1 liter with distilled water.

6.3 Chloroform: Analytical reagent grade.

6.4 Ammonium acetate solution (6.7%): Dissolve 67 g of ammonium acetate in distilled water and dilute to 1 liter.

6.5 Hydrochloric acid (2 N): Dilute 172 ml of conc. HCl to 1 liter.

6.6 Lanthanum alizarin fluoride blue solution[1]: Dissolve 0.18 g of alizarin fluoride blue in a solution containing 0.5 ml of conc. ammonium hydroxide and 15 ml of 6.7% ammonium acetate (6.4). Add a solution that contains 41 g of anhydrous sodium carbonate and 70 ml of glacial acetic acid in 300 ml of distilled water. Add 250 ml of acetone. Dissolve 0.2 g of lanthanum oxide in 12.5 ml of 2 N hydrochloric acid (6.5) and mix with above solution. Dilute to 1 liter.

6.7 Stock solution: Dissolve 2.210 g of sodium fluoride in 100 ml of distilled water and dilute to 1 liter in a volumetric flask. 1.0 ml = 1.0 mg F.

6.8 Standard Solution: Dilute 10.0 ml of stock solution to 1 liter in a volumetric flask. 1.0 ml = 0.01 mg F.

6.8.1 Using standard solution, prepare the following standards in 100 ml volumetric flasks:

| mg F/l | ml Standard Solution/100 ml |
|---|---|
| 0.05 | 0.5 |
| 0.10 | 1.0 |
| 0.20 | 2.0 |
| 0.40 | 4.0 |
| 0.60 | 6.0 |
| 0.80 | 8.0 |
| 1.00 | 10.0 |
| 1.20 | 12.0 |
| 1.50 | 15.0 |

7. Procedure

7.1 Set up manifold as shown in Figure 1.

7.2 Allow both colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on colorimeter to obtain stable baseline.

7.3 Place distilled water wash tubes in alternate openings in Sampler and set sample timing at 2.5 minutes.

7.4 Arrange fluoride standards in Sampler in order of decreasing concentration. Complete loading of Sampler tray with unknown samples.

7.5 Switch sample line from distilled water to Sampler and begin analysis.

8. Calculation

8.1 Prepare standard curve by plotting peak heights of processed fluoride standards against concentration values. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (MDQARL), using surface water samples at concentrations of 0.06, 0.15, and 1.08 mg F/l, the standard deviation was ±0.018.

9.2 In a single laboratory (MDQARL), using surface water samples at concentrations of 0.14 and 1.25 mg F/l, recoveries were 89% and 102%, respectively.

## Bibliography


1. J. T. Baker Laboratory Chemical No. J112 or equivalent.
2. Greenhaigh, R., and Riley, J. P., "The Determination of Fluorides in Natural Waters, with Particular Reference to Sea Water". Anal. Chim. Acta, 25, 179 (1961).
3. Chan, K. M., and Riley, J. P., "The Automatic Determination of Fluoride in Sea Water and Other Natural Waters". Anal. Chim. Acta, *35*, 365 (1966).

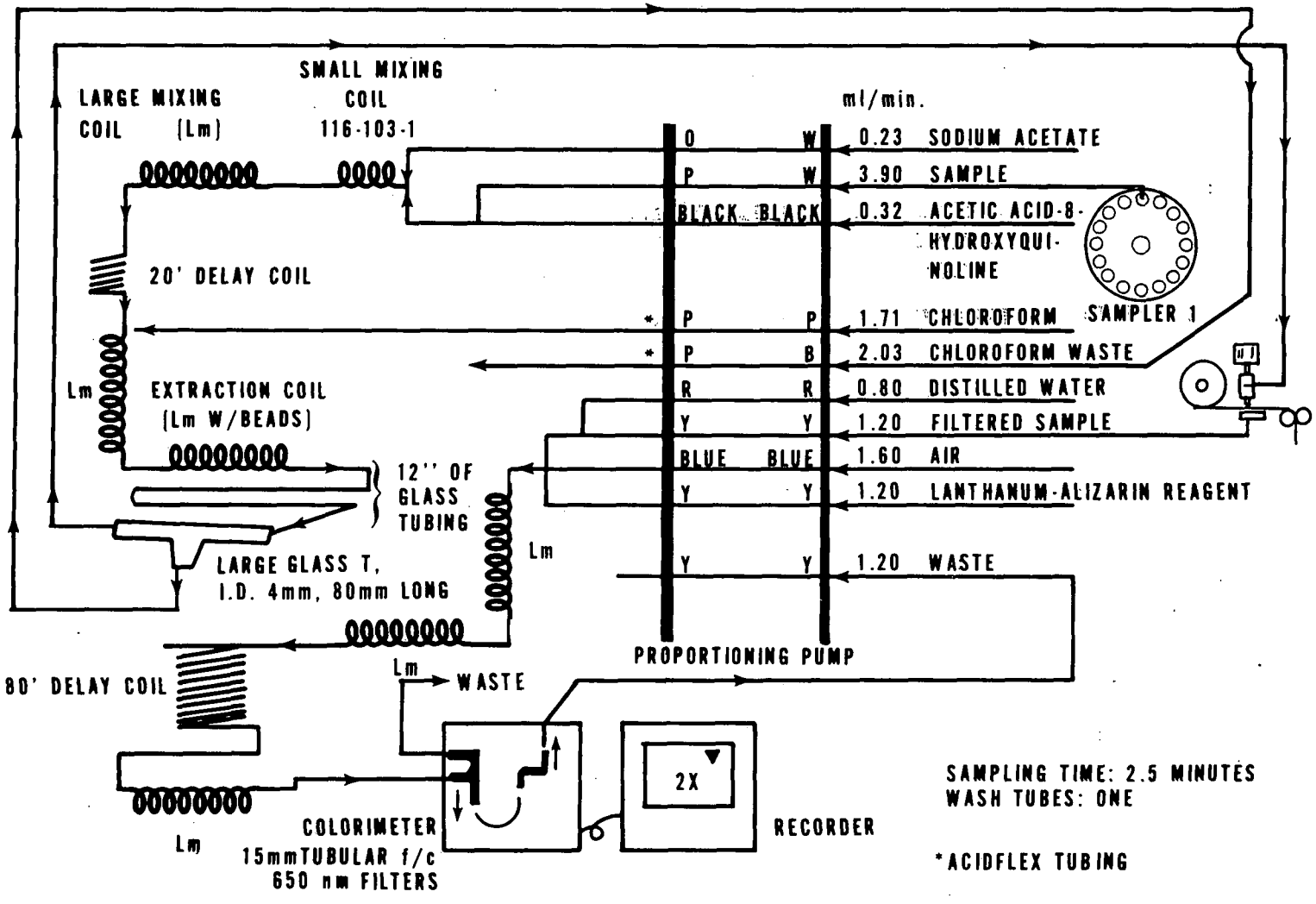



FIGURE 1. FLUORIDE MANIFOLD AA-I

# FLUORIDE

(Electrode)

STORET NO: Total 00951

Dissolved 00950

1. Scope and Application
   1.1 This method is applicable to the measurement of fluoride in drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 Concentration of fluoride from 0.1 up to 1000 mg/liter may be measured.
   1.3 For Total or Total Dissolved Fluoride, the Bellack distillation must be performed on the samples prior to electrode analysis.
2. Summary of Method
   2.1 The fluoride is determined potentiometrically using a selective ion fluoride electrode in conjunction with a standard single junction sleeve-type reference electrode and a pH meter having an expanded millivolt scale or a selective ion meter having a direct concentration scale for fluoride.
   2.2 The fluoride electrode consists of a lanthanum fluoride crystal across which a potential is developed by fluoride ions. The cell may be represented by Ag/Ag Cl, $Cl^-$ (0.3), $F^-$ (0.001) LaF/test solution/SCE/.
3. Interferences
   3.1 Extremes of pH interfere; sample pH should be between 5 and 9. Polyvalent cations of $Si^{+4}$, $Fe^{+3}$ and $Al^{+3}$ interfere by forming complexes with fluoride. The degree of interference depends upon the concentration of the complexing cations, the concentration of fluoride and the pH of the sample. The addition of a pH 5.0 buffer (described below) containing a strong, chelating agent preferentially complexes aluminum (the most common interference), silicon, and iron and eliminates the pH problem.
4. Sampling Handling and Preservation
   4.1 No special requirements.
5. Apparatus
   5.1 Electrometer (pH meter), with expanded mv scale, or a selective ion meter such as the Orion 400 Series.
   5.2 Fluoride Ion Activity Electrode, such as Orion No. 94-09(1).
   5.3 Reference electrode, single junction, sleeve-type, such as Orion No. 90-01, Beckman No. 40454, or Corning No. 476010.
   5.4 Magnetic Mixer, Teflon-coated stirring bar.

6. Reagents

6.1 Buffer solution, pH 5.0-5.5: To approximately 500 ml of distilled water in a 1 liter beaker add 57 ml of glacial acetic acid, 58 g of sodium chloride and 2 g of CDTA[2]. Stir to dissolve and cool to room temperature. Adjust pH of solution to between 5.0 and 5.5 with 5 N sodium hydroxide (about 150 ml will be required). Transfer solution to a 1 liter volumetric flask and dilute to the mark with distilled water. For work with brines, additional NaCl should be added to raise the chloride level to twice the highest expected level of chloride in the sample.

6.2 Sodium fluoride, stock solution: 1.0 ml = 0.1 mg F. Dissolve 0.2210 g of sodium fluoride in distilled water and dilute to 1 liter in a volumetric flask. Store in chemical-resistant glass or polyethylene.

6.3 Sodium fluoride, standard solution: 1.0 ml = 0.01 mg F. Dilute 100.0 ml of sodium fluoride stock solution (6.2) to 1000 ml with distilled water.

7. Calibration

7.1 Prepare a series of standards using the fluoride standard solution (6.3) in the range of 0 to 2.00 mg/l by diluting appropriate volumes to 50.0 ml. The following series may be used:

| Milimeters of Standard (1.0 ml = 0.01 mg/F) | Concentration when Diluted to 50 ml, mg F/liter |
|---|---|
| 0.00 | 0.00 |
| 1.00 | 0.20 |
| 2.00 | 0.40 |
| 3.00 | 0.60 |
| 4.00 | 0.80 |
| 5.00 | 1.00 |
| 6.00 | 1.20 |
| 8.00 | 1.60 |
| 10.00 | 2.00 |

7.2 Calibration of Electrometer: Proceed as described in (8.1). Using semilogarithmic graph paper, plot the concentration of fluoride in mg/liter on the log axis vs. the electrode potential developed in the standard on the linear axis, starting with the lowest concentration at the bottom of the scale. Calibration of a selective ion meter: Follow the directions of the manufacturer for the operation of the instrument.

8. Procedure

8.1 Place 50.0 ml of sample or standard solution and 50.0 ml of buffer (See Note) in a 150 ml beaker. Place on a magnetic stirrer and mix at medium speed. Immerse the electrodes in the solution and observe the meter reading while mixing. The electrodes must remain in the solution for at least three minutes or until the reading has stabilized. At concentrations under 0.5 mg/liter F, it may require as long as five minutes to reach a stable meter reading; higher concentrations stabilize more quickly. If a pH meter is used, record the potential measurement for each unknown sample and convert the potential reading to the fluoride ion concentration of the unknown using the standard curve. If a selective ion meter is used, read the fluoride level in the unknown sample directly in mg/l on the fluoride scale.

NOTE: For industrial waste samples, this amount of buffer may not be adequate. Analyst should check pH first. If highly basic (>11), add 1 N HCl to adjust pH to 8.3.

9. Precision and Accuracy

9.1 A synthetic sample prepared by the Analytical Reference Service, PHS, containing 0.85 mg/l fluoride and no interferences was analyzed by 111 analysts; a mean of 0.84 mg/l with a standard deviation of ±0.03 was obtained.

9.2 On the same study, a synthetic sample containing 0.75 mg/l fluoride, 2.5 mg/l polyphosphate and 300 mg/l alkalinity, was analyzed by the same 111 analysts; a mean of 0.75 mg/l fluoride with a standard deviation of ±0.036 was obtained.

## Bibliography


1. Patent No. 3,431,182 (March 4, 1969).
2. CDTA is the abbreviated designation of 1, 2-cyclohexylene dinitrilo tetraacetic acid, (Mathieson, Coleman & Bell, Cat. No. P8661) or cyclohexane diamine tetraacetic acid (Merck-Titriplex IV or Baker Cat. No. G083).
3. Standard Methods for the Examination of Water and Wastewaters, p 171, Method No. 121A, Preliminary Distillation Step (Bellack), 13th Edition, 1971.

HARDNESS, Total

STORET NO. 00900

1. Scope and Application

   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.

   1.2 The method is suitable for all concentration ranges of hardness; however, in order to avoid large titration volumes, use a sample aliquot containing not more than 25 mg $CaCO_3$.

   1.3 Automated titration may be used.

2. Summary of Method

   2.1 Calcium and magnesium ions in the sample are sequestered upon the addition of disodium ethylenediamine tetraacetate ($Na_2EDTA$). The end point of the reaction is detected by means of Calmagite Indicator, which has a red color in the presence of calcium and magnesium and a blue color when the cations are sequestered.

3. Comments

   3.1 Excessive amounts of heavy metals can interfere. This is usually overcome by complexing the metals with cyanide.

      3.1.1 Routine addition of sodium cyanide solution (*Caution:* deadly poison) to prevent potential metallic interference is recommended.

4. Precision and Accuracy

   4.1 Forty-three analysts in nineteen laboratories analyzed six synthetic water samples containing exact increments of calcium and magnesium salts, with the following results:

| Increment as Total Hardness mg/liter, $CaCO_3$ | Precision as Standard Deviation mg/liter, $CaCO_3$ | Accuracy as Bias, % | Accuracy as Bias, mg/liter, $CaCO_3$ |
|---|---|---|---|
| 31 | 2.87 | -0.87 | -0.003 |
| 33 | 2.52 | -0.73 | -0.24 |
| 182 | 4.87 | -0.19 | -0.4 |
| 194 | 2.98 | -1.04 | -2.0 |
| 417 | 9.65 | -3.35 | -13.0 |
| 444 | 8.73 | -3.23 | -14.3 |

(FWPCA Method Study 1, Mineral and Physical Analyses)

4.2 In a single laboratory (MDQARL), using surface water samples at an average concentration of 194 mg $CaCO_3$/1, the standard deviation was ±3.

5. References

5.1 The procedure to be used for this determination is found in:

Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 179, Method 122B (1971).

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 168, Method D1126-67 (1973).

HARDNESS, Total

(Automated)

STORET NO. 00900

1. Scope and Application
   1.1 This automated method is applicable to drinking, surface, and saline waters. The applicable range is 10 to 400 mg/l as $CaCO_3$. Approximately 12 samples per hour can be analyzed.
2. Summary of Method
   2.1 The magnesium EDTA exchanges magnesium on an equivalent basis for any calcium and/or other cations to form a more stable EDTA chelate than magnesium. The free magnesium reacts with calmagite at a pH of 10 to give a red-violet complex. Thus, by measuring only magnesium concentration in the final reaction stream, an accurate measurement of total hardness is possible.
3. Sample Handling and Preservation
   3.1 No special requirements.
4. Interferences
   4.1 No significant interferences.
5. Apparatus
   5.1 Technicon AutoAnalyzer consisting of:
      5.1.1 Sampler I.
      5.1.2 Continuous Filter.
      5.1.3 Manifold.
      5.1.4 Proportioning Pump.
      5.1.5 Colorimeter equipped with 15 mm tubular flow cell and 520 nm filters.
      5.1.6 Recorder equipped with range expander.
6. Reagents
   6.1 Buffer: Dissolve 67.6 g $NH_4Cl$ in 572 ml of $NH_4OH$ and dilute to 1 liter with distilled water.
   6.2 Calmagite Indicator: Dissolve 0.25 g in 500 ml of distilled water by stirring approximately 30 minutes on a magnetic stirrer. Filter.
   6.3 Magnesium ethylenediamine-tetraacetate (MgEDTA): Dissolve 0.2 g of MgEDTA in 1 liter of distilled water.
   6.4 Stock Solution: Weigh 1.000 g of calcium carbonate (pre-dried at 105°C) into 500 ml Erlenmeyer flask; add 1:1 HCl until all $CaCO_3$ has dissolved. Add 200 ml

of distilled water and boil for a few minutes. Cool, add a few drops of methyl red indicator, and adjust to the orange color with 3N $NH_4OH$ and dilute to 1000 ml with distilled water. 1.0 ml = 1.0 mg $CaCO_3$.

6.4.1 Dilute each of the following volumes of stock solutions to 250 ml in a volumetric flask for appropriate standards:

| Stock Solution, ml | $CaCO_3$, mg/l |
|---|---|
| 2.5 | 10.0 |
| 5.0 | 20.0 |
| 10.0 | 40.0 |
| 15.0 | 60.0 |
| 25.0 | 100.0 |
| 35.0 | 140.0 |
| 50.0 | 200.0 |
| 75.0 | 300.0 |
| 100.0 | 400.0 |

7. Procedure

7.1 Set up manifold as shown in Figure 1.

7.2 Allow both colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on colorimeter to obtain stable baseline.

7.3 Place distilled water wash tubes in alternate openings in Sampler and set sample timing at 2.5 minutes.

7.4 Arrange working standards in Sampler in order of decreasing concentrations. Complete loading of Sampler tray with unknown samples.

7.5 Switch sample line from distilled water to Sampler and begin analysis.

8. Calculation

8.1 Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (MDQARL), using surface water samples at concentrations of 19, 120, 385, and 366 mg/l as $CaCO_3$, the standard deviations were ±1.5, ±1.5, ±4.5, and ±5.0, respectively.

9.2 In a single laboratory (MDQARL), using surface water samples at concentrations of 39 and 296 mg/l as $CaCO_3$, recoveries were 89% and 93%, respectively.

## Bibliography


1. Technicon AutoAnalyzer Methodology, Bulletin No. 2, Technicon Controls, Inc., Chauncey, New York (July 1960).
2. Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 179, Method 122B (1971).

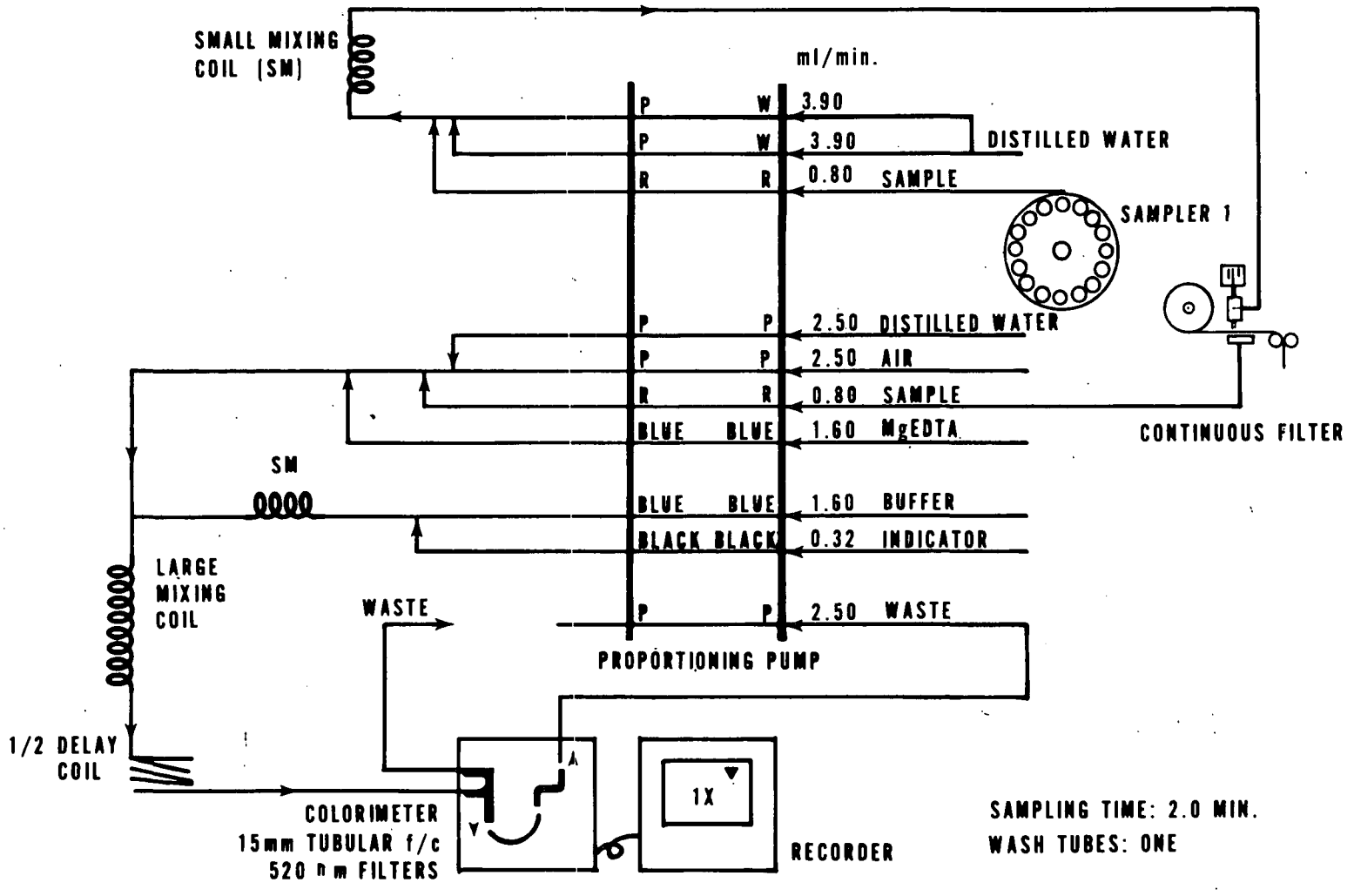



FIGURE 1. HARDNESS MANIFOLD AA-I

# IODIDE

(Titrimetric)

STORET NO. 71865

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, sewage and industrial waste effluents.
   1.2 The concentration range for this method is 2-20 mg/l of iodide.
2. Summary of Method
   2.1 After pretreatment to remove interferences, the sample is analyzed for iodide by converting the iodide to iodate with bromine water and titrating with phenylarsine oxide (PAO) or sodium thiosulfate.
3. Sample Handling and Preservation
   3.1 Store at 4°C and analyze as soon as possible.
4. Interferences
   4.1 Iron, manganese and organic matter can interfere; however, the calcium oxide pretreatment removes or reduces these to insignificant concentrations.
   4.2 Color interferes with the observation of indicator and bromine-water color changes. This interference is eliminated by the use of a pH meter instead of a pH indicator and the use of standardized amounts of bromine water and sodium formate solution instead of observing the light yellow color changes.
5. Reagents
   5.1 Acetic Acid Solution (1:8): Mix 100 ml of glacial acetic acid with 800 ml of distilled water.
   5.2 Bromine Water: In a fume hood, add 0.2 ml bromine to 500 ml distilled water. Stir with a magnetic stirrer and a Teflon-coated stirring bar for several hours or until the bromine dissolves. Store in a glass-stoppered colored bottle.
   5.3 Calcium Oxide (CaO): Anhydrous, powdered.
   5.4 Potassium Iodide (KI): Crystals, ACS Reagent Grade.
   5.5 Sodium Acetate Solution (275 g/l): Dissolve 275 g of sodium acetate trihydrate ($NaC_2H_3O_2 \cdot 3H_2O$) in distilled water. Dilute to 1 liter and filter.
   5.6 Sodium Formate Solution (500 g/l): Dissolve 50 g of sodium formate ($NaCHO_2$) in hot distilled water and dilute to 100 ml.
   5.7 Nitrogen Gas: cylinder.
   5.8 Sulfuric Acid Solution (1:4): Slowly add 200 ml of $H_2SO_4$ (sp. gr. 1.84) to 800 ml of distilled water.

5.9 Phenylarsine Oxide (0.0375 N): Hach Chemical Co. or equivalent. Standardize with 0.0375 N potassium biiodate (5.15, 5.18).

5.10 Phenylarsine Oxide Working Standard (0.0075 N): Transfer 100 ml of commercially available 0.0375 N phenylarsine oxide (5.9) to a 500 ml volumetric flask and dilute to the mark with distilled water. This solution should be prepared fresh daily.

5.11 Amylose Indicator: Mallinckrodt Chemical Works or equivalent.

5.12 Sodium Thiosulfate, Stock Solution, 0.75 N: Dissolve 186.15 g ($Na_2S_2O_3 \cdot 5H_2O$) in boiled and cooled distilled water and dilute to 1.0 liter. Preserve by adding 5 ml chloroform.

5.13 Sodium Thiosulfate Standard Titrant, 0.0375 N: Prepare by diluting 50.0 ml of stock solution to 1.0 liter. Preserve by adding 5 ml of chloroform. Standardize with 0.0375 N potassium biiodate (5.15, 5.18).

5.14 Sodium Thiosulfate Working Standard (0.0075 N): Transfer 100 ml of sodium thiosulfate standard titrant (5.13) to a 500 ml volumetric flask and dilute to the mark with distilled water. This solution should be prepared fresh daily.

5.15 Potassium Biiodate Standard, 0.0375 N: Dissolve 4.387 g potassium biiodate, previously dried 2 hours at 103°C, in distilled water and dilute to 1.0 liter. Dilute 250 ml to 1.0 liter for 0.0375 N biiodate solution.

5.16 Starch Solution: Prepare an emulsion of 10 g of soluble starch in a mortar or beaker with a small quantity of distilled water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 ml per liter of chloroform and storage in a 10°C refrigerator. Commercially available, powdered starch indicators may be used in place of starch solution.

5.17 Potassium Fluoride ($KF \cdot 2H_2O$): ACS Reagent Grade

5.18 Standardization of 0.0375 N Phenylarsine Oxide and 0.0375 N sodium thiosulfate: Dissolve approximately 2 g (±1.0 g) KI (5.4) in 100 to 150 ml distilled water; add 10 ml $H_2SO_4$ solution (5.8) followed by 20 ml standard potassium biiodate solution (5.15). Place in dark for 5 minutes, dilute to 300 ml and titrate with phenylarsine oxide (5.9) or sodium thiosulfate standard titrant (5.13) to a pale straw color. Add a small scoop of indicator (5.11). Wait until homogeneous color develops and continue the titration drop by drop until the blue color disappears. Run in duplicate. Duplicate determinations should agree within ±0.05 ml.

6. Procedure

6.1 Pretreatment

6.1.1 Add a visible excess of CaO (5.3) to 400 ml of sample. Stir or shake vigorously for approximately 5 minutes. Filter through a dry, moderately retentive filter paper, discarding the first 75 ml.

6.2 Iodide Determination

6.2.1 Place 100 ml of pretreated sample (6.1) or a fraction thereof diluted to that volume, into a 150 ml beaker. Add a Teflon-coated stirring bar and place on a magnetic stirrer. Insert a pH electrode and adjust the pH to approximately 7 or slightly less by the dropwise addition of $H_2SO_4$ solution (5.8).

6.2.2 Transfer the sample to a 250 ml wide-mouthed conical flask. Wash beaker with small amounts of distilled water and add washings to the flask.

NOTE: A 250 ml iodine flask would increase accuracy and precision by preventing possible loss of the iodine generated upon addition of potassium iodide and sulfuric acid (6.3.1).

6.2.3 Add 15 ml sodium acetate solution (5.5) and 5 ml acetic acid solution (5.1). Mix well. Add 40 ml bromine water solution (5.2); mix well. Wait 5 minutes.

6.2.4 Add 2 ml sodium formate solution (5.6); mix well. Wait 5 minutes.

6.2.5 Purge the space above the sample with a gentle stream of nitrogen (5.7) for approximately 30 seconds to remove bromine fumes.

6.2.6 If a precipitate forms (iron), add 0.5 g $KF \cdot 2H_2O$ (5.17).

6.2.7 A distilled water blank must be run with each set of samples because of iodide in reagents. If a blank is consistently shown to be zero for a particular "lot" of chemicals it can then be ignored.

6.3 Titration

6.3.1 Dissolve approximately 1 g potassium iodide (5.4) in sample. Add 10 ml of $H_2SO_4$ solution (5.8) and place in dark for 5 minutes.

6.3.2 Titrate with phenylarsine oxide working standard (5.10) or sodium thiosulfate working standard solution (5.14) adding indicator (5.11 or 5.15) as end point is approached (light straw color). Titrate to colorless solution. Disregard returning blue color.

7. Calculations

$$I^- \text{ (mg/l)} = 21{,}150 \left[\frac{\text{ml} \times \text{N}}{\text{V}}\right]$$

ml = the number of ml of PAO need to titrate the sample.

N = the normality of the PAO used to titrate the sample.

V = the volume of sample taken (100 ml or a fraction thereof)

21,150 was calculated from the number of equivalents of iodine produced when the potassium iodide was added and from the rearrangement of the equation to produce the value in terms of mg/l.

8. Precision and Accuracy

8.1 In a single laboratory (MDQARL), using a mixed domestic and industrial waste effluent, at concentrations of 1.6, 4.1, 6.6, 11.6 and 21.6 mg/l of iodide, the standard deviations were ±0.23, ±0.17, ±0.10, ±0.06 and ±0.50 mg/1, respectively.

8.2 In a single laboratory (MDQARL), using a mixed domestic and industrial waste effluent at concentrations of 4.1, 6.6, 11.6 and 21.6 mg/l of iodide, recoveries were 80, 97, 97, and 92%, respectively.

## Bibliography

1. ASTM Standards, Part 23, Water; Atmospheric Analysis, p 331-333, Method D1246 C (1973).

# METALS

## (Atomic Absorption Methods)

1. Scope and Application

1.1 Metals in solution may be readily determined by atomic absorption spectroscopy. The method is simple, rapid, and applicable to a large number of metals in drinking, surface, and saline waters, and domestic and industrial wastes. While drinking waters may be analyzed directly, domestic and industrial wastes require processing to solubilize suspended material. Sludges, sediments and other solid type samples may also be analyzed after proper pretreatment.

1.2 Detection limits, sensitivity and optimum ranges of the metals will vary with the various makes and models of satisfactory atomic absorption spectrophotometers. The data shown in Table 1, however, provide some indication of the actual concentration ranges measurable with conventional atomization. In the majority of instances the concentration range shown in the table may be extended much lower with scale expansion and conversely extended upwards by using a less sensitive wavelength or by rotating the burner 90 degrees. Detection limits may also be extended through concentration of the sample, through solvent extraction techniques and/or the use of the so called furnace techniques. The latter includes the heated graphite atomizer, the carbon rod and the tantalum strip accessories. When using furnace techniques, however, the analyst should be cautioned as to possible chemical reactions occurring at elevated temperatures which may result in either suppression or enhancement of the analysis element. Methods of standard addition are mandatory with these furnace techniques to insure valid data.

1.3 Where conventional, atomic absorption techniques do not provide adequate sensitivity, reference is made to colorimetric or specialized procedures. Examples of these specialized techniques would be the gaseous hydride method for arsenic and selenium and the cold vapor technique for mercury.

1.4 Atomic absorption procedures are provided as the methods of choice; however, other instrumental methods have also been shown to be capable of producing precise and accurate analytical data. These instrumental techniques include emission spectroscopy, X-ray fluorescence, spark source mass spectroscopy, and anodic stripping to name but a few. The analyst should be cautioned that these methods are highly specialized techniques requiring a high degree of skill to interpret results and obtain valid data.

TABLE 1
Atomic Absorption Concentration Ranges With Conventional Atomization***

| Metal | Detection Limit mg/l | Sensitivity mg/l | Optimum Concentration Range mg/l | | |
|---|---|---|---|---|---|
| Aluminum | 0.1 | 1 | 5 | – | 100 |
| Antimony | 0.2 | 0.5 | 1 | – | 40 |
| Arsenic* | 0.002 | – | 0.002 | – | 0.02 |
| Barium | 0.03 | 0.4 | 1 | – | 20 |
| Beryllium | 0.005 | 0.025 | 0.05 | – | 2 |
| Cadmium | 0.002 | 0.025 | 0.05 | – | 2 |
| Calcium | 0.003 | 0.08 | 0.2 | – | 20 |
| Chromium | 0.02 | 0.1 | 0.2 | – | 10 |
| Cobalt | 0.03 | 0.2 | 0.5 | – | 10 |
| Copper | 0.01 | 0.1 | 0.2 | – | 10 |
| Iron | 0.02 | 0.12 | 0.3 | – | 10 |
| Lead | 0.05 | 0.5 | 1 | – | 20 |
| Magnesium | 0.0005 | 0.007 | 0.02 | – | 2 |
| Manganese | 0.01 | 0.05 | 0.1 | – | 10 |
| Mercury** | 0.0002 | – | 0.0002 | – | 0.01 |
| Molybdenum | 0.1 | 0.3 | 0.5 | – | 20 |
| Nickel | 0.02 | 0.15 | 0.3 | – | 10 |
| Potassium | 0.005 | 0.04 | 0.1 | – | 2 |
| Selenium* | 0.002 | – | 0.002 | – | 0.02 |
| Silver | 0.01 | 0.06 | 0.1 | – | 4 |
| Sodium | 0.002 | 0.015 | 0.03 | – | 1 |
| Thallium | 0.1 | 0.5 | 1 | – | 20 |
| Tin | 0.8 | 4 | 10 | – | 200 |
| Titanium | 0.3 | 2 | 5 | – | 100 |
| Vanadium | 0.2 | 0.8 | 1 | – | 100 |
| Zinc | 0.005 | 0.02 | 0.05 | – | 2 |

*Gaseous hydride method.

**Cold vapor technique.

***The concentrations shown above are not contrived values and should be obtainable with conventional aspiration on any satisfactory atomic absorption spectrophotometer.

2. Summary of Method

2.1 Atomic absorption spectroscopy is similar to flame emission photometry in that a sample is atomized and aspirated into a flame. Flame photometry, however, measures the amount of light emitted, whereas, in atomic absorption spectrophotometry a light beam is directed through the flame into a monochromator, and onto a detector that measures the amount of light absorbed. In many instances absorption is more sensitive because it depends upon the presence of free unexcited atoms and generally the ratio of unexited to excited atoms at a given moment is very high. Since the wavelength of the light beam is characteristic of only the metal being determined, the light energy absorbed by the flame is a measure of the concentration of that metal in the sample. This principle is the basis of atomic absorption spectroscopy.

2.2 Although methods have been reported for the analysis of solids by atomic absorption spectroscopy (Spectrochim Acta, *24B* 53, 1969) the technique generally is limited to metals in solution or solubilized through some form of sample processing.

2.2.1 Preliminary treatment of wastewater and/or industrial effluents is usually necessary because of the complexity and variability of the sample matrix. Suspended material must be solubilized through some form of digestion. This may vary because of the metals to be determined but generally will include a wet digestion with nitric acid.

2.2.2 In those instances where complete characterization of a sample is desired, the suspended material must be analyzed separately. This may be accomplished by filtration and acid digestion of the suspended material. Metallic constituents in this acid digest are subsequently determined and the sum of the dissolved plus suspended concentrations will then provide the total concentrations present. The sample should be filtered as soon as possible after collection and the filtrate acidified immediately.

2.2.3 The total sample may also be treated with acid before filtration to measure what may be termed "extractable" concentrations.

3. Definition of Terms

3.1 Sensitivity: The concentration in milligrams of metal per liter that produces an absorption of 1%.

3.2 Detection Limit: The concentration that produces absorption equivalent to twice the magnitude of the fluctuation in the background (zero absorption).

3.3 Dissolved Metals: Those constituents (metals) which will pass through a 0.45 $\mu$ membrane filter.

3.4 Suspended Metals: Those constituents (metals) which are retained by a 0.45 $\mu$ membrane filter.

3.5 Total Metals: The concentration of metals determined on an unfiltered sample following vigorous digestion (Section 4.1.3), or the sum of the concentrations of metals in both the dissolved and suspended fractions.

3.6 Extractable Metals: The concentration of metals in an unfiltered sample following treatment with hot dilute mineral acid (Section 4.1.4).

4. Sample Handling and Preservation

4.1 For the determination of trace metals, contamination and loss are of prime concern. Dust in the laboratory environment, impurities in reagents and impurities on laboratory apparatus which the sample contacts are all sources of potential contamination. For liquid samples, containers can introduce either positive or negative errors in the measurement of trace metals by (a) contributing contaminants through leaching or surface desorption and (b) by depleting concentrations through adsorption. Thus the collection and treatment of the sample prior to analysis requires particular attention. The sample bottle should be thoroughly washed with detergent and tap water; rinsed with 1:1 nitric acid, tap water, 1:1 hydrochloric acid, tap water and finally deionized distilled water in that order.

NOTE 1: Chromic acid may be useful to remove organic deposits from glassware; however, the analyst should be cautioned that the glassware must be thoroughly rinsed with water to remove the last traces of chromium. This is especially important if chromium is to be included in the analytical scheme. Chromic acid should not be used with plastic bottles.

Before collection of the sample a decision must be made as to the type of data desired, i.e., dissolved, suspended, total or extractable.

4.1.1 For the determination of dissolved constituents the sample must be filtered through a 0.45 $\mu$ membrane filter as soon as practical after collection. (Glass or plastic filtering apparatus are recommended to avoid possible contamination.) Use the first 50-100 ml to rinse the filter flask. Discard this portion and collect the required volume of filtrate. Acidify the filtrate with 1:1 redistilled $HNO_3$ to a pH of 2. Normally, 3 ml of (1:1) acid per liter should be sufficient to preserve the sample (See Note

2). Analyses performed on a sample so treated shall be reported as "dissolved" concentrations.

NOTE 2: It has been suggested (International Biological Program, Symposium on Analytical Methods, Amsterdam, Oct. 1966) that additional acid, as much as 25 ml of conc. HCl/liter, may be required to stabilize certain types of highly buffered samples if they are to be stored for any length of time. Therefore, special precautions should be observed for preservation and storage of unusual samples intended for metal analysis.

4.1.2 For the determination of suspended metals a representative volume of unpreserved sample must be filtered through a 0.45 $\mu$ membrane filter. When considerable suspended material is present, as little as 100 ml of a well mixed sample is filtered.

Record the volume filtered and transfer the membrane filter containing the insoluble material to a 250 ml Griffin beaker and add 3 ml conc. redistilled $HNO_3$. Cover the beaker with a watch glass and heat gently. The warm acid will soon dissolve the membrane. Increase the temperature of the hot plate and digest the material. When the acid has evaporated, cool the beaker and watch glass and add another 3 ml of conc. redistilled $HNO_3$.

Cover and continue heating until the digestion is complete, generally indicated by a light colored residue. Add distilled 1:1 HCl (2 ml) to the dry residue and again warm the beaker gently to dissolve the material. Wash down the watch glass and beaker walls with deionized distilled water and filter the sample to remove silicates and other insoluble material that could clog the atomizer. Adjust the volume to some predetermined value based on the expected concentrations of metals present. This volume will vary depending on the metal to be determined. The sample is now ready for analysis. Concentrations so determined shall be reported as "suspended".

4.1.3 For the determination of total metals the sample is acidified with 1:1 redistilled $HNO_3$ to a pH of 2 at the time of collection. The sample is not filtered before processing. Choose a volume of sample appropriate for the expected level of metals. If much suspended material is present, as little as 50-100 ml of well mixed sample will most probably be

sufficient. (The sample volume required may also vary proportionally with the number of metals to be determined).

Transfer a representative aliquot of the well mixed sample to a Griffin beaker and add 3 ml of conc. redistilled $HNO_3$. Place the beaker on a hot plate and evaporate to dryness cautiously, making certain that the sample does not boil. Cool the beaker and add another 3 ml portion of conc. redistilled $HNO_3$. Cover the beaker with a watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated by a light colored residue). Add sufficient distilled 1:1 HCl and again warm the beaker to dissolve the residue. Wash down the beaker walls and watch glass with distilled water and filter the sample to remove silicates and other insoluble material that could clog the atomizer. Adjust the volume to some predetermined value based on the expected metal concentrations. The sample is now ready for analysis. Concentrations so determined shall be reported as "total" (See Note 3). STORET parameter numbers for reporting this type of data have been assigned and are given for each metal.

NOTE 3: Certain metals such as titanium, silver, mercury, and arsenic require modification of the digestion procedure and the individual sheets for these metals should be consulted.

4.1.4 To determine metals soluble in hot, dilute, HCl – $HNO_3$, acidify the entire sample at the time of collection with conc. redistilled $HNO_3$, 5 ml/l. At the time of analysis a 100 ml aliquot of well mixed sample is transferred to a beaker or flask. Five ml of distilled HCl (1:1) is added and the sample heated for 15 minutes at 95°C on a steam bath or hot plate. After this treatment the sample is filtered and the volume adjusted to 100 ml. The sample is then ready for analysis.

The data so obtained are significant in terms of "total" metals in the sample, with the reservation that something less than "total" is probably measured. Concentrations of metal found, especially in heavily silted samples, will be substantially higher than data obtained on only the soluble fraction. STORET parameter numbers for the storage of this type data are not available at this time.

5. Interferences

5.1 The most troublesome type of interference in atomic absorption spectrophotometry is usually termed "chemical" and is caused by lack of absorption of atoms bound in molecular combination in the flame. This phenomenon can occur when the flame is not sufficiently hot to dissociate the molecule, as in the case of phosphate interference with magnesium, or because the dissociated atom is immediately oxidized to a compound that will not dissociate further at the temperature of the flame. The addition of lanthanum will overcome the phosphate interference in the magnesium, calcium and barium determinations. Similarly, silica interference in the determination of manganese can be eliminated by the addition of calcium.

5.1.1 Chemical interferences may also be eliminated by separating the metal from the interfering material. While complexing agents are primarily employed to increase the sensitivity of the analysis, they may also be used to eliminate or reduce interferences.

5.2 The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked. Preferably, high solids type samples should be extracted (See 5.1.1 and 9.2).

5.3 Ionization interferences occur where the flame temperature is sufficiently high to generate the removal of an electron from a neutral atom, giving a positively charged ion. This type of interference can generally be controlled by the addition, to both standard and sample solutions, of a large excess of an easily ionized element.

5.4 Spectral interference can occur when an absorbing wavelength of an element present in the sample but not being determined falls within the width of the absorption line of the element of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal. Spectral interference may sometimes be reduced by narrowing the slit width.

6. Apparatus

6.1 Atomic absorption spectrophotometer: Any commercial atomic absorption instrument having an energy source, an atomizer burner system, a monochromator, and a detector is suitable.

6.2 Burner: The burner recommended by the particular instrument manufacturer should be used. For certain elements the nitrous oxide burner is required.

6.3 Separatory flasks: 250 ml, or larger, for extraction with organic solvents.

6.4 Glassware: All glassware, including sample bottles, should be washed with detergent, rinsed with tap water, 1:1 nitric acid, tap water, 1:1 hydrochloric acid, tap water and deionized distilled water in that order. [See Note 1 under (4.1) concerning the use of chromic acid.]

6.5 Borosilicate glass distillation apparatus.

7. Reagents

7.1 Deionized distilled water: Prepare by passing distilled water through a mixed bed of cation and anion exchange resins. Use deionized distilled water for the preparation of all reagents, calibration standards, and as dilution water.

7.2 Nitric acid (conc.): If metal impurities are found to be present, distill reagent grade nitric acid in a borosilicate glass distillation apparatus. Prepare a 1:1 dilution with deionized distilled water.

*Caution:* Distillation should be performed in hood with protective sash in place.

7.3 Hydrochloric acid (1:1): Prepare a 1:1 solution of reagent grade hydrochloric acid and deionized distilled water. If metal impurities are found to be present, distill this mixture from a borosilicate glass distillation apparatus.

7.4 Stock metal solutions: Prepare as directed in (8.1) and under the individual metal procedures. Commercially available stock standard solutions may also be used.

7.5 Standard metal solutions: Prepare a series of standards of the metal by dilution of the appropriate stock metal solution to cover the concentration range desired.

7.6 Fuel and oxidant: Commercial grade acetylene is generally acceptable. Air may be supplied from a compressed air line, a laboratory compressor, or from a cylinder of compressed air. Reagent grade nitrous oxide is also required for certain determinations.

7.7 Special reagents for the extraction procedure.

7.7.1 Pyrrolidine dithiocarbamic acid (PDCA): Prepare by adding 18 ml of analytical reagent grade pyrrolidine to 500 ml of chloroform in a liter flask. Cool and add 15 ml of carbon disulfide in small portions and with swirling. Dilute to 1 liter with chloroform. The solution can be used for several months if stored in a brown bottle in a refrigerator.

7.7.2 Ammonium hydroxide, 2N: Dilute 3 ml conc. $NH_4OH$ to 100 ml with deionized distilled water.

7.7.3 Bromphenol blue indicator.

7.7.4 HCl: Dilute 2 ml redistilled HCl to 40 ml with deionized distilled water.

8. Preparation of Standards and Calibration

8.1 Stock solutions are prepared from high purity metals, oxides or nonhygroscopic reagent grade salts using redistilled nitric or hydrochloric acids. Sulfuric or phosphoric acids should be avoided as they produce an adverse effect on many elements. The stock solutions are prepared at concentrations of 1000 mg of the metal per liter.

8.2 Standard solutions are prepared by diluting the stock metal solutions at the time of analysis. For best results, calibration standards should be prepared fresh each time an analysis is to be made and discarded after use. Prepare a blank and calibration standards in graduated amounts in the appropriate range. The *calibration standards* should be prepared using the same type of acid (HCl, $HNO_3$ or $H_2SO_4$) and at the same concentration as will result in the samples following processing. As filtered water samples are preserved with 1:1 redistilled $HNO_3$ (3 ml per liter), calibration standards for these analyses should be similarly prepared with $HNO_3$. Samples processed for suspended metals (4.1.2) or total metals (4.1.3) should be analyzed using calibration standards prepared in HCl. Beginning with the blank and working toward the highest standard, aspirate the solutions and record the readings. Repeat the operation with both the calibration standards and the samples a sufficient number of times to secure a reliable average reading for each solution.

8.3 Where the sample matrix is so complex that viscosity, surface tension and components cannot be accurately matched with standards, the method of standard addition must be used. This technique relies on the addition of small, known amounts of the analysis element to portions of the sample – the absorbance difference between those and the original solution giving the slope of the calibration curve. The method of standard addition is described in greater detail in (8.5).

8.4 For those instruments which do not read out directly in concentration, a calibration curve is prepared to cover the appropriate concentration range. Usually, this means the preparation of standards which produce an absorption of 0 to 80 percent. The correct method is to convert the percent absorption readings to absorbance and plot that value against concentration. The following relationship is used to convert absorption values to absorbance:

$$\text{absorbance} = \log (100/\%T) = 2 - \log \%T$$

where $\%T = 100 - \%$ absorption

As the curves are frequently nonlinear, especially at high absorption values, the number of standards should be increased in that portion of the curve.

8.5 Standard Addition Method: In this method, equal volumes of sample are added to a deionized distilled water blank and to three standards containing different known amounts of the test element. The volume of the blank and the standards must be the same. The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate. An example of a plot so obtained is shown in Fig. 1.

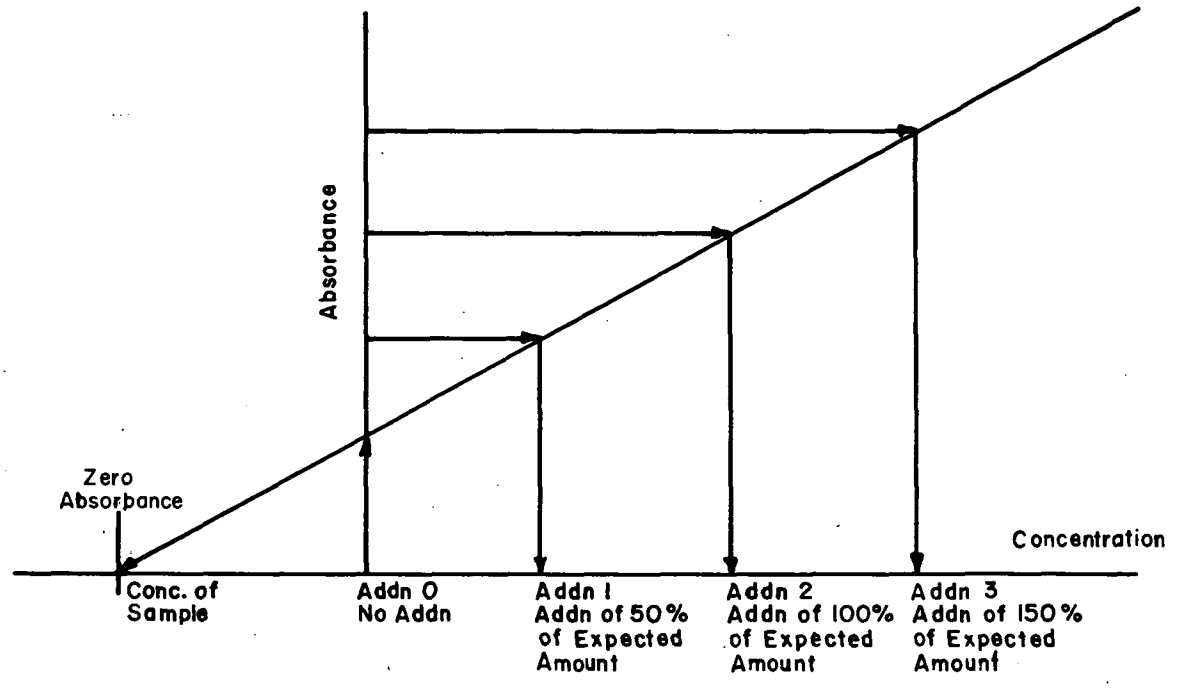



FIGURE 1. STANDARD ADDITION PLOT

9. General Procedure for Analysis by Atomic Absorption

9.1 Differences between the various makes and models of satisfactory atomic absorption spectrophotometers prevent the formulation of detailed instructions applicable to every instrument. The analyst should follow the manufacturer's operating instructions for his particular instrument. In general, after choosing the proper hollow cathode lamp for the analysis, the lamp should be allowed to warm up for a minimum of 15 minutes. During this period, align the instrument, position the monochromator at the correct wavelength, select the proper monochromator slit width, and adjust the hollow cathode current according to the manufacturer's recommendation. Subsequently, light the flame and regulate the flow of fuel and oxidant, adjust the burner and nebulizer flow rate for maximum percent absorption and stability, and balance the photometer. Run a series of standards of the element under analysis and construct working curves by plotting the concentrations of the standards against the absorbance. For those instruments which read directly in concentration set the curve corrector to read out the proper concentration. Aspirate the samples and determine the concentrations either directly or from the calibration curve. For best results run standards each time a sample or series of samples are run.

9.2 Special Extraction Procedure: When the concentration of the metal is not sufficiently high to determine directly, or when considerable dissolved solids are present in the sample, certain of the metals may be chelated and extracted with organic solvents. Ammonium pyrrolidine dithiocarbamate (APDC) in methyl isobutyl ketone (MIBK) is widely used for this purpose and is particularly useful for zinc, cadmium, iron, manganese, copper, silver, lead and chromium$^{+6}$. Tri-valent chromium does not react with APDC unless it has first been converted to the hexavalent form [Atomic Absorption Newsletter *6*, p 128 (1967)]. Aluminum, beryllium, barium and strontium also do not react with APDC. While the APDC-MIBK chelating-solvent system can be used satisfactorily, it is possible to experience difficulties. Also, when multiple metals are to be determined either larger sample volumes must be extracted or individual extractions made for each metal being determined. The acid form of APDC-pyrrolidine dithiocarbamic acid prepared directly in chloroform as described by Lakanen, [Atomic Absorption Newsletter 5, p 17 (1966)], has been found to be most advantageous. It is very stable and may be stored in a brown bottle in the refrigerator for months. Because chloroform is used as the solvent, it may not be aspirated into the flame. The following procedure is suggested.

9.2.1 Extraction Procedure with pyrrolidine dithiocarbamic acid (PDCA) in chloroform.

a. Transfer 200 ml of sample into a 250 ml separatory funnel, add 2 drops bromphenol blue indicator solution (7.7.3) and mix.

b. Prepare a blank and sufficient standards in the same manner and adjust the volume of each to approximately 200 ml with deionized distilled water. All of the metals to be determined may be combined into single solutions at the appropriate concentration levels.

c. Adjust the pH by addition of 2N $NH_4OH$ solution (7.7.2) until a blue color persists. Add HCl (7.7.4) dropwise until the blue color just disappears; then add 2.0 ml HCl (7.7.4) in excess. The pH at this point should be 2.3. (The pH adjustment may be made with a pH meter instead of using indicator).

d. Add 5 ml of PDCA-chloroform reagent (7.7.1) and shake vigorously for 2 minutes. Allow the phases to separate and drain the chloroform layer into a 100 ml beaker.

e. Add a second portion of 5 ml PDCA-chloroform reagent (7.7.1) and shake vigorously for 2 minutes. Allow the phases to separate and combine the chloroform phase with that obtained in step (d).

f. Determine the pH of the aqueous phase and adjust to 4.5.

g. Repeat step (d) again combining the solvent extracts.

h. Readjust the pH to 5.5, extract, readjust to 6.5 and extract a fifth time. Combine all extracts and evaporate to dryness on a steam bath.

i. Hold the beaker at a 45 degree angle, and slowly add 2 ml of conc. distilled nitric acid, rotating the beaker to effect thorough contact of the acid with the residue.

j. Place the beaker on a low temperature hotplate and evaporate just to dryness.

k. Add 2 ml of nitric acid (1:1) to the beaker and heat for 1 minute. Cool, quantitatively transfer the solution to a 10 ml volumetric flask and bring to volume with distilled water. The sample is now ready for analysis.

9.3 General-purpose electrically heated devices (flameless atomization) have recently been introduced as a means of extending detection limits. These techniques are generally acceptable but the analyst should be cautioned as to possible suppression or enhancement effects. With flameless atomization, background correction becomes of high importance. This is because certain samples, when atomized, may absorb or scatter light from the hollow cathode lamp. It can be caused by the presence of gaseous molecular species, salt particles, or smoke in the sample beam. If no correction is made, sample absorbance will be greater than it should be, and the analytical result will be erroneously high.

10. Calculation

10.1 Direct determination of liquid samples: Read the metal value in mg/l from the calibration curve or directly from the readout system of the instrument.

10.1.1 If dilution of sample was required:

$$\text{mg/l metal in sample} = (\text{mg/l of metal in the diluted aliquot}) \times D$$

$$\text{where } D = \frac{\left(\begin{array}{c}\text{ml of}\\ \text{aliquot}\end{array}\right) + \left(\begin{array}{c}\text{ml of deionized}\\ \text{distilled water}\end{array}\right)}{\text{ml of aliquot}}$$

10.2 For samples containing particulates:

$$\text{mg/l metal in sample} = \frac{A \times B}{C}$$

where:

A = mg/l of metal in processed sample

B = final volume of processed sample in ml

C = volume of sample aliquot processed in ml

10.3 For solid samples: Report all concentrations as mg/kg dry weight.

10.3.1 Dry sample

$$\text{mg/kg} = \frac{\left(\begin{array}{c}\text{mg/l of constituent}\\ \text{in prepared sample}\end{array}\right) \times \left(\begin{array}{c}\text{volume of prepared}\\ \text{sample in ml}\end{array}\right)}{\text{weight of dry sample in g}}$$

10.3.2 Wet sample

$$\text{mg/kg} = \frac{\left(\begin{array}{c}\text{mg/l of constituent}\\ \text{in prepared sample}\end{array}\right) \times \left(\begin{array}{c}\text{volume of prepared}\\ \text{sample in ml}\end{array}\right)}{(\text{weight of wet sample in g}) \times (\% \text{ solids})}$$

# ALUMINUM

(Standard Conditions)

STORET NO. Total 01105

**Optimum Concentration Range:** 5-100 mg/l using a wavelength of 309.2 nm

**Sensitivity:** 1.0 mg/l

**Detection Limit:** 0.1 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Carefully weigh 1.000 gram of aluminum metal (analytical reagent grade). Add 15 ml of conc. HCl to the metal in a covered beaker and warm gently. When solution is complete, transfer quantitatively to a 1 liter volumetric flask and make up to volume with deionized distilled water. 1 ml = 1 mg Al (1000 mg/l).
2. Potassium Chloride Solution: Dissolve 95 g potassium chloride (KCl) in deionized distilled water and make up to 1 liter.
3. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis. To each 100 ml of standard and sample alike add 2.0 ml potassium chloride solution.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Aluminum hollow cathode lamp
2. Wavelength: 309.2 nm
3. Fuel: Acetylene
4. Oxidant: Nitrous oxide
5. Type of flame: Fuel rich

**Interferences**

1. Aluminum is partially ionized in the nitrous oxide-acetylene flame. This problem may be controlled by the addition of an alkali metal (potassium, 1000 $\mu$g/ml) to both sample and standard solutions.

Notes

1. The following lines may also be used:
   308.2 nm Relative Sensitivity 1
   396.2 nm Relative Sensitivity 2
   394.4 nm Relative Sensitivity 2.5
2. Data to be entered into STORET must be reported as µg/l.
3. For concentrations of aluminum below 0.3 mg/l, the Eriochrome cyanine R method may be used (Standard Methods, 13th Edition, p 57).

Precision and Accuracy

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of MDQARL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, iron, manganese, lead and zinc were added to natural water samples. The statistical results for aluminum were as follows:

| Number of Labs | True values µg/liter | Mean Value µg/liter | Standard Deviation µg/liter | Accuracy as % Bias |
|---|---|---|---|---|
| 38 | 1205 | 1281 | 299 | 6.3 |
| 38 | 1004 | 1003 | 391 | −0.1 |
| 37 | 500 | 463 | 202 | −7.4 |
| 37 | 625 | 582 | 272 | −6.8 |
| 22 | 35 | 96 | 108 | 175 |
| 21 | 15 | 109 | 168 | 626 |

# ANTIMONY

(Standard Conditions)

STORET NO. Total 01097

**Optimum Concentration Range:** 1-40 mg/l using a wavelength of 217.6 nm

**Sensitivity:** 0.5 mg/l

**Detection Limit:** 0.2 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Carefully weigh 2.7426 g of antimony potassium tartrate (analytical reagent grade) and dissolve in deionized distilled water. Dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Sb (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Antimony hollow cathode lamp
2. Wavelength: 217.6 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Fuel lean

**Interferences**

1. In the presence of lead (1000 mg/l), a spectral interference may occur at the 217.6 nm resonance line. In this case the 231.1 nm antimony line should be used.
2. Increasing acid concentrations decrease antimony absorption. To avoid this effect, the acid concentration in the samples and in the standards should be matched.

**Notes**

1. Data to be entered into STORET must be reported as µg/l.

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 5.0 and 15 mg Sb/1, the standard deviations were ±0.08 and ±0.1, respectively. Recoveries at these levels were 96% and 97%, respectively.

# ARSENIC

## (Gaseous Hydride Method)

STORET NO. Total 01002

1. Scope and Application

   1.1 The gaseous hydride method determines inorganic arsenic when present in concentrations at or above 2 μg/l. The method is applicable to most fresh and saline waters in the absence of high concentrations of chromium, cobalt, copper, mercury, molybdenum, nickel and silver.

2. Summary of Method

   2.1 Arsenic in the sample is first reduced to the trivalent form using $SnCl_2$ and converted to arsine, $AsH_3$, using zinc metal. The gaseous hydride is swept into an argon-hydrogen flame of an atomic absorption spectrophotometer. The working range of the method is 2-20 μg/l. The 193.7 nm wavelength line is used.

3. Comments

   3.1 In analyzing most surface and ground waters, interferences are rarely encountered. Industrial waste samples should be spiked with a known amount of arsenic to establish adequate recovery.

   3.2 Organic forms of arsenic must be converted to inorganic compounds and organic matter must be oxidized before beginning the analysis. The oxidation procedure given in Standard Methods, 13th Edition, Method 104B, p 65, Procedure 4.a has been found suitable.

   3.3 The silver diethyldithiocarbamate colorimetric procedure may also be used (Standard Methods, 13th Edition, p 62) with the digestion described in (3.2). l-Ephedrine in chloroform has been found to be a suitable solvent for silver diethyldithiocarbamate if the analyst finds the odor of pyridine objectionable [Anal. Chem. *45*, 1786 (1973)].

   3.4 Data to be entered into STORET must be reported as μg/l.

4. Precision and Accuracy

   4.1 Ten replicate solutions of o-arsenilic acid at the 5, 10 and 20 μg/l level were analyzed by a single laboratory (Caldwell, et. al.). Standard deviations were ±0.3, ±0.9 and ±1.1 with recoveries of 94, 93 and 85%, respectively.

## Bibliography

1. Caldwell, J. S., Lishka, R. J., and McFarren, E. F., "Evaluation of a Low Cost Arsenic and Selenium Determination at Microgram per Liter Levels", JAWWA., vol 65, p 731, Nov., 1973.

# BARIUM

(Standard Conditions)

STORET NO. Total 01007

**Optimum Concentration Range:** 1-20 mg/l using a wavelength of 553.6 nm

**Sensitivity:** 0.4 mg/l

**Detection Limit:** 0.03 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 1.7787 g barium chloride ($BaCl_2 \cdot 2H_2O$, analytical reagent grade) in deionized distilled water and dilute to 1 liter.
   1 ml = 1 mg Ba (1000 mg/l).
2. Potassium chloride solution: Dissolve 95 g potassium chloride, KCl, in deionized distilled water and make up to 1 liter.
3. Prepare dilutions of the stock barium solution to be used as calibration standards at the time of analysis. To each 100 ml of standard and sample alike add 2.0 ml potassium chloride solution. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Barium hollow cathode lamp
2. Wavelength: 553.6 nm
3. Fuel: Acetylene
4. Oxidant: Nitrous oxide
5. Type of flame: Fuel rich

**Interferences**

1. The use of a nitrous oxide-acetylene flame virtually eliminates chemical interference; however, barium is easily ionized in this flame and potassium must be added (1000 mg/l) to standards and samples alike to control this effect.
2. If the nitrous oxide flame is not available and acetylene-air is used, phosphate, silicon and aluminum will severely depress the barium absorbance. This may be overcome by the addition of 2000 mg/l lanthanum.

**Notes**

1. Data to be entered into STORET must be reported as $\mu$g/l.

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 0.40 and 2.0 mg Ba/l, the standard deviations were ±0.043 and ±0.13, respectively. Recoveries at these levels were 94% and 113%, respectively.

# BERYLLIUM

(Standard Conditions)

STORET NO. Total 01012

**Optimum Concentration Range:** 0.05-2 mg/l using a wavelength of 234.9 nm

**Sensitivity:** 0.025 mg/l

**Detection Limit:** 0.005 mg/l

**Preparation of Standard Solution**

1. Stock solution: Dissolve 11.6586 g beryllium sulfate, $BeSO_4$, in deionized distilled water containing 2 ml conc. nitric acid and dilute to 1 liter. 1 ml = 1 mg Be (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Beryllium hollow cathode lamp
2. Wavelength: 234.9 nm
3. Fuel: Acetylene
4. Oxidant: Nitrous oxide
5. Type of flame: Fuel rich

**Interferences:**

1. Sodium and silicon at concentrations in excess of 1000 mg/l have been found to severely depress the beryllium absorbance.
2. Bicarbonate ion is reported to interfere; however, its effect is eliminated when samples are acidified to a pH of 1.5.
3. Aluminum at concentrations of >500 µg/l is reported to depress the sensitivity of beryllium [Spectrochim Acta *22*, 1325 (1966)].

**Notes**

1. Data to be entered into STORET must be reported as µg/l.
2. The "aluminon method" may also be used (Standard Methods, 13th Edition, p 67).

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 0.01, 0.05 and 0.25 mg Be/l, the standard deviations were ±0.001, ±0.001 and ±0.002, respectively. Recoveries at these levels were 100%, 98% and 97%, respectively.

# CADMIUM

(Standard Conditions)

STORET NO. Total 01027

**Optimum Concentration Range:** 0.05-2 mg/l using a wavelength of 228.8 nm

**Sensitivity:** 0.025 mg/l

**Detection Limit:** 0.002 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Carefully weigh 2.282 g of cadmium sulfate ($CdSO_4 \cdot 8H_2O$, analytical reagent grade) and dissolve in deionized distilled water. 1 ml = 1 mg Cd (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Cadmium hollow cathode lamp
2. Wavelength: 228.8 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

**Notes**

1. For levels of cadmium below 20 $\mu$g/l, the extraction procedure is recommended.
2. Data to be entered into STORET must be reported as $\mu$g/l.
3. The dithizone colorimetric procedure may be used (Standard Methods, 13th Edition, p 422).

**Precision and Accuracy**

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of MDQARL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, iron, manganese, lead and zinc were added to natural water samples. The statistical results for cadmium were as follows:

| Number of Labs | True Values µg/liter | Mean Value µg/liter | Standard Deviation µg/liter | Accuracy as % Bias |
|---|---|---|---|---|
| 74 | 71 | 70 | 21 | −2.2 |
| 73 | 78 | 74 | 18 | −5.7 |
| 63 | 14 | 16.8 | 11.0 | 19.8 |
| 68 | 18 | 18.3 | 10.3 | 1.9 |
| 55 | 1.4 | 3.3 | 5.0 | 135 |
| 51 | 2.8 | 2.9 | 2.8 | 4.7 |

# CALCIUM

(Standard Conditions)

STORET NO. Total 00916

**Optimum Concentration Range:** 0.2-20 mg/l using a wavelength of 422.7 nm

**Sensitivity:** 0.08 mg/l

**Detection Limit:** 0.003 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Suspend 1.250 g of $CaCO_3$ (analytical reagent grade), dried at 180°C for 1 hour before weighing, in deionized distilled water and dissolve cautiously with a minimum of dilute HCl. Dilute to 1000 ml with deionized distilled water. 1 ml = 0.5 mg Ca (500 mg/l).
2. Lanthanum chloride solution: Dissolve 29 g of $La_2O_3$, slowly and in small portions, in 250 ml conc. HCl (*Caution*: Reaction is violent) and dilute to 500 ml with deionized distilled water.
3. Prepare dilutions of the stock calcium solutions to be used as calibration standards at the time of analysis. To each *calibration standard* solution, add 1.0 ml of $LaCl_3$ solution for each 10 ml of volume of working standard, ie., 20 ml working standard + 2 ml $LaCl_3$ = 22 ml.

**Sample Preparation**

1. For the analysis of total calcium in domestic and industrial effluents, the procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.
2. For ambient waters, a representative aliquot of a well-mixed sample may be used directly for analysis. If suspended solids are present in sufficient amounts to clog the nebulizer, the sample may be allowed to settle and the supernatant liquid analyzed directly.

**Instrumental Parameters (General)**

1. Calcium hollow cathode lamp
2. Wavelength: 422.7 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Reducing

**Notes**

1. Phosphate, sulfate and aluminum interfere but are masked by the addition of lanthanum. Since low calcium values result if the pH of the sample is above 7, both standards and samples are prepared in dilute hydrochloric acid solution. Concentrations of magnesium greater than 1000 mg/l also cause low calcium values. Concentrations of up to 500 mg/l each of sodium, potassium and nitrate cause no interference.
2. Anionic chemical interferences can be expected if lanthanum is not used in samples and standards.
3. The nitrous oxide-acetylene flame will provide two to five times greater sensitivity and freedom from chemical interferences. Ionization interferences should be controlled by adding a large amount of alkali to the sample and standards. The analysis appears to be free from chemical suppressions in the nitrous oxide-acetylene flame.
4. The 239.9 nm line may also be used. This line has a sensitivity of 20 mg/l.
5. Data to be entered into STORET must be reported as mg/l.
6. The EDTA titrimetric method may be used (Standard Methods, 13th Edition, p 84).

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using distilled water at concentrations of 9.0 and 36 mg/l, the standard deviations were ±0.3 and ±0.6, respectively. Recoveries at both these levels were 99%.

CHROMIUM

(Standard Conditions)

STORET NO. Total 01034

**Optimum Concentration Range:** 0.2-10 mg/l using a wavelength of 357.9 nm

**Sensitivity:** 0.1 mg/l

**Detection Limit:** 0.02 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 1.923 g of chromium trioxide ($CrO_3$, reagent grade) in deionized distilled water. When solution is complete, acidify with redistilled $HNO_3$ and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Cr (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Chromium hollow cathode lamp
2. Wavelength: 357.9 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Slightly fuel rich

**Notes**

1. The following wavelengths may also be used:
   360.5 nm Relative Sensitivity 1.2
   359.3 nm Relative Sensitivity 1.4
   425.4 nm Relative Sensitivity 2
   427.5 nm Relative Sensitivity 3
   428.9 nm Relative Sensitivity 4
2. The nitrous oxide-acetylene flame provides greater sensitivity and freedom from chemical interference.

3. The absorption of chromium is suppressed by iron and nickel. If the analysis is performed in a lean flame the interference can be lessened but the sensitivity will also be reduced. The interference does not exist in a nitrous oxide-acetylene flame.
4. For levels of chromium below 50 μg/l, the extraction procedure is recommended. Only hexavalent chromium will react with APDC, thus, to measure trivalent chromium an oxidation step must be included. [Atomic Absorption Newsletter, *6*, p 128 (1967)].
5. Data to be entered into STORET must be reported as μg/l.
6. The diphenylcarbazide colorimetric procedure may be used (Standard Methods, 13th Edition, p 426).

**Precision and Accuracy**

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of MDQARL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, iron, manganese, lead and zinc were added to natural water samples. The statistical results for chromium were as follows:

| Number of Labs | True Values μg/liter | Mean Value μg/liter | Standard Deviation μg/liter | Accuracy as % Bias |
|---|---|---|---|---|
| 74 | 370 | 353 | 105 | −4.5 |
| 76 | 407 | 380 | 128 | −6.5 |
| 72 | 74 | 72 | 29 | −3.1 |
| 70 | 93 | 84 | 35 | −10.2 |
| 47 | 7.4 | 10.2 | 7.8 | 37.7 |
| 47 | 15.0 | 16.0 | 9.0 | 6.8 |

# COBALT

(Standard Conditions)

STORET NO. Total 01037

**Optimum Concentration Range:** 0.5-10 mg/l using a wavelength of 240.7 nm

**Sensitivity:** 0.2 mg/l

**Detection Limit:** 0.03 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 4.307 g of cobaltous chloride, $CoCl_2 \cdot 6H_2O$ (analytical reagent grade), in deionized distilled water. Add 10 ml of concentrated nitric acid and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Co (1000 mg/l).

2. Prepare dilutions of the stock cobalt solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Cobalt hollow cathode lamp
2. Wavelength: 240.7 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of Flame: Stoichiometric

**Notes**

1. For levels of cobalt below 50 µg/l, the extraction procedure is recommended.
2. Data to be entered into STORET must be reported as µg/l.

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 0.20, 1.0 and 5.0 mg Co/l, the standard deviations were ±0.013, ±0.1 and ±0.05, respectively. Recoveries at these levels were 98%, 98% and 97%, respectively.

# COPPER

(Standard Conditions)

STORET NO. Total 01042

**Optimum Concentration Range:** 0.2-10 mg/l using a wavelength of 324.7 nm

**Sensitivity:** 0.1 mg/l

**Detection Limit:** 0.01 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Carefully weigh 1.00 g of electrolyte copper (analytical reagent grade). Dissolve in 5 ml redistilled $HNO_3$ and make up to 1 liter with deionized distilled water. Final concentration is 1 mg Cu per ml (1000 mg/1).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Copper hollow cathode lamp
2. Wavelength: 324.7 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

**Notes**

1. For levels of copper below 20 $\mu$g/l, the extraction procedure is recommended.
2. Copper atoms are distributed over a wider area in laminar flow-flames than that normally found. Consequently, the burner parameters are not as critical as for most other elemental determinations.
3. Because of the spectral intensity of the 324.7 nm line, the P.M. tube may become saturated. If this situation occurs the current should be decreased.
4. Numerous absorption lines are available for the determination of copper. By selecting a suitable absorption wavelength, copper samples may be analyzed over a very wide range of concentration. The following lines may be used:
   327.4 nm Relative Sensitivity 2

217.8 nm Relative Sensitivity 4

216.5 nm Relative Sensitivity 7

218.1 nm Relative Sensitivity 9

222.5 nm Relative Sensitivity 20

249.2 nm Relative Sensitivity 90

5. Data to be entered into STORET must be reported as μg/l.
6. The 2,9-dimethyl-1, 10-phenanthroline colorimetric method may be used (Standard Methods, 13th Edition, p. 430).

**Precision and Accuracy**

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of MDQARL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, iron, manganese, lead and zinc were added to natural water samples. The statistical results for copper were as follows:

| Number of Labs | True Values μg/liter | Mean Value μg/liter | Standard Deviation μg/liter | Accuracy as % Bias |
|---|---|---|---|---|
| 91 | 302 | 305 | 56 | 0.9 |
| 92 | 332 | 324 | 56 | −2.4 |
| 86 | 60 | 64 | 23 | 7.0 |
| 84 | 75 | 76 | 22 | 1.3 |
| 66 | 7.5 | 9.7 | 6.1 | 29.7 |
| 66 | 12.0 | 13.9 | 9.7 | 15.5 |

# IRON

(Standard Conditions)

STORET NO. Total 01045

**Optimum Concentration Range:** 0.3-10 mg/l using a wavelength of 248.3 nm

**Sensitivity:** 0.12 mg/l

**Detection Limit:** 0.02 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Carefully weigh 1.000 g of pure iron wire (analytical reagent grade) and dissolve in 5 ml redistilled $HNO_3$, warming if necessary. When solution is complete make up to 1 liter with deionized distilled water. 1 ml = 1 mg Fe (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Iron hollow cathode lamp
2. Wavelength: 248.3 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

**Notes**

1. The following lines may also be used:
   248.8 nm Relative Sensitivity 2
   271.9 nm Relative Sensitivity 4
   302.1 nm Relative Sensitivity 5
   252.7 nm Relative Sensitivity 6
   372.0 nm Relative Sensitivity 10
   386.0 nm Relative Sensitivity 20
   344.1 nm Relative Sensitivity 30
2. Absorption is strongly dependent upon the lamp current.

3. Better signal-to-noise can be obtained from a neon-filled hollow cathode lamp than an argon filled lamp.
4. Data to be reported into STORET must be reported as μg/l.
5. The 1,10-phenanthroline colorimetric method may be used (Standard Methods, 13th Edition, p 433).

**Precision and Accuracy**

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of MDQARL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, iron, manganese, lead and zinc were added to natural water samples. The statistical results for iron were as follows:

| Number of Labs | True Values μg/liter | Mean Value μg/liter | Standard Deviation μg/liter | Accuracy as % Bias |
|---|---|---|---|---|
| 82 | 840 | 855 | 173 | 1.8 |
| 85 | 700 | 680 | 178 | −2.8 |
| 78 | 350 | 348 | 131 | −0.5 |
| 79 | 438 | 435 | 183 | −0.7 |
| 57 | 24 | 58 | 69 | 141 |
| 54 | 10 | 48 | 69 | 382 |

# LEAD

(Standard Conditions)

STORET NO. Total 01051

**Optimum Concentration Range:** 1-20 mg/l using a wavelength of 283.3 nm

**Sensitivity:** 0.5 mg/l

**Detection Limit:** 0.05 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Carefully weigh 1.599 g of lead nitrate, $Pb(NO_3)_2$ (analytical reagent grade), and dissolve in deionized distilled water. When solution is complete acidify with 10 ml redistilled $HNO_3$ and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Pb (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Lead hollow cathode lamp
2. Wavelength: 283.3 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Slightly oxidizing

**Notes**

1. The analysis of this metal is exceptionally sensitive to turbulence and absorption bands in the flame. Therefore, some care should be taken to position the light beam in the most stable, center portion of the flame. To do this, first adjust the burner to maximize the absorbance reading with a lead standard. Then, aspirate a water blank and make minute adjustments in the burner alignment to minimize the signal.
2. For levels of lead below 100 $\mu$g/l, the extraction procedure is recommended. The optimum pH for the extraction of lead is 2.8.

3. The following lines may also be used:
   217.0 nm Relative Sensitivity 0.4
   261.4 nm Relative Sensitivity 30
4. Data to be entered into STORET must be reported as μg/l.
5. The dithizone colorimetric method may be used (Standard Methods, 13th Edition, p 436).

**Precision and Accuracy**

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of MDQARL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, iron, manganese, lead and zinc were added to natural water samples. The statistical results for lead were as follows:

| Number of Labs | True Values μg/liter | Mean Value μg/liter | Standard Deviation μg/liter | Accuracy as % Bias |
|---|---|---|---|---|
| 74 | 367 | 377 | 128 | 2.9 |
| 74 | 334 | 340 | 111 | 1.8 |
| 64 | 101 | 101 | 46 | −0.2 |
| 64 | 84 | 85 | 40 | 1.1 |
| 61 | 37 | 41 | 25 | 9.6 |
| 60 | 25 | 31 | 22 | 25.7 |

# MAGNESIUM

(Standard Conditions)

STORET NO. Total 00927

**Optimum Concentration Range:** 0.02-2 mg/l using a wavelength of 285.2 nm

**Sensitivity:** 0.007 mg/l

**Detection Limit:** 0.0005 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 0.829 g of magnesium oxide, MgO (analytical reagent grade), in 10 ml of redistilled $HNO_3$ and dilute to 1 liter with deionized distilled water. 1 ml = 0.50 mg Mg (500 mg/l).
2. Lanthanum chloride solution: Dissolve 29 g of $La_2O_3$, slowly and in small portions in 250 ml conc. HCl, (*Caution*: Reaction is violent) and dilute to 500 ml with deionized distilled water.
3. Prepare dilutions of the stock magnesium solution to be used as calibration standards at the time of analysis. To each *calibration standard* solution, add 1.0 ml of $LaCl_3$ solution for each 10 ml of volume of working standard, ie., 20 ml working standard + 2 ml $LaCl_3$ = 22 ml.

**Sample Preparation**

1. For the analysis of total magnesium in domestic and industrial effluents, the procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.
2. For ambient waters, a representative aliquot of a well-mixed sample may be used directly for analysis. If suspended solids are present in sufficient amounts to clog the nebulizer, the sample may be allowed to settle and the supernatant liquid analyzed directly.
3. Samples should be preserved with (1:1) nitric acid to a pH of 2 at the time of collection.

**Instrumental Parameters (General)**

1. Magnesium hollow cathode lamp
2. Wavelength: 285.2 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Reducing

Notes

1. Analytical sensitivity decreases with increased lamp current.
2. The interference caused by aluminum at concentrations greater than 2 mg/l is masked by addition of lanthanum. Sodium, potassium and calcium cause no interference at concentrations less than 400 mg/l.
3. Because of the spectral intensity of the 285.2 nm line, the P.M. tube may become saturated. If this situation occurs, the current should be decreased.
4. The following line may also be used:
   202.5 nm Relative Sensitivity 25
5. To cover the range of magnesium values normally observed in surface waters (0.1-20 mg/l), it is suggested that the burner be rotated 55°.
6. Data to be entered into STORET must be reported as mg/l.
7. The gravimetric method may be used (Standard Methods, 13th Edition, p 201).

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a distilled water sample at concentrations of 2.1 and 8.2 mg/l, the standard deviations were ±0.1 and ±0.2, respectively. Recoveries at both of these levels were 100%.

# MANGANESE

(Standard Conditions)

STORET NO. Total 01055

**Optimum Concentration Range:** 0.1-10 mg/l using a wavelength of 279.5 nm

**Sensitivity:** 0.05 mg/l

**Detection Limit:** 0.01 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Carefully weigh 1.000 g of manganese metal (analytical reagent grade) and dissolve in 10 ml of redistilled $HNO_3$. When solution is complete dilute to 1 liter with 1% (V/V) HCl. 1 ml = 1 mg Mn (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Manganese hollow cathode lamp
2. Wavelength: 279.5 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

**Notes**

1. For levels of manganese below 25 $\mu$g/l, the extraction procedure is recommended. The extraction is carried out at a pH of 4.5 to 5. The manganese chelate is very unstable and the analysis must be made without delay to prevent its re-solution in the aqueous phase.
2. Analytical sensitivity is somewhat dependent on lamp current.
3. The following line may also be used:
   403.1 nm Relative Sensitivity 10.
4. Data to be entered into STORET must be reported as $\mu$g/l.

Precision and Accuracy

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of MDQARL. Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, iron, manganese, lead and zinc were added to natural water samples. The statistical results for manganese were as follows:

| Number of Labs | True Values µg/liter | Mean Value µg/liter | Standard Deviation µg/liter | Accuracy as % Bias |
|---|---|---|---|---|
| 77 | 426 | 432 | 70 | 1.5 |
| 78 | 469 | 474 | 97 | 1.2 |
| 71 | 84 | 86 | 26 | 2.1 |
| 70 | 106 | 104 | 31 | −2.1 |
| 55 | 11 | 21 | 27 | 93 |
| 55 | 17 | 21 | 20 | 22 |

MERCURY IN WATER

(Manual Cold Vapor Technique)

STORET NO. Total 71900

1. Scope and Application

   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.

   1.2 In addition to inorganic forms of mercury, organic mercurials may also be present. These organo-mercury compounds will not respond to the flameless atomic absorption technique unless they are first broken down and converted to mercuric ions. Potassium permanganate oxidizes many of these compounds, but recent studies have shown that a number of organic mercurials, including phenyl mercuric acetate and methyl mercuric chloride, are only partially oxidized by this reagent. Potassium persulfate has been found to give approximately 100% recovery when used as the oxidant with these compounds. Therefore, a persulfate oxidation step following the addition of the permanganate has been included to insure that organomercury compounds, if present, will be oxidized to the mercuric ion before measurement. A heat step is required for methyl mercuric chloride when present in or spiked to a natural system. For distilled water the heat step is not necessary.

   1.3 The range of the method may be varied through instrument and/or recorder expansion. Using a 100 ml sample, a detection limit of 0.2 $\mu$g Hg/l can be achieved; concentrations below this level should be reported as $<0.2$ (see Appendix 11.2).

2. Summary of Method

   2.1 The flameless AA procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapor. The mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration and recorded in the usual manner.

3. Sample Handling and Preservation

   3.1 Until more conclusive data are obtained, samples should be preserved by acidification with nitric acid to a pH of 2 or lower immediately at the time of collection. If only dissolved mercury is to be determined, the sample should be

filtered through an all glass apparatus before the acid is added. For total mercury the filtration is omitted.

4. Interference

4.1 Possible interference from sulfide is eliminated by the addition of potassium permanganate. Concentrations as high as 20 mg/l of sulfide as sodium sulfide do not interfere with the recovery of added inorganic mercury from distilled water.

4.2 Copper has also been reported to interfere; however, copper concentrations as high as 10 mg/l had no effect on recovery of mercury from spiked samples.

4.3 Sea waters, brines and industrial effluents high in chlorides require additional permanganate (as much as 25 ml). During the oxidation step, chlorides are converted to free chlorine which will also absorb radiation at 253 nm. Care must be taken to assure that free chlorine is absent before the mercury is reduced and swept into the cell. This may be accomplished by using an excess of hydroxylamine sulfate reagent (25 ml). In addition, the dead air space in the BOD bottle must be purged before the addition of stannous sulfate. Both inorganic and organic mercury spikes have been quantitatively recovered from sea water using this technique.

4.4 Interference from certain volatile organic materials which will absorb at this wavelength is also possible. A preliminary run without reagents should determine if this type of interference is present (see Appendix 11.1).

5. Apparatus

5.1 Atomic Absorption Spectrophotometer: (See Note 1) Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed. Note 1: Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.

5.2 Mercury Hollow Cathode Lamp: Westinghouse WL-22847, argon filled, or equivalent.

5.3 Recorder: Any multi-range variable speed recorder that is compatible with the UV detection system is suitable.

5.4 Absorption Cell: Standard spectrophotometer cells 10 cm long, having quartz end windows may be used. Suitable cells may be constructed from plexiglass tubing, 1" O.D. X 4-1/2". The ends are ground perpendicular to the longitudinal axis and quartz windows (1" diameter X 1/16" thickness) are cemented in place. Gas inlet and outlet ports (also of plexiglass but 1/4" O.D.) are attached approximately

1/2" from each end. The cell is strapped to a burner for support and aligned in the light beam by use of two 2" by 2" cards. One inch diameter holes are cut in the middle of each card; the cards are then placed over each end of the cell. The cell is then positioned and adjusted vertically and horizontally to give the maximum transmittance.

5.5 Air Pump: Any peristaltic pump capable of delivering 1 liter of air per minute may be used. A Masterflex pump with electronic speed control has been found to be satisfactory.

5.6 Flowmeter: Capable of measuring an air flow of 1 liter per minute.

5.7 Aeration Tubing: A straight glass frit having a coarse porosity. Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return.

5.8 Drying Tube: 6" X 3/4" diameter tube containing 20 g of magnesium perchlorate (see Note 2). The apparatus is assembled as shown in Figure 1.

NOTE 2: In place of the magnesium perchlorate drying tube, a small reading lamp with 60W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about 10°C above ambient.

6. Reagents

6.1 Sulfuric Acid, Conc: Reagent grade.

6.1.1 Sulfuric acid, 0.5 N: Dilute 14.0 ml of conc. sulfuric acid to 1.0 liter.

6.2 Nitric Acid, Conc: Reagent grade of low mercury content (See Note 3).

NOTE 3: If a high reagent blank is obtained, it may be necessary to distill the nitric acid.

6.3 Stannous Sulfate: Add 25 g stannous sulfate to 250 ml of 0.5 N sulfuric acid. This mixture is a suspension and should be stirred continuously during use. (Stannous chloride may be used in place of stannous sulfate.)

6.4 Sodium Chloride-Hydroxylamine Sulfate Solution: Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in distilled water and dilute to 100.0 ml. (Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.)

6.5 Potassium Permanganate: 5% solution, w/v. Dissolve 5 g of potassium permanganate in 100 ml of distilled water.

6.6 Potassium Persulfate: 5% solution, w/v. Dissolve 5 g of potassium persulfate in 100 ml of distilled water.

6.7 Stock Mercury Solution: Dissolve 0.1354 g of mercuric chloride in 75 ml of distilled water. Add 10 ml of conc. nitric acid and adjust the volume to 100.0 ml. 1 ml = 1 mg Hg.

6.8 Working Mercury Solution: Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 $\mu$g per ml. This working standard and the dilutions of the stock mercury solution should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot.

7. Calibration

7.1 Transfer 0, 0.5, 1.0, 2.0, 5.0 and 10.0 ml aliquots of the working mercury solution containing 0 to 1.0 $\mu$g of mercury to a series of 300 ml BOD bottles. Add enough distilled water to each bottle to make a total volume of 100 ml. Mix thoroughly and add 5 ml of conc. sulfuric acid (6.1) and 2.5 ml of conc. nitric acid (6.2) to each bottle. Add 15 ml of $KMnO_4$ (6.5) solution to each bottle and allow to stand at least 15 minutes. Add 8 ml of potassium persulfate (6.6) to each bottle and heat for 2 hours in a water bath maintained at 95°C. Cool and add 6 ml of sodium chloride-hydroxylamine sulfate solution (6.4) to reduce the excess permanganate. When the solution has been decolorized wait 30 seconds, add 5 ml of the stannous sulfate solution (6.3) and immediately attach the bottle to the aeration apparatus forming a closed system. At this point the sample is allowed to stand quietly without manual agitation. The circulating pump, which has previously been adjusted to a rate of 1 liter per minute, is allowed to run continuously (See Note 4). The absorbance will increase and reach maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass valve and continue the aeration until the absorbance returns to its minimum value (see Note 5). Close the bypass valve, remove the stopper and frit from the BOD bottle and continue the aeration. Proceed with the standards and construct a standard curve by plotting peak height versus micrograms of mercury.

NOTE 4: An open system where the mercury vapor is passed through the absorption cell only once may be used instead of the closed system.

NOTE 5: Because of the toxic nature of mercury vapor precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system to either vent the mercury vapor into an exhaust hood or pass the vapor through some absorbing media, such as:

a) equal volumes of 0.1 M $KMnO_4$ and 10% $H_2SO_4$

b) 0.25% iodine in a 3% KI solution

A specially treated charcoal that will adsorb mercury vapor is also available from Barnebey and Cheney, E. 8th Ave. and N. Cassidy St., Columbus, Ohio 43219, Cat. #580-13 or #580-22.

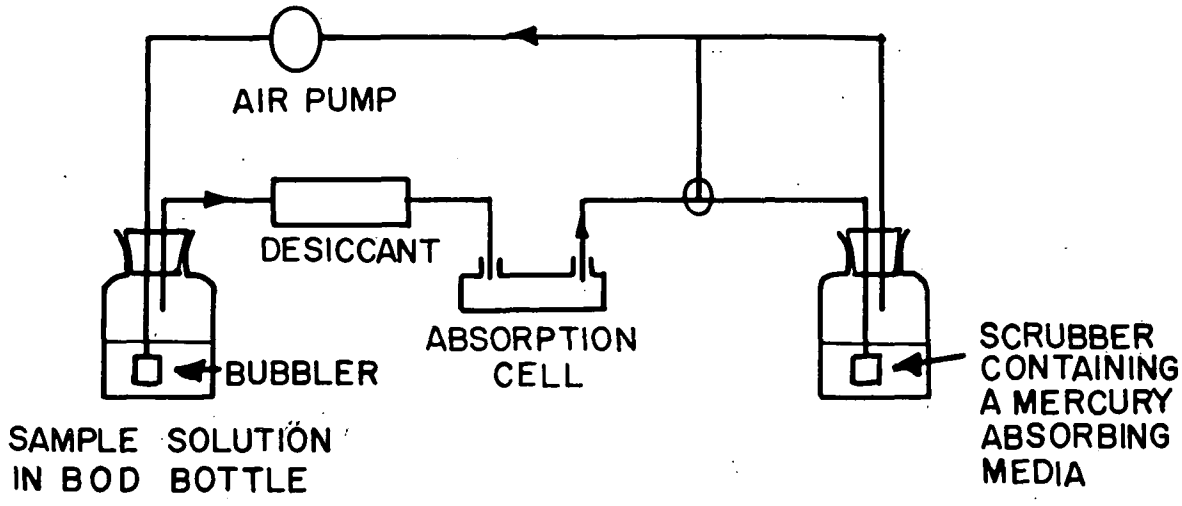



**FIGURE 1. APPARATUS FOR FLAMELESS MERCURY DETERMINATION**

8. Procedure

8.1 Transfer 100 ml or an aliquot diluted to 100 ml, containing not more than 1.0μg of mercury, to a 300 ml BOD bottle. Add 5 ml of sulfuric acid (6.1) and 2.5 ml of conc. nitric acid (6.2) mixing after each addition. Add 15 ml of potassium permanganate solution (6.5) to each sample bottle. For sewage samples additional permanganate may be required. Shake and add additional portions of potassium permanganate solution, if necessary, until the purple color persists for at least 15 minutes. Add 8 ml of potassium persulfate (6.6) to each bottle and heat for 2 hours in a water bath at 95°C. Cool and add 6 ml of sodium chloride-hydroxylamine sulfate (6.4) to reduce the excess permanganate. After a delay of at least 30 seconds add 5 ml of stannous sulfate (6.3) and immediately attach the bottle to the aeration apparatus. Continue as described under Calibration.

9. Calculation

9.1 Determine the peak height of the unknown from the chart and read the mercury value from the standard curve.

9.2 Calculate the mercury concentration in the sample by the formula:

$$\mu gHg/l = \left(\begin{array}{c}\mu g\ Hg\ in \\ aliquot\end{array}\right)\left(\frac{1000}{\text{vol. of aliquot in ml.}}\right)$$

9.3 Report mercury concentrations as follows: Below 0.2 μg/l, <0.2; between 1 and 10 μg/l, one decimal; above 10 μg/l, whole numbers.

10. Precision and Accuracy

10.1 In a single laboratory (MDQARL), using an Ohio River composite sample with a background mercury concentration of 0.35 μg/l, spiked with concentrations of 1, 3 and 4 μg/l, the standard deviations were ±0.14, ±0.10 and ±0.08, respectively. Standard deviation at the 0.35 level was ±0.16. Percent recoveries at the three levels were 89, 87, and 87%, respectively.

10.2 In a joint EPA/ASTM interlaboratory study of the cold vapor technique for total mercury in water, increments of organic and inorganic mercury were added to natural waters. Recoveries were determined by difference. A statistical summary of this study follows:

| Number of Labs | True Values µg/liter | Mean Value µg/liter | Standard Deviation µg/liter | Accuracy as % Bias |
|---|---|---|---|---|
| 76 | 0.21 | 0.349 | 0.276 | 66 |
| 80 | 0.27 | 0.414 | 0.279 | 53 |
| 82 | 0.51 | 0.674 | 0.541 | 32 |
| 77 | 0.60 | 0.709 | 0.390 | 18 |
| 82 | 3.4 | 3.41 | 1.49 | 0.34 |
| 79 | 4.1 | 3.81 | 1.12 | −7.1 |
| 79 | 8.8 | 8.77 | 3.69 | −0.4 |
| 78 | 9.6 | 9.10 | 3.57 | −5.2 |

## 11. Appendix

11.1 While the possibility of absorption from certain organic substances actually being present in the sample does exist, the MDQAR Laboratory has not encountered such samples. This is mentioned only to caution the analyst of the possibility. A simple correction that may be used is as follows: If an interference has been found to be present (4.4), the sample should be analyzed both by using the regular procedure and again under oxidizing conditions only, that is without the reducing reagents. The true mercury value can then be obtained by subtracting the two values.

11.2 If additional sensitivity is required, a 200 ml sample with recorder expansion may be used provided the instrument does not produce undue noise. Using a Coleman MAS-50 with a drying tube of magnesium perchlorate and a variable recorder, 2 mv was set to read full scale. With these conditions, and distilled water solutions of mercuric chloride at concentrations of 0.15, 0.10, 0.05 and 0.025 µg/l the standard deviations were ±0.027, ±0.006, ±0.01 and ±0.004. Percent recoveries at these levels were 107, 83, 84 and 96%, respectively.

11.3 Directions for the disposal of mercury-containing wastes are given in ASTM Standards, Part 23, Water and Atmospheric Analysis, p 352, Method D3223 (1973).

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# MERCURY IN WATER

(Automated Cold Vapor Technique)

STORET NO. 71900

1. Scope and Application
   1.1 This method is applicable to surface waters. It may be applicable to saline waters, wastewaters, effluents, and domestic sewages providing potential interferences are not present (See Interference 4).
   1.2 The working range is 0.2 to 20.0 μg Hg/l.
2. Summary of Method
   2.1 The flameless AA procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapor. The mercury is reduced to the elemental state and aerated from solution. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration and recorded in the usual manner.
   2.2 In addition to inorganic forms of mercury, organic mercurials may also be present. These organo-mercury compounds will not respond to the flameless atomic absorption technique unless they are first broken down and converted to mercuric ions. Potassium permanganate oxidizes many of these compounds but recent studies have shown that a number of organic mercurials, including phenyl mercuric acetate and methyl mercuric chloride, are only partially oxidized by this reagent. Potassium persulfate has been found to give approximately 100% recovery when used as the oxidant with these compounds. Therefore, an automated persulfate oxidation step following the automated addition of the permanganate has been included to insure that organo-mercury compounds, if present, will be oxidized to the mercuric ion before measurement.
3. Sample Handling and Preservation
   3.1 Until more conclusive data are obtained, samples should be preserved by acidification with nitric acid to a pH of 2 or lower immediately at the time of collection.[1] If only dissolved mercury is to be determined, the sample should be filtered before the acid is added. For total mercury the filtration is omitted.
4. Interference (See NOTE 1)
   4.1 Some sea waters and wastewaters high in chlorides have shown a positive interference, probably due to the formation of free chlorine.

4.2 Interference from certain volatile organic materials which will absorb at this wavelength is also possible. A preliminary run under oxidizing conditions, without stannous sulfate, would determine if this type of interference is present.

4.3 Formation of a heavy precipitate, in some wastewaters and effluents, has been reported upon addition of concentrated sulfuric acid. If this is encountered, the problem sample cannot be analyzed by this method.

4.4 Samples containing solids must be blended and then mixed while being sampled if total mercury values are to be reported.

NOTE 1: All the above interferences can be overcome by use of the Manual Mercury method in this manual.

5. Apparatus

5.1 Technicon AutoAnalyzer consisting of:

5.1.1 Sampler II with provision for sample mixing.

5.1.2 Manifold.

5.1.3 Proportioning Pump II or III.

5.1.4 High temperature heating bath with two distillation coils (Technicon Part #116-0163) in series.

5.2 Vapor-liquid separator (Figure 1).

5.3 Absorption cell, 100 mm long, 10 mm diameter with quartz windows.

5.4 Atomic Absorption Spectrophotometer (See Note 2): Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed.

NOTE 2: Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.

5.5 Mercury Hollow Cathode Lamp: Westinghouse WL-22847, argon filled, or equivalent.

5.6 Recorder: Any multi-range variable speed recorder that is compatible with the UV detection system is suitable.

5.7 Source of cooling water for jacketed mixing coil and connector A-7.

5.8 Heat lamp: A small reading lamp with 60W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about 10°C above ambient.

6. Reagents

6.1 Sulfuric Acid, Conc: Reagent grade

6.1.1 Sulfuric acid, 2 N: Dilute 56 ml of conc. sulfuric acid to 1 liter with distilled water.

6.1.2 Sulfuric acid, 10%: Dilute 100 ml conc. sulfuric acid to 1 liter with distilled water.

6.2 Nitric acid, Conc: Reagent grade of low mercury content.

6.2.1 Nitric Acid, 0.5% Wash Solution: Dilute 5 ml of conc. nitric acid to 1 liter with distilled water.

6.3 Stannous Sulfate: Add 50 g stannous sulfate to 500 ml of 2 N sulfuric acid (6.1.1). This mixture is a suspension and should be stirred continuously during use.

NOTE 3: Stannous chloride may be used in place of stannous sulfate.

6.4 Sodium Chloride-Hydroxylamine Sulfate Solution: Dissolve 30 g of sodium chloride and 30 g of hydroxylamine sulfate in distilled water to 1 liter.

NOTE 4: Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.

6.5 Potassium Permanganate: 0.5% solution, w/v. Dissolve 5 g of potassium permanganate in 1 liter of distilled water.

6.6 Potassium Permanganate, 0.1 N: Dissolve 3.16 g of potassium permanganate in distilled water and dilute to 1 liter.

6.7 Potassium Persulfate: 0.5% solution, w/v. Dissolve 5 g of potassium persulfate in 1 liter of distilled water.

6.8 Stock Mercury Solution: Dissolve 0.1354 g of mercuric chloride in 75 ml of distilled water. Add 10 ml of conc. nitric acid and adjust the volume to 100.0 ml. 1.0 ml = 1.0 mg Hg.

6.9 Working Mercury Solution: Make successive dilutions of the stock mercury solution (6.8) to obtain a working standard containing 0.1 $\mu$g per ml. This working standard and the dilutions of the stock mercury solution should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot. From this solution prepare standards containing 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 $\mu$g Hg/l.

6.10 Air Scrubber Solution: Mix equal volumes of 0.1 N potassium permanganate (6.6) and 10% sulfuric acid (6.1.2).

7. Procedure

7.1 Set up manifold as shown in Figure 2.

7.2 Feeding all the reagents through the system with acid wash solution (6.2.1) through the sample line, adjust heating bath to 105°C.

7.3 Turn on atomic absorption spectrophotometer, adjust instrument settings as recommended by the manufacturer, align absorption cell in light path for maximum transmittance and place heat lamp directly over absorption cell.

7.4 Arrange working mercury standards from 0.2 to 20.0 μg Hg/l in sampler and start sampling. Complete loading of sample tray with unknown samples.

7.5 Prepare standard curve by plotting peak height of processed standards against concentration values. Determine concentration of samples by comparing sample peak height with standard curve.

NOTE 5: Because of the toxic nature of mercury vapor, precaution must be taken to avoid its inhalation. Venting the mercury vapor into an exhaust hood or passing the vapor through some absorbing media such as:

a) equal volumes of 0.1 N $KMnO_4$ (6.6) and 10% $H_2SO_4$ (6.1.2).

b) 0.25% iodine in a 3% KI solution, is recommended.

A specially treated charcoal that will adsorb mercury vapor is also available from Barnebey and Cheney, E. 8th Ave. and North Cassidy St., Columbus, Ohio 43219, Cat. #580-13 or #580-22.

7.6 After the analysis is complete put all lines except the $H_2SO_4$ line in distilled water to wash out system. After flushing, wash out the $H_2SO_4$ line. Also flush the coils in the high temperature heating bath by pumping stannous sulfate (6.3) through the sample lines followed by distilled water. This will prevent build-up of oxides of manganese.

8. Precision and Accuracy

8.1 In a single laboratory (SEWL), using distilled water standards at concentrations of 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg Hg/l, the standard deviations were ±0.04, ±0.07, ±0.09, ±0.20, ±0.40 and ±0.84 μg/l, respectively.

8.2 In a single laboratory (SEWL), using surface water samples spiked with ten organic mercurials at the 10 μg/l level, recoveries ranged from 87 to 117%. Recoveries of the same ten organic mercurials in distilled water at the 10 μg/l level, ranged from 92 to 125%.

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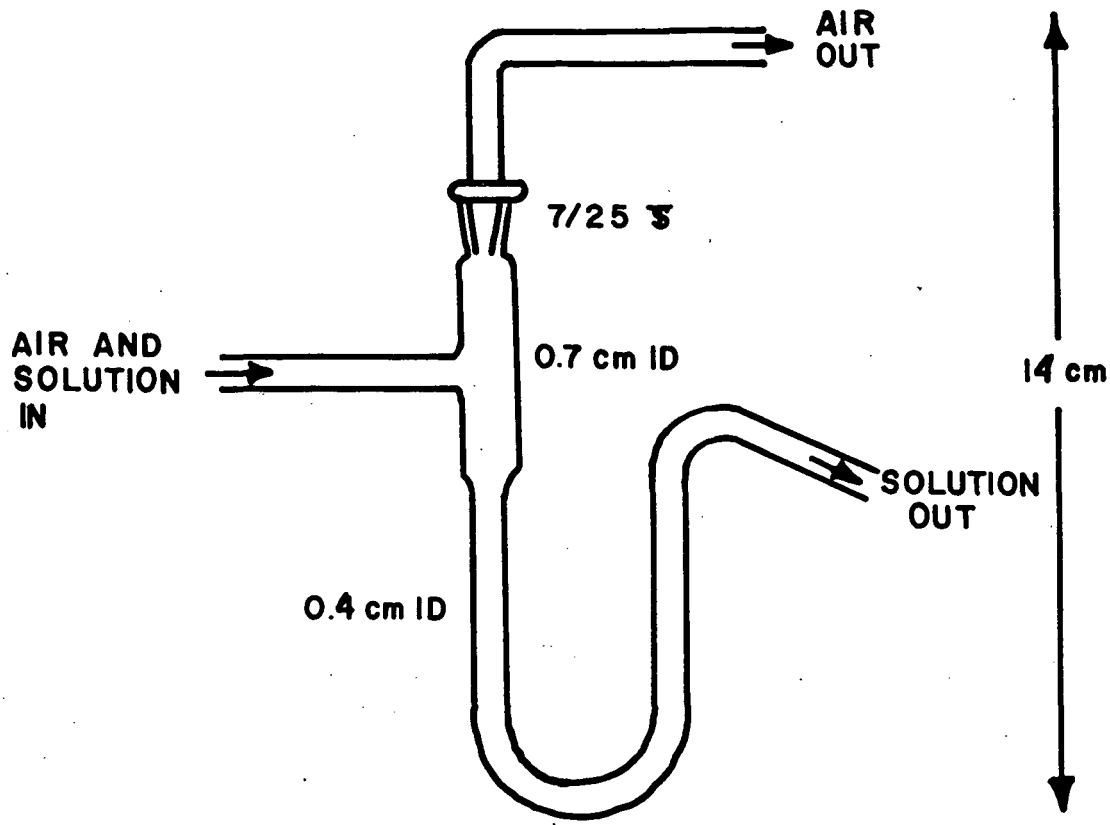


FIGURE 1. VAPOR LIQUID SEPARATOR

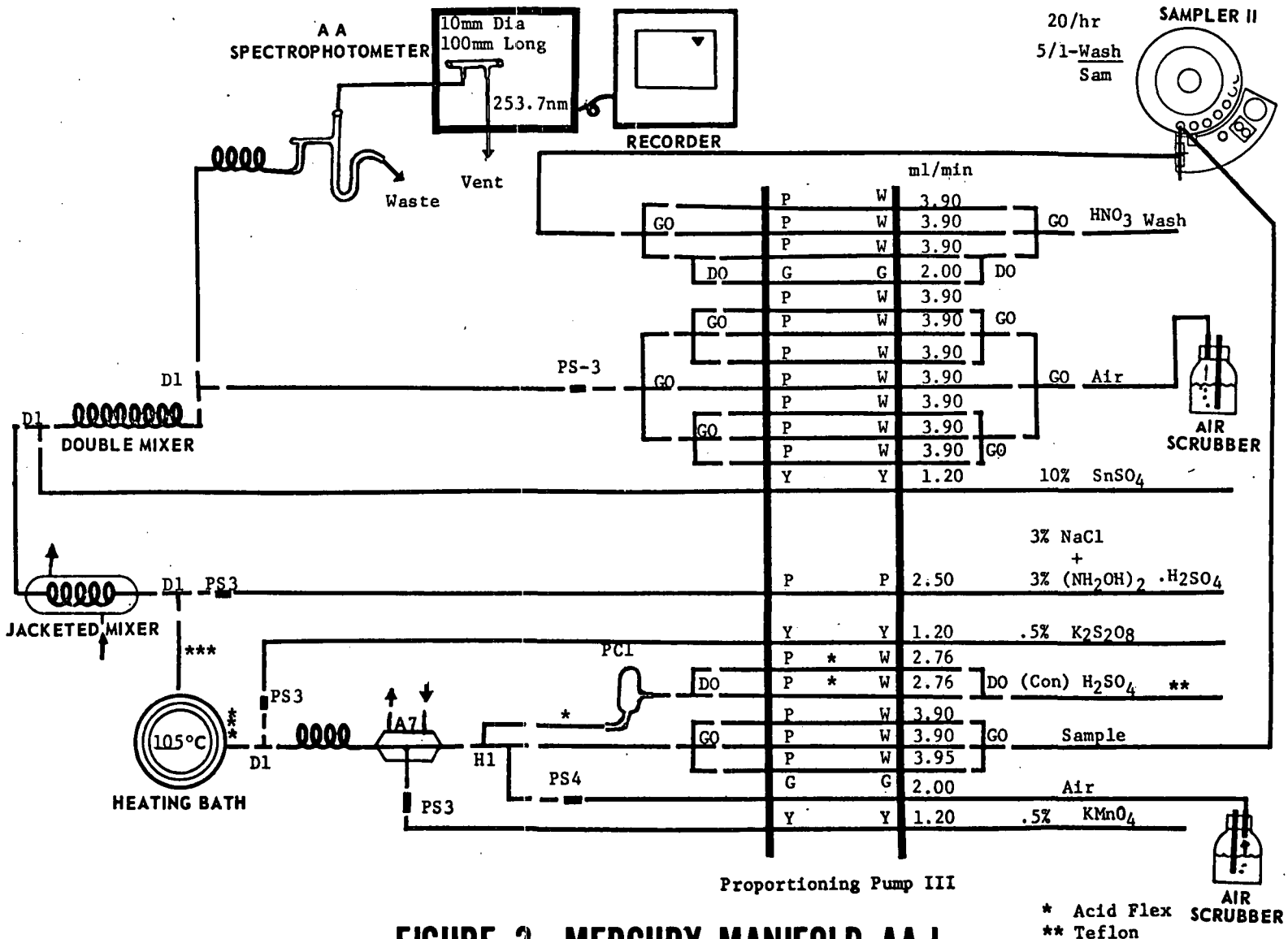


**FIGURE 2 MERCURY MANIFOLD AA-I**

# MERCURY IN SEDIMENT

(Manual Cold Vapor Technique)

1. Scope and Application

   1.1 This procedure[1] measures total mercury (organic + inorganic) in soils, sediments, bottom deposits and sludge type materials.

   1.2 The range of the method is 0.2 to 5 μg/g. The range may be extended above or below the normal range by increasing or decreasing sample size or through instrument and recorder control.

2. Summary of Method

   2.1 A weighed portion of the sample is digested in aqua regia for 2 minutes at 95°C, followed by oxidation with potassium permanganate. Mercury in the digested sample is then measured by the conventional cold vapor technique.

   2.2 An alternate digestion[2] involving the use of an autoclave is described in (8.2).

3. Sample Handling and Preservation

   3.1 Because of the extreme sensitivity of the analytical procedure and the omnipresence of mercury, care must be taken to avoid extraneous contamination. Sampling devices and sample containers should be ascertained to be free of mercury; the sample should not be exposed to any condition in the laboratory that may result in contact or air-borne mercury contamination.

   3.2 While the sample may be analyzed without drying, it has been found to be more convenient to analyze a dry sample. Moisture may be driven off in a drying oven at a temperature of 60°C. No mercury losses have been observed by using this drying step. The dry sample should be pulverized and thoroughly mixed before the aliquot is weighed.

4. Interferences

   4.1 The same types of interferences that may occur in water samples are also possible with sediments, ie., sulfides, high copper, high chlorides, etc.

   4.2 Volatile materials which absorb at 253.7 nm will cause a positive interference. In order to remove any interfering volatile materials, the dead air space in the BOD bottle should be purged before the addition of stannous sulfate.

5. Apparatus

   5.1 Atomic Absorption Spectrophotometer (See Note 1): Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed.

NOTE 1: Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.

5.2 Mercury Hollow Cathode Lamp: Westinghouse WL-22847, argon filled, or equivalent.

5.3 Recorder: Any multi-range variable speed recorder that is compatible with the UV detection system is suitable.

5.4 Absorption Cell: Standard spectrophotometer cells 10 cm long, having quartz end windows may be used. Suitable cells may be constructed from plexiglass tubing, 1" O.D. X 4-1/2". The ends are ground perpendicular to the longitudinal axis and quartz windows (1" diameter X 1/16" thickness) are cemented in place. Gas inlet and outlet ports (also of plexiglass but 1/4" O.D.) are attached approximately 1/2" from each end. The cell is strapped to a burner for support and aligned in the light beam to give the maximum transmittance.

NOTE 2: Two 2" X 2" cards with one inch diameter holes may be placed over each end of the cell to assist in positioning the cell for maximum transmittance.

5.5 Air Pump: Any peristaltic pump capable of delivering 1 liter of air per minute may be used. A Masterflex pump with electronic speed control has been found to be satisfactory. (Regulated compressed air can be used in an open one-pass system.)

5.6 Flowmeter: Capable of measuring an air flow of 1 liter per minute.

5.7 Aeration Tubing: Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return. Straight glass tubing terminating in a coarse porous frit is used for sparging air into the sample.

5.8 Drying Tube: 6" X 3/4" diameter tube containing 20 g of magnesium perchlorate (See Note 3). The apparatus is assembled as shown in the accompanying diagram.

NOTE 3: In place of the magnesium perchlorate drying tube, a small reading lamp with 60W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about 10°C above ambient.

6. Reagents

6.1 Aqua Regia: Prepare immediately before use by carefully adding three volumes of conc. HCl to one volume of conc. $HNO_3$.

6.2 Sulfuric Acid, 0.5 N: Dilute 14.0 ml of conc. sulfuric acid to 1 liter.

6.3 Stannous Sulfate: Add 25 g stannous sulfate to 250 ml of 0.5 N sulfuric acid (6.2). This mixture is a suspension and should be stirred continuously during use.

6.4 Sodium Chloride-Hydroxylamine Sulfate Solution: Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in distilled water and dilute to 100 ml.

NOTE 4: A 10% solution of stannous chloride may be substituted for (6.3) and hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate in (6.4).

6.5 Potassium Permanganate: 5% solution, w/v. Dissolve 5 g of potassium permanganate in 100 ml of distilled water.

6.6 Stock Mercury Solution: Dissolve 0.1354 g of mercuric chloride in 75 ml of distilled water. Add 10 ml of conc. nitric acid and adjust the volume to 100.0 ml. 1.0 ml = 1.0 mg Hg.

6.7 Working Mercury Solution: Make successive dilutions of the stock mercury solution (6.6) to obtain a working standard containing 0.1 $\mu$g/ml. This working standard and the dilution of the stock mercury solutions should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot.

7. Calibration

7.1 Transfer 0, 0.5, 1.0, 2.0, 5.0 and 10 ml aliquots of the working mercury solution (6.7) containing 0 to 1.0 $\mu$g of mercury to a series of 300 ml BOD bottles. Add enough distilled water to each bottle to make a total volume of 10 ml. Add 5 ml of aqua regia (6.1) and heat 2 minutes in a water bath at 95°C. Allow the sample to cool and add 50 ml distilled water and 15 ml of $KMnO_4$ solution (6.5) to each bottle and return to the water bath for 30 minutes. Cool and add 6 ml of sodium chloride-hydroxylamine sulfate solution (6.4) to reduce the excess permanganate. Add 50 ml of distilled water. Treating each bottle individually, add 5 ml of stannous sulfate solution (6.3) and immediately attach the bottle to the aeration apparatus. At this point, the sample is allowed to stand quietly without manual agitation. The circulating pump, which has previously been adjusted to a rate of 1 liter per minute, is allowed to run continuously. The absorbance, as exhibited either on the spectrophotometer or the recorder, will increase and reach maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass valve and continue the aeration until the absorbance returns to its minimum value (See Note 5). Close the bypass valve, remove the fritted tubing from the BOD bottle and continue the aeration. Proceed with the standards and construct a standard curve by plotting peak height versus micrograms of mercury.

NOTE 5: Because of the toxic nature of mercury vapor precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system to either vent the mercury vapor into an exhaust hood or pass the vapor through some absorbing media, such as:

a) equal volumes of 0.1 N $KMnO_4$ and 10% $H_2SO_4$

b) 0.25% iodine in a 3% KI solution.

A specially treated charcoal that will absorb mercury vapor is also available from Barnebey and Cheney, E. 8th Ave. and North Cassidy St., Columbus, Ohio 43219, Cat. #580-13 or #580-22.

8. Procedure

8.1 Weigh triplicate 0.2 g portions of dry sample and place in bottom of a BOD bottle. Add 5 ml of distilled water and 5 ml of aqua regia (6.1). Heat 2 minutes in a water bath at 95°C. Cool, add 50 ml distilled water and 15 ml potassium permanganate solution (6.5) to each sample bottle. Mix thoroughly and place in the water bath for 30 minutes at 95°C. Cool and add 6 ml of sodium chloride-hydroxylamine sulfate (6.4) to reduce the excess permanganate. Add 55 ml of distilled water. Treating each bottle individually, add 5 ml of stannous sulfate (6.3) and immediately attach the bottle to the aeration apparatus. Continue as described under (7.1).

8.2 An alternate digestion procedure employing an autoclave may also be used. In this method 5 ml of conc. $H_2SO_4$ and 2 ml of conc. $HNO_3$ are added to the 0.2 g of sample. 5 ml of saturated $KMnO_4$ solution is added and the bottle covered with a piece of aluminum foil. The samples are autoclaved at 121°C and 15 lbs. for 15 minutes. Cool, make up to a volume of 100 ml with distilled water and add 6 ml of sodium chloride-hydroxylamine sulfate solution (6.4) to reduce the excess permanganate. Purge the dead air space and continue as described under (7.1).

9. Calculation

9.1 Measure the peak height of the unknown from the chart and read the mercury value from the standard curve.

9.2 Calculate the mercury concentration in the sample by the formula:

$$\mu g\ Hg/g = \frac{\mu g\ Hg \text{ in the aliquot}}{\text{wt of the aliquot in gms.}}$$

9.3 Report mercury concentrations as follows: Below 0.1 $\mu$g/gm, <0.1; between 0.1 and 1 $\mu$g/gm, to the nearest 0.01 $\mu$g; between 1 and 10 $\mu$g/gm, to nearest 0.1 $\mu$g; above 10 $\mu$g/gm, to nearest $\mu$g.

10. Precision and Accuracy

10.1 The following standard deviations on replicate sediment samples were recorded at the indicated levels; 0.29 $\mu$g/g ±0.02 and 0.82 $\mu$g/g ±0.03. Recovery of mercury at these levels, added as methyl mercuric chloride, was 97 and 94%, respectively.

## Bibliography


1. Bishop, J. N., "Mercury in Sediments", Ontario Water Resources Comm., Toronto, Ontario, Canada, 1971.
2. Salma, M., private communication, EPA Cal/Nev Basin Office, Almeda, California.

# MOLYBDENUM

(Standard Conditions)

STORET NO. Total 01062

**Optimum Concentration Range:** 0.5-20 mg/l using a wavelength of 313.3 nm

**Sensitivity:** 0.3 mg/l

**Detection Limit:** 0.1 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 1.840 g of ammonium molybdate $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$ (analytical reagent grade) in deionized distilled water and dilute to 1 liter. 1 ml = 1 mg Mo (1000 mg/l).
2. Aluminum nitrate solution: Dissolve 139 g aluminum nitrate, $Al(NO_3)_3 \cdot 9H_2O$, in 150 ml of deionized distilled water; heat to effect solution. Allow to cool and make up to 200 ml.
3. Prepare dilutions of the stock molybdenum solution to be used as calibration standards at the time of analysis. To each 100 ml of standard and sample alike, add 2 ml of the aluminum nitrate solution. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Molybdenum hollow cathode lamp
2. Wavelength: 313.3 nm
3. Fuel: Acetylene
4. Oxidant: Nitrous Oxide
5. Type of flame: Fuel rich

**Interferences**

1. With the recommended nitrous oxide-acetylene flame, interferences of calcium and other ions may be controlled by adding 1000 mg/l of a refractory metal such as aluminum [Anal. Chem. Acta *44*, 437 (1969)]. This should be done to both samples and standards alike.

**Notes**

1. For low levels of molybdenum an oxine extraction procedure may be useful. (Ref: Chau et.al., Anal. Chem. Acta *48,* 205, 1969).
2. Data to be entered into STORET must be reported as µg/l.

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 0.30, 1.5 and 7.5 mg Mo/l, the standard deviations were ±0.007, ±0.02 and ±0.07, respectively. Recoveries at these levels were 100%, 96% and 95%, respectively.

# NICKEL

(Standard Conditions)

STORET NO. Total 01067

**Optimum Concentration Range:** 0.3-10 mg/l using a wavelength of 232.0 nm

**Sensitivity:** 0.15 mg/l

**Detection List:** 0.02 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 4.953 g of nickel nitrate, $Ni(NO_3)_2 \cdot 6H_2O$ (analytical reagent grade) in deionized distilled water. Add 10 ml of conc. nitric acid and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Ni (1000 mg/l).
2. Prepare dilutions of the stock nickel solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Nickel hollow cathode lamp
2. Wavelength: 232.0 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of Flame: Oxidizing

**Interferences**

1. The 352.4 nm wavelength is less susceptible to nonatomic absorbance and may be used. The calibration curve is more linear at this wavelength; however, there is some loss of sensitivity.

**Notes**

1. For levels of nickel below 50 µg/l, the extraction procedure is recommended.
2. Data to be entered into STORET must be reported as µg/l.
3. The heptoxime method may be used (Standard Methods, 13th Edition, p 443).

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 0.20, 1.0 and 5.0 mg Ni/l, the standard deviations were ±0.011, ±0.02 and ±0.04, respectively. Recoveries at these levels were 100%, 97% and 93%, respectively.

# POTASSIUM

(Standard Conditions)

STORET NO. Total 00937

**Optimum Concentration Range:** 0.1-2 mg/l using a wavelength of 766.5 nm

**Sensitivity:** 0.04 mg/l

**Detection Limit:** 0.005 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 0.1907 g of KCl (analytical reagent grade), dried at 110°C, in deionized distilled water and make up to 1 liter. 1 ml = 0.10 mg K (100 mg/1).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis.

**Sample Preparation**

1. For the analysis of total postassium in domestic and industrial effluents, the procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.
2. For ambient waters, a representative aliquot of a well-mixed sample may also be used directly for analysis. If suspended solids are present in sufficient amounts to clog the nebulizer, the sample may be allowed to settle and the supernatant liquid analyzed directly.
3. Samples should be preserved with (1:1) nitric acid to a pH of 2 at the time of collection.

**Instrumental Parameters (General)**

1. Potassium hollow cathode lamp
2. Wavelength: 766.5 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Slightly oxidizing

**Notes**

1. The Osram potassium vapor-discharge lamp may also be used in the Perkin-Elmer 303. In this case the current should be 350 ma or the optimum operating current.
2. Sodium may interfere if present at much higher levels than the potassium. This effect can be compensated by approximately matching the sodium content of the potassium standards with that of the sample.

3. Potassium absorption is enhanced in the presence of Na, Li and Cs, especially in a high-temperature flame. This enhancement effect of sodium can be eliminated by changing the burner height and the type of flame used. The burner assembly is set approximately 0.05 cm below the optical light path so that the optical light path is sliced at the bottom by the burner head. A fuel-rich flame is used.
4. The 404.4 nm line may also be used. This line has a sensitivity of 5 mg/l for 1% absorption.
5. To cover the range of potassium values normally observed in surface waters (0.1-20 mg/l), it is suggested that the burner be rotated 75°.
6. The flame photometric or colorimetric methods may be used (Standard Methods, 13th Edition, p 283 & 285).
7. Data to be entered into STORET must be reported as mg/l.

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using distilled water samples at concentrations of 1.6 and 6.3 mg/l, the standard deviations were ±0.2 and ±0.5, respectively. Recoveries at these levels were 103% and 102%.

# SELENIUM

## (Gaseous Hydride Method)

STORET NO. Total 01147

1. Scope and Application

   1.1 The gaseous hydride method determines inorganic selenium when present in concentrations at or above 2 μg/l. The method is applicable to most fresh and saline waters, in the absence of high concentrations of chromium, cobalt, copper, mercury, molybdenum, nickel and silver.

2. Summary of Method

   2.1 Selenium in the sample is reduced from the +6 oxidation state to the +4 oxidation state by the addition of $SnCl_2$. Zinc is added to the acidified sample, producing hydrogen and converting the selenium to the hydride, $SeH_2$. The gaseous selenium hydride is swept into an argon-hydrogen flame of an atomic absorption spectrophotometer. The working range of the method is 2-20 μg/l using the 196.0 nm wavelength.

3. Comments

   3.1 In analyzing most surface and ground waters, interferences are rarely encountered. Industrial waste samples should be spiked with a known amount of selenium to establish adequate recovery.

   3.2 Organic forms of selenium must be converted to an inorganic form and organic matter must be oxidized before beginning the analysis.

   3.3 Data to be entered into STORET must be reported as μg/l.

4. Precision and Accuracy

   4.1 Ten replicate solutions of selenium oxide at the 5, 10 and 15 μg/l level were analyzed by a single laboratory (Caldwell, Et.Al.). Standard deviations at these levels were ±0.6, ±1.1 and ±2.9 with recoveries of 100, 100 and 101%.

### Bibliography

1. Caldwell, J. S., Lishka, R. J., and McFarren, E. F., "Evaluation of a Low-Cost Arsenic and Selenium Determination at Microgram per Liter Levels", JAWWA, vol 65, p. 731, Nov. 1973.

# SILVER

(Standard Conditions)

STORET NO. Total 01077

**Optimum Concentration Range:** 0.1-4 mg/l using a wavelength of 328.1 nm

**Sensitivity:** 0.06 mg/l

**Detection Limit:** 0.01 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 1.575 g of $AgNO_3$ (analytical reagent grade) in deionized distilled water, add 10 ml conc. $HNO_3$ and make up to 1 liter. 1 ml = 1 mg Ag (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. Maintain an acid strength of 0.15% $HNO_3$ in all calibration standards.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory; however, the residue must be taken up in dilute nitric acid rather than hydrochloric to prevent precipitation of AgCl.

**Instrumental Parameters (General)**

1. Silver hollow cathode lamp
2. Wavelength: 328.1 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

**Notes**

1. For levels of silver below 20 $\mu$g/l, the extraction procedure is recommended.
2. Silver nitrate standards are light sensitive. Dilutions of the stock should be discarded after use as concentrations below 10 mg/l are not stable over long periods of time.
3. The 338.2 nm wavelength may also be used. This has a relative sensitivity of 3.
4. Data to be entered into STORET must be reported as $\mu$g/l.
5. The dithizone colorimetric method may be used (Standard Methods, 13th Edition, p 310).

# SODIUM

(Standard Conditions)

STORET NO. Total 00929

**Optimum Concentration Range:** 0.03-1.0 mg/l using a wavelength of 589.6 nm
**Sensitivity:** 0.015 mg/l
**Detection Limit:** 0.002 mg/l

**Preparation of Standard Solutions**

1. Stock Solution: Dissolve 2.542 g of NaCl (analytical reagent grade), dried at 140°C, in deionized distilled water and make up to 1 liter. 1 ml = 1 mg Na (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis.

**Sample Preparation**

1. For the analysis of total sodium in domestic and industrial effluents, the procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.
2. For ambient waters, a representative aliquot of a well-mixed sample may be used directly for analysis. If suspended solids are present in sufficient amounts to clog the nebulizer, the sample may be allowed to settle and the supernatant liquid analyzed directly.
3. Samples should be preserved with (1:1) nitric acid to a pH of 2 at the time of collection.

**Instrumental Parameters (General)**

1. Sodium hollow cathode lamp
2. Wavelength: 589.6 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

**Notes**

1. The 330.2 nm resonance line of sodium yields a sensitivity of about 3 mg/l sodium for 1% absorption and provides a convenient way to avoid the need to dilute more concentrated solutions of sodium.

2. Low-temperature flames increase sensitivity by reducing the extent of ionization of this easily ionized metal. Ionization may also be controlled by adding potassium (1000 mg/l) to both standards and samples.
3. Data to be entered into STORET must be reported as mg/l.
4. The flame photometric method may be used (Standard Methods, 13th Edition, p 317).

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using distilled water samples at levels of 8.2 and 52 mg/l, the standard deviations were ±0.1 and ±0.8, respectively. Recoveries at these levels were 102% and 100%.

# THALLIUM

(Standard Conditions)

STORET NO. Total 01059

**Optimum Concentration Range:** 1-20 mg/l using a wavelength of 276.8 nm

**Sensitivity:** 0.5 mg/l

**Detection Limit:** 0.1 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 1.303 g of thallium nitrate, $TlNO_3$ (analytical reagent grade) in deionized distilled water. Add 10 ml of conc. nitric acid and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Tl (1000 mg/l).
2. Prepare dilutions of the stock thallium solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Thallium hollow cathode lamp
2. Wavelength: 276.8 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

**Notes**

1. Data to be entered into STORET must be reported as $\mu$g/l.

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 0.60, 3.0 and 15 mg Tl/l, the standard deviations were ±0.018, ±0.05 and ±0.2, respectively. Recoveries at these levels were 100%, 98% and 98%, respectively.

# TIN

(Standard Conditions)

STORET NO. Total 01102

**Optimum Concentration Range:** 10-200 mg/l using a wavelength of 286.3 nm

**Sensitivity:** 4 mg/l

**Detection Limit:** 0.8 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 1.000 g of tin metal (analytical reagent grade) in 100 ml of conc. HCl and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg Sn (1000 mg/l).
2. Prepare dilutions of the stock tin solution to be used as calibration standards at the time of analysis. Maintain an acid concentration of 10% HCl in all solutions.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Tin hollow cathode lamp
2. Wavelength: 286.3 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Fuel rich

**Notes**

1. Data to be entered into STORET must be reported as $\mu$g/l.

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 4.0, 20 and 60 mg Sn/l, the standard deviations were ±0.25, ±0.5 and ±0.5, respectively. Recoveries at these levels were 96%, 101% and 101%, respectively.

# TITANIUM

(Standard Conditions)

STORET NO. Total 01152

**Optimum Concentration Range:** 5-100 mg/l using a wavelength of 365.3 nm

**Sensitivity:** 2.0 mg/l

**Detection Limit:** 0.3 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 4.008 g of titanium sulfate, $Ti_2(SO_4)_3$, in dilute HCl and make up to 1 liter with deionized distilled water. 1 ml = 1 mg Ti (1000 mg/l).
2. Potassium chloride solution: Dissolve 95 g potassium chloride, KCl, in distilled water and make up to 1 liter.
3. Prepare dilutions of the stock titanium solution to be used as calibration standards at the time of analysis. To each 100 ml of standard and sample alike, add 2 ml of potassium chloride solution.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual must be modified by the addition of 3 ml of conc. sulfuric acid in addition to the nitric acid. This is necessary to keep any titanium that may be present in solution.

**Instrumental Parameters (General)**

1. Titanium hollow cathode lamp
2. Wavelength: 365.3 nm
3. Fuel: Acetylene
4. Oxidant: Nitrous Oxide
5. Type of flame: Fuel rich

**Interferences**

1. A number of elements increase the sensitivity of titanium. To control this problem, potassium (1000 mg/l) must be added to standards and samples alike. [Atomic Absorption Newsletter *6*, p 86 (1967)]

**Notes**

1. Data to be entered into STORET must be reported as µg/l.

**Precision and Accuracy**

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 2.0, 10 and 50 mg Ti/l, the standard deviations were ±0.07, ±0.1 and ±0.4, respectively. Recoveries at these levels were 97%, 91% and 88%, respectively.

# VANADIUM

(Standard Conditions)

STORET NO. Total 01087

**Optimum Concentration Range:** 1-100 mg/l using a wavelength of 318.4 nm

**Sensitivity:** 0.8 mg/l

**Detection Limit:** 0.2 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Dissolve 1.7854 g of vanadium pentoxide, $V_2O_5$ (analytical reagent) in 10 ml of conc. nitric acid and dilute to 1 liter with deionized distilled water. 1 ml = 1 mg V (1000 mg/l).
2. Aluminum nitrate solution: Dissolve 139 g aluminum nitrate, $Al(NO_3)_3 \cdot 9H_2O$, in 150 ml of deionized distilled water; heat to effect solution. Allow to cool and make up to 200 ml.
3. Prepare dilutions of the stock vanadium solution to be used as calibration standards at the time of analysis. To each 100 ml of standard and sample alike, add 2 ml of the aluminum nitrate solution. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters (General)**

1. Vanadium hollow cathode lamp
2. Wavelength: 318.4 nm
3. Fuel: Acetylene
4. Oxidant: Nitrous oxide
5. Type of flame: Fuel rich

**Interferences**

1. It has been reported that high concentrations of aluminum and titanium increase the sensitivity of vanadium. This interference can be controlled by adding excess aluminum (1000 ppm) to both samples and standards. [Talanta *15*, 871 (1968)].

Notes

1. Data to be entered into STORET must be reported as µg/l.
2. The gallic acid colorimetric method may be used (Standard Methods, 13th Edition, p 357).

Precision and Accuracy

1. In a single laboratory (MDQARL), using a mixed industrial-domestic waste effluent at concentrations of 2.0, 10 and 50 mg V/l, the standard deviations were ±0.10, ±0.1 and ±0.2, respectively. Recoveries at these levels were 100%, 95% and 97%, respectively.

# ZINC

(Standard Conditions)

STORET NO. Total 01092

**Optimum Concentration Range:** 0.05-2 mg/l using a wavelength of 213.9 nm

**Sensitivity:** 0.02 mg/l

**Detection Limit:** 0.005 mg/l

**Preparation of Standard Solution**

1. Stock Solution: Carefully weigh 1.00 g of zinc metal (analytical reagent grade) and dissolve cautiously in 10 ml $HNO_3$. When solution is complete make up to 1 liter with deionized distilled water. 1 ml = 1 mg Zn (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. The *calibration standards* should be prepared using the same type of acid (HCl or $HNO_3$) and at the same concentration as the samples for analysis.

**Sample Preparation**

1. The procedure for the determination of total metals as given in part 4.1.3 of the Atomic Absorption Methods section of this manual has been found to be satisfactory.

**Instrumental Parameters**

1. Zinc hollow cathode lamp
2. Wavelength: 213.9 nm
3. Fuel: Acetylene
4. Oxidant: Air
5. Type of flame: Oxidizing

**Notes**

1. High levels of silicon may interfere.
2. The air-acetylene flame absorbs about 25% of the energy at the 213.9 nm line.
3. The sensitivity may be increased by the use of low-temperature flames.
4. Data to be entered into STORET must be reported as $\mu$g/l.
5. The dithizone colorimetric method may be used (Standard Methods, 13th Edition, p 444).

**Precision and Accuracy**

1. An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of MDQARL. Six synthetic concentrates containing varying levels of aluminum.

cadmium, chromium, iron, manganese, lead and zinc were added to natural water samples. The statistical results for zinc were as follows:

| Number of Labs | True Values μg/liter | Mean Value μg/liter | Standard Deviation μg/liter | Accuracy as % Bias |
|---|---|---|---|---|
| 86 | 281 | 284 | 97 | 1.2 |
| 89 | 310 | 308 | 114 | –0.7 |
| 82 | 56 | 62 | 28 | 11.3 |
| 81 | 70 | 75 | 28 | 6.6 |
| 62 | 7 | 22 | 26 | 206 |
| 61 | 11 | 17 | 18 | 56.6 |

# METHYLENE BLUE ACTIVE SUBSTANCES (MBAS)

(Methylene Blue Method)

STORET NO. 38260

1. Scope and Application
   1.1 This method is applicable to the measurement of methylene blue active substances (MBAS) in drinking waters, surface waters, domestic and industrial wastes. It is not applicable to measurement of surfactant-type materials in saline waters.
   1.2 It is not possible to differentiate between linear alkyl sulfonate (LAS) and alkyl benzene sulfonate (ABS) or other isomers of these types of compounds. However, LAS has essentially replaced ABS on the surfactant market so that measurable surfactant materials will probably be LAS type materials.
   1.3 The method is applicable over the range of 0.025 to 100 mg/l LAS.
2. Summary of Method
   2.1 The dye, methylene blue, in aqueous solution reacts with anionic-type surface active materials to form a blue colored salt. The salt is extractable with chloroform and the intensity of color produced is proportional to the concentration of MBAS.
3. Comments
   3.1 Materials other than man-made surface active agents which react with methylene blue are organically bound sulfates, sulfonates, carboxylates, phosphates, phenols, cyanates, thiocyanates and some inorganic ions such as nitrates and chlorides. However, the occurrence of these materials at interference levels is relatively rare and with the exception of chlorides may generally be disregarded.
   3.2 Chlorides at concentration of about 1000 mg/l show a positive interference but the degree of interference has not been quantified. For this reason the method is not applicable to brine samples.
   3.3 Naturally occurring organic materials that react with methylene blue are relatively insignificant. Except under highly unusual circumstances, measurements of MBAS in finished waters, surface waters and domestic sewages may be assumed to be accurate measurements of man-made surface active agents.
4. Precision and Accuracy
   4.1 On a sample of filtered river water, spiked with 2.94 mg LAS/liter, 110 analysts obtained a mean of 2.98 mg/liter with a standard deviation of ±0.272.

4.2 On a sample of tap water spiked with 0.48 mg LAS/liter, analysts obtained a mean of 0.49 mg/l with a standard deviation of ±0.048.

4.3 On a sample of distilled water spiked with 0.27 mg LAS/liter, 110 analysts obtained a mean of 0.24 mg/l with a standard deviation of ±0.036.

4.4 Analytical Reference Service, Water Surfactant No. 3, Study No. 32, (1968).

5. References

5.1 The procedure to be used for this determination is found in:

Standard Methods for the Examination of Water and Wastewaters, 13th Edition, p 339-342, Method No. 159A (1971).

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 492, Method D2330-68 (1973).

# NITROGEN, AMMONIA
## (Distillation Procedure)

STORET NO. 00610

1. Scope and Application
   1.1 This distillation method covers the determination of ammonia-nitrogen exclusive of total Kjeldahl nitrogen, in drinking, surface, and saline waters, domestic and industrial wastes. It is the method of choice where economics and sample load do not warrant the use of automated equipment.
   1.2 The method covers the range from about 0.05 to 1.0 mg/l $NH_3$–N/l for the colorimetric procedures, from 1.0 to 25 mg/l for the titrimetric procedure, and from 0.05 to 1400 mg/l for the electrode method.
   1.3 This method is described for macro glassware; however, micro distillation equipment may also be used.
2. Summary of Method
   2.1 The sample is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and is then distilled into a solution of boric acid. The ammonia in the distillate can be determined colorimetrically by nesslerization, titrimetrically with standard sulfuric acid with the use of a mixed indicator, or potentiometrically by the ammonia electrode. The choice between the first two procedures depends on the concentration of the ammonia.
3. Sample Handling and Preservation
   3.1 Samples may be preserved with 2 ml of conc. $H_2SO_4$ or 40 mg $HgCl_2$ per liter and stored at 4°C.
4. Interferences
   4.1 A number of aromatic and aliphatic amines, as well as other compounds, both organic and inorganic, will cause turbidity upon the addition of Nessler reagent, so direct nesslerization (i.e., without distillation), has been discarded as an official method.
   4.2 Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at the pH of 9.5 at which distillation is carried out. Volatile alkaline compounds, such as certain ketones, aldehydes, and alcohols, may cause an off-color upon nesslerization in the distillation method. Some of these, such as formaldehyde, may be eliminated by boiling off at a low pH (approximately 2 to 3) prior to distillation and nesslerization.

4.3 Residual chlorine must also be removed by pretreatment of the sample with sodium thiosulfate before distillation.

4.4 *If the sample has been preserved with a mercury salt, the mercury ion must be complexed with sodium thiosulfate (0.2 g) prior to distillation.*

5. Apparatus

5.1 An all-glass distilling apparatus with an 800-1000 ml flask.

5.2 Spectrophotometer or filter photometer for use at 425 nm and providing a light path of 1 cm or more.

5.3 Nessler tubes: Matched Nessler tubes (APHA Standard) about 300 mm long, 17 mm inside diameter, and marked at 225 mm ±1.5 mm inside measurement from bottom.

5.4 Erlenmeyer flasks: The distillate is collected in 500 ml glass-stoppered flasks. These flasks should be marked at the 350 and the 500 ml volumes. With such marking, it is not necessary to transfer the distillate to volumetric flasks.

6. Reagents

6.1 Distilled water should be free of ammonia. Such water is best prepared by passage through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.

NOTE 1: All solutions must be made with ammonia-free water.

6.2 Ammonium chloride, stock solution:

1.0 ml = 1.0 mg $NH_3$–N. Dissolve 3.819 g $NH_4Cl$ in distilled water and bring to volume in a 1 liter volumetric flask.

6.3 Ammonium chloride, standard solution:

1.0 ml = 0.01 mg. Dilute 10.0 ml of stock solution (6.2) to 1 liter in a volumetric flask.

6.4 Boric acid solution (20 g/l): Dissolve 20 g $H_3BO_3$ in distilled water and dilute to 1 liter.

6.5 Mixed indicator: Mix 2 volumes of 0.2% methyl red in 95% ethyl alcohol with 1 volume of 0.2% methylene blue in 95% ethyl alcohol. This solution should be prepared fresh every 30 days.

NOTE 2: Specially denatured ethyl alcohol conforming to Formula 3A or 30 of the U.S. Bureau of Internal Revenue may be substituted for 95% ethanol.

6.6 Nessler reagent: Dissolve 100 g of mercuric iodide and 70 g of potassium iodide in a small amount of water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of NaOH in 500 ml of water. Dilute the mixture to 1 liter. If

this reagent is stored in a Pyrex bottle out of direct sunlight, it will remain stable for a period of up to 1 year.

NOTE 3: This reagent should give the characteristic color with ammonia within 10 minutes after addition, and should not produce a precipitate with small amounts of ammonia (0.04 mg in a 50 ml volume).

6.7 Borate buffer: Add 88 ml of 0.1 N NaOH solution to 500 ml of 0.025 M sodium tetraborate solution (5.0 g anhydrous $Na_2B_4O_7$ or 9.5 g $Na_2B_4O_7 \cdot 10H_2O$ per liter) and dilute to 1 liter.

6.8 Sulfuric acid, standard solution: (0.02 N, 1 ml = 0.28 mg $NH_3$–N). Prepare a stock solution of approximately 0.1 N acid by diluting 3 ml of conc. $H_2SO_4$ (sp. gr. 1.84) to 1 liter with $CO_2$-free distilled water. Dilute 200 ml of this solution to 1 liter with $CO_2$-free distilled water.

NOTE 4: An alternate and perhaps preferable method is to standardize the approximately 0.1 N $H_2SO_4$ solution against a 0.100 N $Na_2CO_3$ solution. By proper dilution the 0.02 N acid can then be prepared.

6.8.1 Standardize the approximately 0.02 N acid against 0.0200 N $Na_2CO_3$ solution. This last solution is prepared by dissolving 1.060 g anhydrous $Na_2CO_3$, oven-dried at 140°C, and diluting to 1000 ml with $CO_2$-free distilled water.

6.9 Sodium hydroxide, 1 N: Dissolve 40 g NaOH in ammonia-free water and dilute to 1 liter.

6.10 Dechlorinating reagents: A number of dechlorinating reagents may be used to remove residual chlorine prior to distillation. These include:

a. Sodium thiosulfate (1/70 N): Dissolve 3.5 g $Na_2S_2O_3$ in distilled water and dilute to 1 liter. One ml of this solution will remove 1 mg/l of residual chlorine in 500 ml of sample.

b. Sodium arsenite (1/70 N): Dissolve 1.0 g $NaAsO_2$ in distilled water and dilute to 1 liter.

7. Procedure

7.1 Preparation of equipment: Add 500 ml of distilled water to an 800 ml Kjeldahl flask. The addition of boiling chips which have been previously treated with dilute NaOH will prevent bumping. Steam out the distillation apparatus until the distillate shows no trace of ammonia with Nessler reagent.

7.2 Sample preparation: Remove the residual chlorine in the sample by adding dechlorinating agent equivalent to the chlorine residual. To 400 ml of sample add 1 N NaOH (6.9), until the pH is 9.5, checking the pH during addition with a pH meter or by use of a short range pH paper.

7.3 Distillation: Transfer the sample, the pH of which has been adjusted to 9.5, to an 800 ml Kjeldahl flask and add 25 ml of the borate buffer (6.7). Distill 300 ml at the rate of 6-10 ml/min. into 50 ml of 2% boric acid (6.4) contained in a 500 ml Erlenmeyer flask.

NOTE 5: The condenser tip or an extention of the condenser tip must extend below the level of the boric acid solution.

Dilute the distillate to 500 ml with distilled water and nesslerize an aliquot to obtain an approximate value of the ammonia-nitrogen concentration. For concentrations above 1 mg/l the ammonia should be determined titrimetrically. For concentrations below this value it is determined colorimetrically. The electrode method may also be used.

7.4 Determination of ammonia in distillate: Determine the ammonia content of the distillate titrimetrically, colorimetrically or potentiometrically as described below.

7.4.1 Titrimetric determination: Add 3 drops of the mixed indicator to the distillate and titrate the ammonia with the 0.02 N $H_2SO_4$, matching the end point against a blank containing the same volume of distilled water and $H_3BO_3$ solution.

7.4.2 Colorimetric determination: Prepare a series of Nessler tube standards as follows:

| ml of Standard 1.0 ml = 0.01 mg $NH_3$–N | mg $NH_3$–N/50.0 ml |
|---|---|
| 0.0 | 0.0 |
| 0.5 | 0.005 |
| 1.0 | 0.01 |
| 2.0 | 0.02 |
| 3.0 | 0.03 |
| 4.0 | 0.04 |
| 5.0 | 0.05 |
| 8.0 | 0.08 |
| 10.0 | 0.10 |

Dilute each tube to 50 ml with distilled water, add 1.0 ml of Nessler reagent (6.6) and mix. After 20 minutes read the optical densities at 425 nm against the blank. From the values obtained plot optical density (absorbance) vs. mg $NH_3$–N for the standard curve.

7.4.3 Potentiometric determination: Consult the method entitled Nitrogen, Ammonia: Selective Ion Electrode Method in this manual.

7.4.4 It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least two standards (a high and low) be distilled and compared to similar values on the curve to insure that the distillation technique is reliable. If distilled standards do not agree with undistilled standards the operator should find the cause of the apparent error before proceeding.

7.5 Determine the ammonia in the distillate by nesslerizing 50 ml or an aliquot diluted to 50 ml and reading the optical density at 425 nm as described above for the standards. Ammonia-nitrogen content is read from the standard curve.

8. Calculations

8.1 Titrimetric

$$\text{mg/l } NH_3\text{–N} = \frac{A \times 0.28 \times 1000}{S}$$

where:

A = ml 0.02 N $H_2SO_4$ used.

S = ml sample.

8.2 Spectrophotometric

$$\text{mg/l } NH_3\text{–N} = \frac{A \times 1000}{D} \times \frac{B}{C}$$

where:

A = mg $NH_3$–N read from standard curve.

B = ml total distillate collected, including boric acid and dilution.

C = ml distillate taken for nesslerization.

D = ml of original sample taken.

8.3 Potentiometric

$$\text{mg/l } NH_3\text{–N} = \frac{500}{D} \times A$$

where:

A = mg $NH_3$–N/l from electrode method standard curve.

D = ml of original sample taken.

9. Precision and Accuracy

9.1 Twenty-four analysts in sixteen laboratories analyzed natural water samples containing exact increments of an ammonium salt, with the following results:

| Increment as Nitrogen, Ammonia mg N/liter | Precision as Standard Deviation mg N/liter | Accuracy as Bias, % | Accuracy as Bias, mg N/liter |
|---|---|---|---|
| 0.21 | 0.122 | − 5.54 | −0.01 |
| 0.26 | 0.070 | −18.12 | −0.05 |
| 1.71 | 0.244 | + 0.46 | +0.01 |
| 1.92 | 0.279 | − 2.01 | −0.04 |

(FWPCA Method Study 2, Nutrient Analyses)

# NITROGEN, AMMONIA

## (Selective Ion Electrode Method)

STORET NO. 00610

1. Scope and Application
   1.1 This method is applicable to the measurement of ammonia-nitrogen in drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 This method covers the range from 0.03 to 1400 mg $NH_3$–N/l. Color and turbidity have no effect on the measurements and distillation is not necessary.
2. Summary of Method
   2.1 The ammonia is determined potentiometrically using a selective ion ammonia electrode and a pH meter having an expanded millivolt scale or a specific ion meter.
   2.2 The ammonia electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an ammonium chloride internal solution. Ammonia in the sample diffuses through the membrane and alters the pH of the internal solution, which is sensed by a pH electrode. The constant level of chloride in the internal solution is sensed by a chloride selective ion electrode which acts as the reference electrode.
3. Sample Handling and Preservation
   3.1 Preserve by refrigeration at 4°C; analyze within 24 hours. If longer holding times are desired, preserve with 2 ml conc. $H_2SO_4$ per liter (pH<2).
4. Interferences
   4.1 Volatile amines act as a positive interference.
   4.2 Mercury interferes by forming a strong complex with ammonia. Thus the samples cannot be preserved with mercuric chloride.
5. Apparatus
   5.1 Electrometer (pH meter) with expanded mV scale or a specific ion meter.
   5.2 Ammonia selective electrode, such as Orion Model 95-10 or EIL Model 8002-2.
   5.3 Magnetic stirrer, thermally insulated, and Teflon-coated stirring bar.
6. Reagents
   6.1 Distilled water: Special precautions must be taken to insure that the distilled water is free of ammonia. This is accomplished by passing distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin.

6.2 Sodium hydroxide, 10N: Dissolve 400 g of sodium hydroxide in 800 ml of distilled water. Cool and dilute to 1 liter with distilled water (6.1).

6.3 Ammonium chloride, stock solution: 1.0 ml = 1.0 mg $NH_3$–N. Dissolve 3.819 g $NH_4Cl$ in water and bring to volume in a 1 liter volumetric flask using distilled water (6.1).

6.4 Ammonium chloride, standard solution: 1.0 ml = 0.01 mg $NH_3$–N. Dilute 10.0 ml of the stock solution (6.3) to 1 liter with distilled water (6.1) in a volumetric flask.

NOTE 1: When analyzing saline waters, standards must be made up in synthetic ocean water (SOW); found in Nitrogen, Ammonia: Automated Colorimetric Phenate Method.

7. Procedure

7.1 Preparation of standards: Prepare a series of standard solutions covering the concentration range of the samples by diluting either the stock or standard solutions of ammonium chloride.

7.2 Calibration of electrometer: Place 100 ml of each standard solution in clean 150 ml beakers. Immerse electrode into standard of lowest concentration and add 1 ml of 10N sodium hydroxide solution while mixing. Keep electrode in the solution until a stable reading is obtained.

NOTE 2: The pH of the solution after the addition of NaOH *must* be above 11.

*Caution*: Sodium hydroxide must not be added prior to electrode immersion, for ammonia may be lost from a basic solution.

7.3 Repeat this procedure with the remaining standards, going from lowest to highest concentration. Using semilogarithmic graph paper, plot the concentration of ammonia in mg $NH_3$–N/l on the log axis vs. the electrode potential developed in the standard on the linear axis, starting with the lowest concentration at the bottom of the scale.

7.4 Calibration of a specific ion meter: Follow the directions of the manufacturer for the operation of the instrument.

7.5 Sample measurement: Follow the procedure in (7.2) for 100 ml of sample in 150 ml beakers. Record the stabilized potential of each unknown sample and convert the potential reading to the ammonia concentration using the standard curve. If a specific ion meter is used, read the ammonia level directly in mg $NH_3$–N/l.

8. Precision and Accuracy

8.1 In a single laboratory (MDQARL), using surface water samples at concentrations of 1.00, 0.77, 0.19, and 0.13 mg $NH_3$–N/l, standard deviations were ±0.038, ±0.017, ±0.007, and ±0.003, respectively.

8.2 In a single laboratory (MDQARL), using surface water samples at concentrations of 0.19 and 0.13 $NH_3$–N/l, recoveries were 96% and 91%, respectively.

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NITROGEN, AMMONIA

(Automated Colorimetric Phenate Method)

STORET NO. 00610

1. Scope and Application

   1.1 This method covers the determination of ammonia in drinking, surface, and saline waters, domestic and industrial wastes in the range of 0.01 to 2.0 mg/l $NH_3$ as N. This range is for photometric measurements made at 630-660 nm in a 15 mm or 50 mm tubular flow cell. Higher concentrations can be determined by sample dilution. Approximately 20 to 60 samples per hour can be analyzed.

2. Summary of Method

   2.1 Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.

3. Sample Handling and Preservation

   3.1 Preservation by addition of 2 ml conc. $H_2SO_4$ or 40 mg $HgCl_2$ per liter and refrigeration at 4°C. Note $HgCl_2$ interference under (4.2).

4. Interferences

   4.1 In sea water, calcium and magnesium ions are present in concentrations sufficient to cause precipitation problems during the analysis. This problem is eliminated by using 5% EDTA.

   4.2 Mercury chloride, used as a preservative, gives a negative interference by complexing with the ammonia. This is overcome by adding a comparable amount of $HgCl_2$ to the ammonia standards used for the preparation of the standard curve.

   4.3 Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used will also interfere.

5. Apparatus

   5.1 Technicon AutoAnalyzer Unit (AAI or AAII) consisting of:

      5.1.1 Sampler.

      5.1.2 Manifold (AAI) or Analytical Cartridge (AAII).

      5.1.3 Proportioning pump.

      5.1.4 Heating bath with double delay coil (AAI).

      5.1.5 Colorimeter equipped with 15 mm tubular flow cell and 630-660 nm filters.

5.1.6 Recorder.

5.1.7 Digital printer for AAII (optional).

6. Reagents

6.1 Distilled water: Special precaution must be taken to insure that distilled water is free of ammonia. Such water is prepared by passage of distilled water through an ion exchange column comprised of a mixture of both strongly acidic cation and strongly basic anion exchange resins. The regeneration of the ion exchange column should be carried out according to the instruction of the manufacturer. NOTE 1: All solutions must be made using ammonia-free water.

6.2 Sulfuric acid 5N: Air scrubber solution. Carefully add 139 ml of conc. sulfuric acid to approximately 500 ml of ammonia-free distilled water. Cool to room temperature and dilute to 1 liter with ammonia-free distilled water.

6.3 Sodium phenolate: Using a 1 liter Erlenmeyer flask, dissolve 83 g phenol in 500 ml of distilled water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically, cool flask under water faucet. When cool, dilute to 1 liter with distilled water.

6.4 Sodium hypochlorite solution: Dilute 250 ml of a bleach solution containing 5.25% NaOCl (such as "Clorox") to 500 ml with distilled water. Available chlorine level should approximate 2 to 3%. Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.

6.5 Disodium ethylenediamine-tetraacetate (EDTA) (5%): Dissolve 50 g of EDTA (disodium salt) and approximately six pellets of NaOH in 1 liter of distilled water. NOTE 2: On salt water samples where EDTA solution does not prevent precipitation of cations, sodium potassium tartrate solution may be used to advantage. It is prepared as follows:

6.5.1 Sodium potassium tartrate solution: 10% $NaKC_4H_4O_6 \cdot 4H_2O$. To 900 ml of distilled water add 100 g sodium potassium tartrate. Add 2 pellets of NaOH and a few boiling chips, boil gently for 45 minutes. Cover, cool, and dilute to 1 liter with ammonia-free distilled water. Adjust pH to 5.2±.05 with $H_2SO_4$. After allowing to settle overnight in a cool place, filter to remove precipitate. Then add 1/2 ml Brij-35 (available from Technicon Corporation) solution and store in stoppered bottle.

6.6 Sodium nitroprusside (0.05%): Dissolve 0.5 g of sodium nitroprusside in 1 liter of distilled water.

6.7 Stock solution: Dissolve 3.819 g of anhydrous ammonium chloride, $NH_4Cl$, dried at 105°C, in distilled water, and dilute to 1000 ml. 1.0 ml = 1.0 mg $NH_3$–N.

6.8 Standard solution A: Dilute 10.0 ml of stock solution (6.7) to 1000 ml with distilled water. 1.0 ml = 0.01 mg $NH_3$–N.

6.9 Standard solution B: Dilute 10.0 ml of standard solution A (6.8) to 100.0 ml with distilled water. 1.0 ml = 0.001 mg $NH_3$–N.

6.10 Using standard solutions A and B, prepare the following standards in 100 ml volumetric flasks (prepare fresh daily):

| $NH_3$–N, mg/l | ml Standard Solution/100 ml |
|---|---|
| | Solution B |
| 0.01 | 1.0 |
| 0.02 | 2.0 |
| 0.05 | 5.0 |
| 0.10 | 10.0 |
| | Solution A |
| 0.20 | 2.0 |
| 0.50 | 5.0 |
| 0.80 | 8.0 |
| 1.00 | 10.0 |
| 1.50 | 15.0 |
| 2.00 | 20.0 |

NOTE 3: When saline water samples are analyzed, Substitute Ocean Water (SOW) should be used for preparing the above standards used for the calibration curve; otherwise, distilled water is used. If SOW is used, subtract its blank background response from the standards before preparing the standard curve.

Substitute Ocean Water (SOW)

| | g/l | | g/l |
|---|---|---|---|
| NaCl | 24.53 | $NaHCO_3$ | 0.20 |
| $MgCl_2$ | 5.20 | KBr | 0.10 |
| $Na_2SO_4$ | 4.09 | $H_3BO_3$ | 0.03 |
| $CaCl_2$ | 1.16 | $SrCl_2$ | 0.03 |
| KCl | 0.70 | NaF | 0.003 |

7. Procedure

7.1 Any marked variation in acidity or alkalinity among samples should be eliminated, since the intensity of the color used to quantify the concentration is pH dependent. Likewise, the pH of the wash water and the standard ammonia solutions should approximate that of the samples. For example, if the samples have been preserved with 2 ml conc. $H_2SO_4$/liter, the wash water and standards should also contain 2 ml conc. $H_2SO_4$/liter.

7.2 For a working range of 0.01 to 2.00 mg $NH_3$–N/l (AAI), set up the manifold as shown in Figure 1. For a working range of .01 to 1.0 mg $NH_3$–N/l (AAII), set up the manifold as shown in Figure 2. Higher concentrations may be accommodated by sample dilution.

7.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through sample line.

7.4 For the AAI system, sample at a rate of 20/hr, 1:1. For the AAII use a 60/hr 6:1 cam with a common wash.

7.5 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

7.6 Switch sample line from distilled water to sampler and begin analysis.

8. Calculations

8.1 Prepare appropriate standard curve derived from processing ammonia standards through manifold. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory, (MDQARL), using surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg $NH_3$–N/l, the standard deviation was ±0.005.

9.2 In a single laboratory (MDQARL), using surface water samples at concentrations of 0.16 and 1.44 mg $NH_3$–N/l, recoveries were 107% and 99%, respectively.

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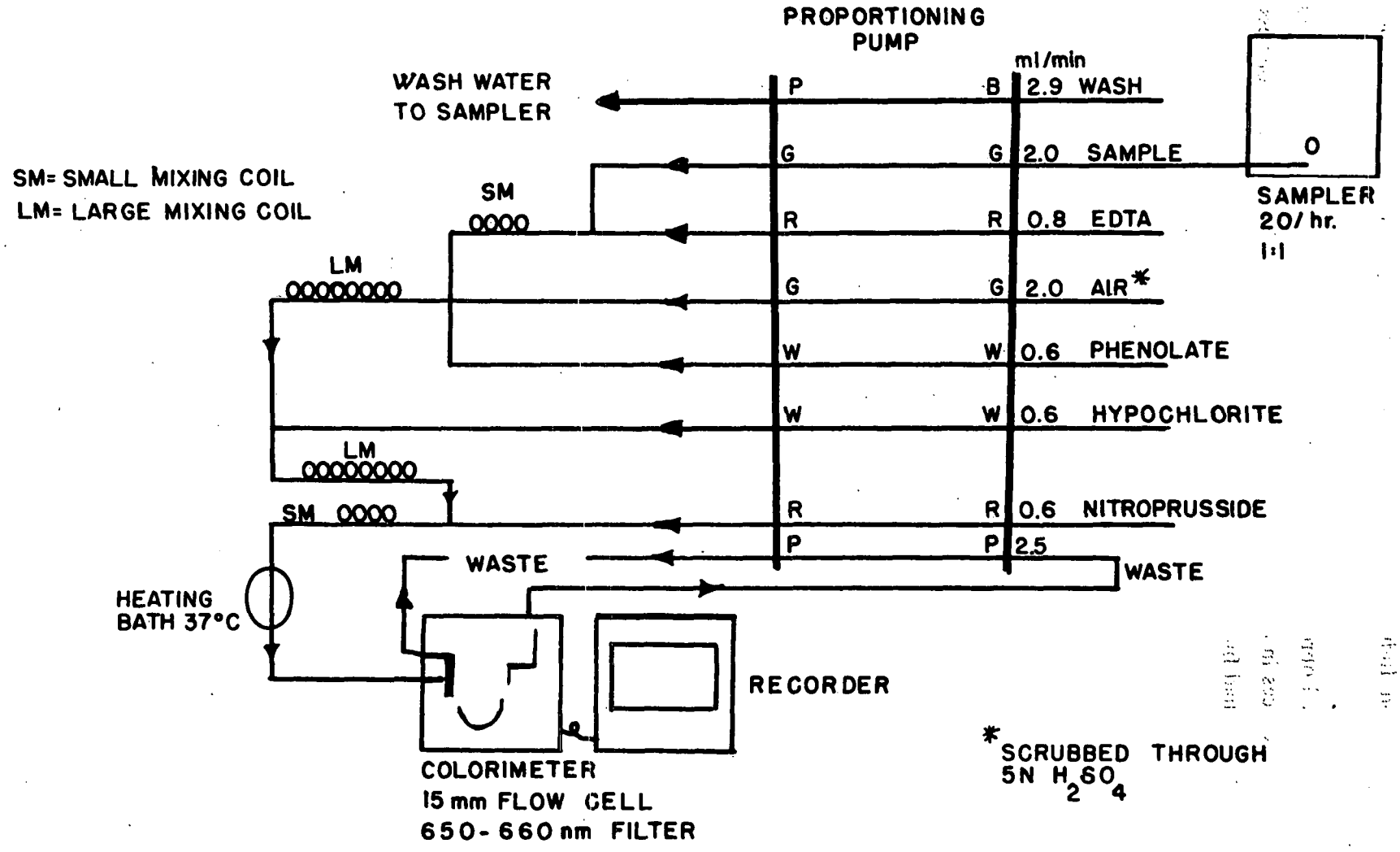


FIGURE 1 AMMONIA MANIFOLD AA I

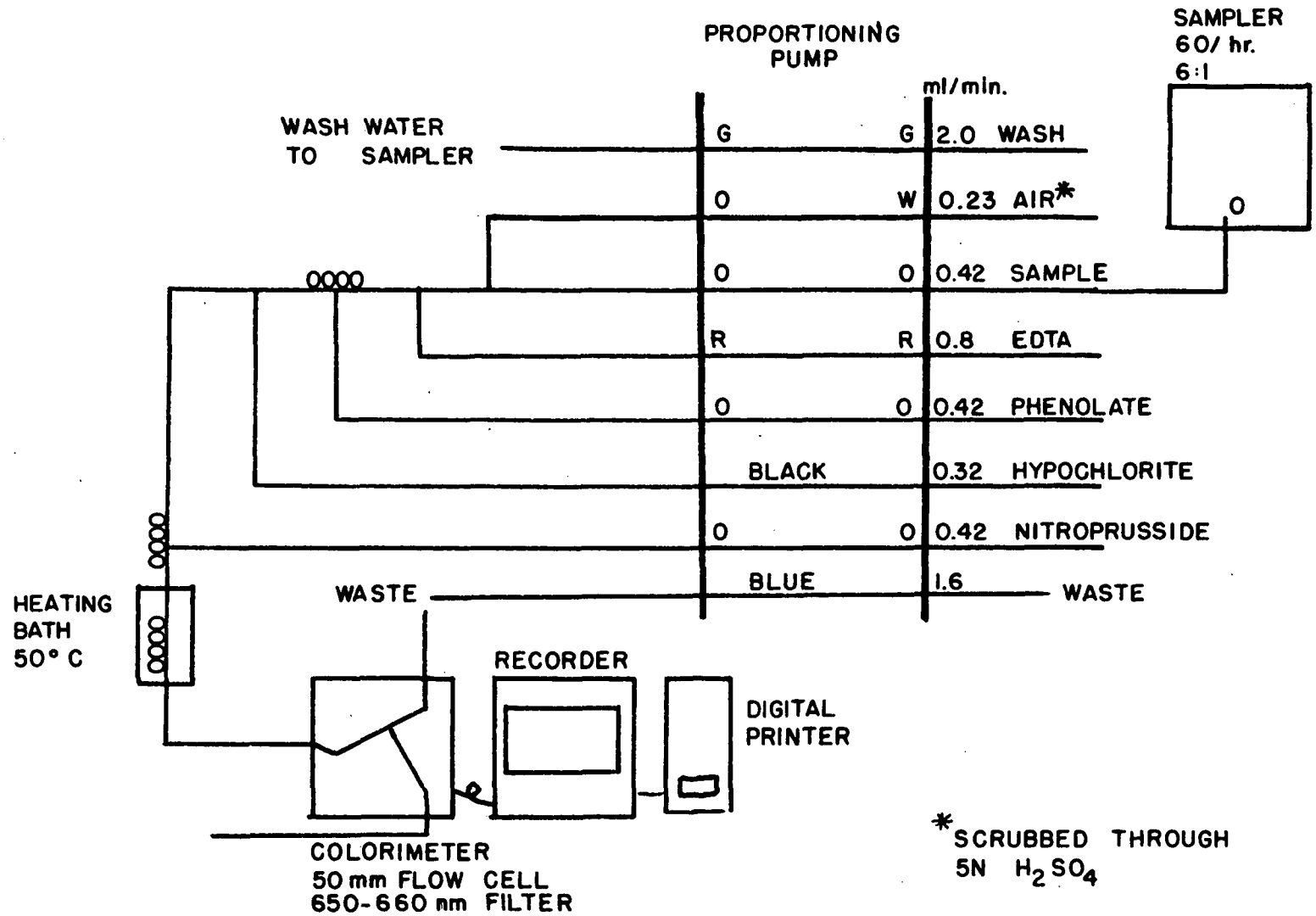


FIGURE 2. AMMONIA MANIFOLD AA II

## NITROGEN, KJELDAHL, Total

STORET NO. 00625

1. Scope and Application

   1.1 This method covers the determination of total Kjeldahl nitrogen in drinking, surface, and saline waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

   1.2 Three alternatives are listed for the determination of ammonia after distillation: the titrimetric method which is applicable to concentrations above 1 mg N/liter; the Nesslerization method which is applicable to concentrations below 1 mg N/liter; and the potentiometric method applicable to the range 0.05 to 1400 mg/l.

   1.3 This method is described for macro and micro glassware systems.

2. Definitions

   2.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below.

   2.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value (cf Nitrogen, Ammonia, this manual) from the total Kjeldahl nitrogen value. This may be determined directly by removal of ammonia before digestion.

3. Summary of Method

   3.1 The sample is heated in the presence of conc. sulfuric acid, $K_2SO_4$ and $HgSO_4$ and evaporated until $SO_3$ fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled, diluted, and is treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled and determined after distillation by Nesslerization, titrimetry, or potentiometrically.

4. Sample Handling and Preservation

   4.1 Samples may be preserved by addition of 2 ml of conc. $H_2SO_4$ or 40 mg $HgCl_2$ per liter and stored at 4°C. Even when preserved in this manner, conversion of organic nitrogen to ammonia may occur. Preserved samples should be analyzed as soon as possible.

5. Apparatus

5.1 Digestion apparatus: A Kjeldahl digestion apparatus with 800 or 100 ml flasks and suction takeoff to remove $SO_3$ fumes and water.

5.2 Distillation apparatus: The macro Kjeldahl flask is connected to a condenser and an adaptor so that the distillate can be collected. Micro Kjeldahl steam distillation apparatus is commercially available.

5.3 Spectrophotometer for use at 400 to 425 nm with a light path of 1 cm or longer.

6. Reagents

6.1 Distilled water should be free of ammonia. Such water is best prepared by the passage of distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.

NOTE 1: All solutions must be made with ammonia-free water.

6.2 Mercuric sulfate solution: Dissolve 8 g red, mercuric oxide (HgO) in 50 ml of 1:5 sulfuric acid (10.0 ml conc. $H_2SO_4$: 40 ml distilled water) and dilute to 100 ml with distilled water.

6.3 Sulfuric acid-mercuric sulfate-potassium sulfate solution: Dissolve 267 g $K_2SO_4$ in 1300 ml distilled water and 400 ml conc. $H_2SO_4$. Add 50 ml mercuric sulfate solution (6.2) and dilute to 2 liters with distilled water.

6.4 Sodium hydroxide-sodium thiosulfate solution: Dissolve 500 g NaOH and 25 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water and dilute to 1 liter.

6.5 Phenolphthalein indicator solution: Dissolve 5 g phenolphthalein in 500 ml 95% ethyl alcohol or isopropanol and add 500 ml distilled water. Add 0.02 N NaOH dropwise until a faint, pink color appears.

6.6 Mixed indicator: Mix 2 volumes of 0.2% methyl red in 95% ethanol with 1 volume of 0.2% methylene blue in ethanol. Prepare fresh every 30 days.

6.7 Boric acid solution: Dissolve 20 g boric acid, $H_3BO_3$, in water and dilute to 1 liter with distilled water.

6.8 Sulfuric acid, standard solution: (0.02 N) 1 ml = 0.28 mg $NH_3$–N. Prepare a stock solution of approximately 0.1 N acid by diluting 3 ml of conc. $H_2SO_4$ (sp. gr. 1.84) to 1 liter with $CO_2$-free distilled water. Dilute 200 ml of this solution to 1 liter with $CO_2$-free distilled water. Standardize the approximately 0.02 N acid so prepared against 0.0200 N $Na_2CO_3$ solution. This last solution is prepared by dissolving 1.060 g anhydrous $Na_2CO_3$, oven-dried at 140°C, and diluting to 1 liter with $CO_2$-free distilled water.

NOTE 2: An alternate and perhaps preferable method is to standardize the approximately 0.1 N $H_2SO_4$ solution against a 0.100 N $NaCO_3$ solution. By proper dilution the .02 N acid can then be prepared.

6.9 Ammonium chloride, stock solution: 1.0 ml = 1.0 mg $NH_3$–N. Dissolve 3.819 g $NH_4Cl$ in water and make up to 1 liter in a volumetric flask with distilled water.

6.10 Ammonium chloride, standard solution: 1.0 ml = 0.01 mg $NH_3$–N. Dilute 10.0 ml of the stock solution (6.9) with distilled water to 1 liter in a volumetric flask.

6.11 Nessler reagent: Dissolve 100 g of mercuric iodide in a small volume of distilled water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of NaOH in 500 ml of distilled water. Dilute the mixture to 1 liter. The solution is stable for at least one year if stored in a pyrex bottle out of direct sunlight.

NOTE 3: Reagents 6.8, 6.9, 6.10, and 6.11 are identical to reagents 6.8, 6.2, 6.3, and 6.6 described under Nitrogen, Ammonia (Distillation Procedure).

7. Procedure

7.1 The distillation apparatus should be pre-steamed before use by distilling a 1:1 mixture of distilled water and sodium hydroxide-sodium thiosulfate solution (6.4) until the distillate is ammonia-free. This operation should be repeated each time the apparatus is out of service long enough to accumulate ammonia (usually 4 hours or more).

7.2 Macro Kjeldahl system

7.2.1 Place a measured sample or the residue from the distillation in the ammonia determination (for Organic Kjeldahl only) into an 800 ml Kjeldahl flask. The sample size can be determined from the following table:

| Kjeldahl Nitrogen in Sample, mg/l | Sample Size ml |
|---|---|
| 0 – 5 | 500 |
| 5 – 10 | 250 |
| 10 – 20 | 100 |
| 20 – 50 | 50.0 |
| 50 – 500 | 25.0 |

Dilute the sample, if required, to 500 ml with distilled water, and add 100 ml sulfuric acid-mercuric sulfate-potassium sulfate solution (6.3)

(Note 4), and evaporate the mixture in the Kjeldahl apparatus until $SO_3$ fumes are given off and the solution turns colorless or *pale yellow*. Continue heating for 30 additional minutes. Cool the residue and add 300 ml distilled water.

NOTE 4: Digesting the sample with 1 Kel-Pac (Olin-Matheson) and 20 ml conc. $H_2SO_4$ is acceptable. Cut the end from the package and empty the contents into the digestion flask; discard the container.

7.2.2 Make the digestate alkaline by careful addition of 100 ml of sodium hydroxide-thiosulfate solution (6.4) without mixing.

NOTE 5: Slow addition of the heavy caustic solution down the tilted neck of the digestion flask will cause heavier solution to underlay the aqueous sulfuric acid solution without loss of free-ammonia. Do not mix until the digestion flask has been connected to the distillation apparatus.

7.2.3 Connect the Kjeldahl flask to the condenser with the tip of condenser (or an extension of the condenser tip) below the level of the boric acid solution (6.7) in the receiving flask.

7.2.4 Distill 300 ml at the rate of 6-10 ml/min., into 50 ml of 2% boric acid (6.7) contained in a 500 ml Erlenmeyer flask.

7.2.5 Dilute the distillate to 500 ml in the flask. These flasks should be marked at the 350 and the 500 ml volumes. With such marking, it is not necessary to transfer the distillate to volumetric flasks. For concentrations above 1 mg/l, the ammonia can be determined titrimetrically. For concentrations below this value, it is determined colorimetrically. The potentiometric method is applicable to the range 0.05 to 1400 mg/l.

7.3 Micro Kjeldahl system

7.3.1 Place 50.0 ml of sample or an aliquot diluted to 50 ml in a 100 ml Kjeldahl flask and add 10 ml sulfuric acid-mercuric sulfate-potassium sulfate solution (6.3). Evaporate the mixture in the Kjeldahl apparatus until $SO_3$ fumes are given off and the solution turns colorless or pale yellow. Then digest for an additional 30 minutes. Cool the residue and add 30 ml distilled water.

7.3.2 Make the digestate alkaline by careful addition of 10 ml of sodium hydroxide-thiosulfate solution (6.4) without mixing. Do not mix until the digestion flask has been connected to the distillation apparatus.

7.3.3 Connect the Kjeldahl flask to the condenser with the tip of condenser or an extension of the condenser tip below the level of the boric acid solution (6.7) in the receiving flask or 50 ml short-form Nessler tube.

7.3.4 Steam distill 30 ml at the rate of 6-10 ml/min., into 5 ml of 2% boric acid (6.7).

7.3.5 Dilute the distillate to 50 ml. For concentrations above 1 mg/l the ammonia can be determined titrimetrically. For concentrations below this value, it is determined colorimetrically. The potentiometric method is applicable to the range 0.05 to 1400 mg/l.

7.4 Determination of ammonia in distillate: Determine the ammonia content of the distillate titrimetrically, colorimetrically, or potentiometrically, as described below.

7.4.1 Titrimetric determination: Add 3 drops of the mixed indicator (6.6) to the distillate and titrate the ammonia with the 0.02 N $H_2SO_4$, (6.8), matching the endpoint against a blank containing the same volume of distilled water and $H_3BO_3$ (6.7) solution.

7.4.2 Colorimetric determination: Prepare a series of Nessler tube standards as follows:

| ml as Standard<br>1.0 ml = 0.01 mg $NH_3$–N | mg $NH_3$–N/50.0 ml |
|---|---|
| 0.0 | 0.0 |
| 0.5 | 0.005 |
| 1.0 | 0.010 |
| 2.0 | 0.020 |
| 4.0 | 0.040 |
| 5.0 | 0.050 |
| 8.0 | 0.080 |
| 10.0 | 0.10 |

To the standards and distilled samples, add 1 ml of Nessler reagent (6.11) and mix. After 20 minutes read the optical densities at 425 nm against the blank. From the values obtained for the standards plot optical density (absorbance) vs. mg $NH_3$–N for the standard curve. Read the ammonia-nitrogen in mg for the samples from the standard curve.

7.4.3 Potentiometric determination: Consult the method entitled Nitrogen, Ammonia: Selective Ion Electrode Method, in this manual.

7.4.4 It is not imperative that all standards be treated in the same manner as

the samples. It is recommended that at least 2 standards (a high and low) be digested, distilled, and compared to similar values on the curve to insure that the digestion-distillation technique is reliable. If treated standards do not agree with untreated standards the operator should find the cause of the apparent error before proceeding.

8. Calculation

8.1 If the titrimetric procedure is used calculate Total Kjeldahl Nitrogen, in mg/l, in the original sample as follows:

$$\text{Total Kjeldahl nitrogen, mg/l} = \frac{(A-B)N \times F \times 1000}{S}$$

where:

A = milliliters of standard 0.020 N $H_2SO_4$ solution used in titrating sample.

B = milliliters of standard 0.020 N $H_2SO_4$ solution used in titrating blank.

N = normality of sulfuric acid solution.

F = milliequivalent weight of nitrogen (14 mg).

S = milliliters of sample digested.

If the sulfuric acid is exactly 0.02 N the formula is shortened to:

$$\text{TKN, mg/l} = \frac{(A-B) \times 280}{S}$$

8.2 If the Nessler procedure is used, calculate the Total Kjeldahl Nitrogen, in mg/l, in the original sample as follows:

$$\text{TKN, mg/l} = \frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$$

where:

A = mg $NH_3$–N read from curve.

B = ml total distillate collected including the $H_3BO_3$.

C = ml distillate taken for Nesslerization.

8.3 Calculate Organic Kjeldahl Nitrogen in mg/l, as follows:

Organic Kjeldahl Nitrogen = TKN –($NH_3$–N.)

8.4 Potentiometric determination: Calculate Total Kjeldahl Nitrogen, in mg/l, in the original sample as follows:

$$\text{TKN, mg/l} = \frac{500}{D} \times A$$

where:

A = mg $NH_3$–N/l from electrode method standard curve.

D = ml of original sample taken.

9. Precision

9.1 Thirty-one analysts in twenty laboratories analyzed natural water samples containing exact increments of organic nitrogen, with the following results:

| Increment as Nitrogen, Kjeldahl mg N/liter | Precision as Standard Deviation mg N/liter | Accuracy as Bias, % | Accuracy as Bias, mg N/liter |
|---|---|---|---|
| 0.20 | 0.197 | +15.54 | +0.03 |
| 0.31 | 0.247 | + 5.45 | +0.02 |
| 4.10 | 1.056 | + 1.03 | +0.04 |
| 4.61 | 1.191 | – 1.67 | –0.08 |

(FWPCA Method Study 2, Nutrient Analyses)

# NITROGEN, KJELDAHL, TOTAL

(Automated Phenate Method)

STORET NO. 00625

1. Scope and Application

   1.1 This automated method may be used to determine Kjeldahl nitrogen in surface and saline waters. The applicable range is 0.05 to 2.0 mg N/l. Approximately 20 samples per hour can be analyzed.

2. Summary of Method

   2.1 The sample is automatically digested with a sulfuric acid solution containing potassium sulfate and mercuric sulfate as a catalyst to convert organic nitrogen to ammonium sulfate. The solution is then automatically neutralized with sodium hydroxide solution and treated with alkaline phenol reagent and sodium hypochlorite reagent. This treatment forms a blue color designated as indophenol. Sodium nitroprusside, which increases the intensity of the color, is added to obtain necessary sensitivity for measurement of low level nitrogen.

3. Definitions

   3.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and of organic nitrogen compounds which are converted to $(NH_4)_2SO_4$ under the conditions of digestion which are specified below.

   3.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value. Also, organic Kjeldahl nitrogen may be determined directly by removal of ammonia before digestion.

4. Sample Handling and Preservation

   4.1 Samples may be preserved by addition of 2 ml of conc. $H_2SO_4$ or 40 mg $HgCl_2$ per liter and refrigeration at 4°C.

5. Interferences

   5.1 Iron and chromium ions tend to catalyze while copper ions tend to inhibit the indophenol color reaction.

6. Apparatus

   6.1 Technicon AutoAnalyzer consisting of:

      6.1.1 Sampler II, equipped with continuous mixer.

      6.1.2 Two proportioning pumps.

6.1.3 Manifold I.

6.1.4 Manifold II.

6.1.5 Continuous digester.

6.1.6 Planetary pump.

6.1.7 Five-gallon Carboy fume-trap.

6.1.8 80°C Heating bath.

6.1.9 Colorimeter equipped with 50 mm tubular flow cell and 630 nm filters.

6.1.10 Recorder equipped with range expander.

6.1.11 Vacuum pump.

7. Reagents

7.1 Distilled water: Special precaution must be taken to insure that distilled water is free of ammonia. Such water is prepared by passage of distilled water through an ion exchange column comprised of a mixture of both strongly acidic cation and strongly basic anion exchange resins. Furthermore, since organic contamination may interfere with this analysis, use of the resin Dowex XE-75 or equivalent which also tends to remove organic impurities is advised. The regeneration of the ion exchange column should be carried out according to the instruction of the manufacturer.

NOTE 1: All solutions must be made using ammonia-free water.

7.2 Sulfuric acid: As it readily absorbs ammonia, special precaution must also be taken with respect to its use. Do not store bottles reserved for this determination in areas of potential ammonia contamination.

7.3 EDTA (2% solution): Dissolve 20 g disodium ethylenediamine tetraacetate in 1 liter of distilled water. Adjust pH to 10.5-11 with NaOH (7.4).

7.4 Sodium hydroxide (30% solution): Dissolve 300 g NaOH in 1 liter of distilled water.

NOTE 2: The 30% sodium hydroxide should be sufficient to neutralize the digestate. In rare cases it may be necessary to increase the concentration of sodium hydroxide in this solution to insure neutralization of the digested sample in the manifold at the water jacketed mixing coil.

7.5 Sodium nitroprusside, (0.05% solution): Dissolve 0.5 g $Na_2Fe(CN)_5NO \cdot 2H_2O$ in 1 liter distilled water.

7.6 Alkaline phenol reagent: Pour 550 ml liquid phenol (88-90%) slowly with mixing into 1 liter of 40% (400 g per liter) NaOH. Cool and dilute to 2 liters with distilled water.

7.7 Sodium hypochlorite (1% solution): Dilute commercial "Clorox"–200 ml to 1 liter with distilled water. Available chlorine level should be approximately 1%. Due to the instability of this product, storage over an extended period should be avoided.

7.8 Digestant mixture: Place 2 g red HgO in a 2 liter container. Slowly add, with stirring, 300 ml of acid water (100 ml $H_2SO_4$ + 200 ml $H_2O$) and stir until cool. Add 100 ml 10% (10 g per 100 ml) $K_2SO_4$. Dilute to 2 liters with conc. sulfuric acid (approximately 500 ml at a time, allowing time for cooling). Allow 4 hours for the precipitate to settle or filter through glass fiber filter.

7.9 Stock solutions: Dissolve 4.7619 g of pre-dried (1 hour at 105°C) ammonium sulfate in distilled water and dilute to 1.0 liter in a volumetric flask. 1.0 ml = 1.0 mg N.

7.10 Standard solution: Dilute 10.0 ml of stock solution (7.9) to 1000 ml. 1.0 ml = 0.01 mg N.

7.11 Using the standard solution (7 .10), prepare the following standards in 100 ml volumetric flasks:

| Conc., mg N/l | ml Standard Solution/100 ml |
|---|---|
| 0.00 | 0.0 |
| 0.05 | 0.5 |
| 0.10 | 1.0 |
| 0.20 | 2.0 |
| 0.40 | 4.0 |
| 0.60 | 6.0 |
| 0.80 | 8.0 |
| 1.00 | 10.0 |
| 1.50 | 15.0 |
| 2.00 | 20.0 |

8. Procedure

8.1 Set up manifolds as shown in Figures 1, 2, and 3.

8.1.1 In the operation of manifold No. 1, the control of four key factors is required to enable manifold No. 2 to receive the mandatory representative feed. First, the digestant flowing into the pulse chamber (PC-1) must be bubble free; otherwise, air will accumulate in A-7, thus altering

the ratio of sample to digestant in digestor. Second, in maintaining even flow from the digestor helix, the peristaltic pump must be adjusted to cope with differences in density of the digestate and the wash water. Third, the sample pick-up rate from the helix must be precisely adjusted to insure that the entire sample is aspirated into the mixing chamber. And finally, the contents of the "Mixing Chamber" must be kept homogeneous by the proper adjustment of the air bubbling rate.

8.1.2 In the operation of manifold No. 2, it is important in the neutralization of the digested sample to adjust the concentration of the NaOH so that the waste from the C-3 debubbler is slightly acid to Hydrion B paper.

8.1.3 The digestor temperature is 390°C for the first stage and 360°C for the second and third stages.

8.2 Allow both colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on colorimeter to obtain stable baseline.

8.3 Set sampling rate of Sampler II at 20 samples per hour, using a sample to wash ratio of 1 to 2 (1 minute sample, 2 minute wash).

8.4 Arrange various standards in sampler cups in order of increasing concentration. Complete loading of sampler tray with unknown samples.

8.5 Switch sample line from distilled water to sampler and begin analysis.

9. Calculation

9.1 Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentration of samples by comparing sample peak heights with standard curve.

9.2 Any sample that has a computed concentration that is less than 10% of the sample run immediately prior to it must be rerun.

10. Precision and Accuracy

10.1 Six laboratories analyzed four natural water samples containing exact increments of organic nitrogen compounds, with the following results:

| Increment as Kjeldahl-Nitrogen mg N/liter | Precision as Standard Deviation Kjeldahl–N mg N/liter | Accuracy as | |
|---|---|---|---|
| | | Bias, % | Bias, mg N/liter |
| 1.89 | 0.54 | −24.6 | −0.46 |
| 2.18 | 0.61 | −28.3 | −0.62 |
| 5.09 | 1.25 | −23.8 | −1.21 |
| 5.81 | 1.85 | −21.9 | −1.27 |

(FWQA Method Study 4, Automated Methods – In preparation).

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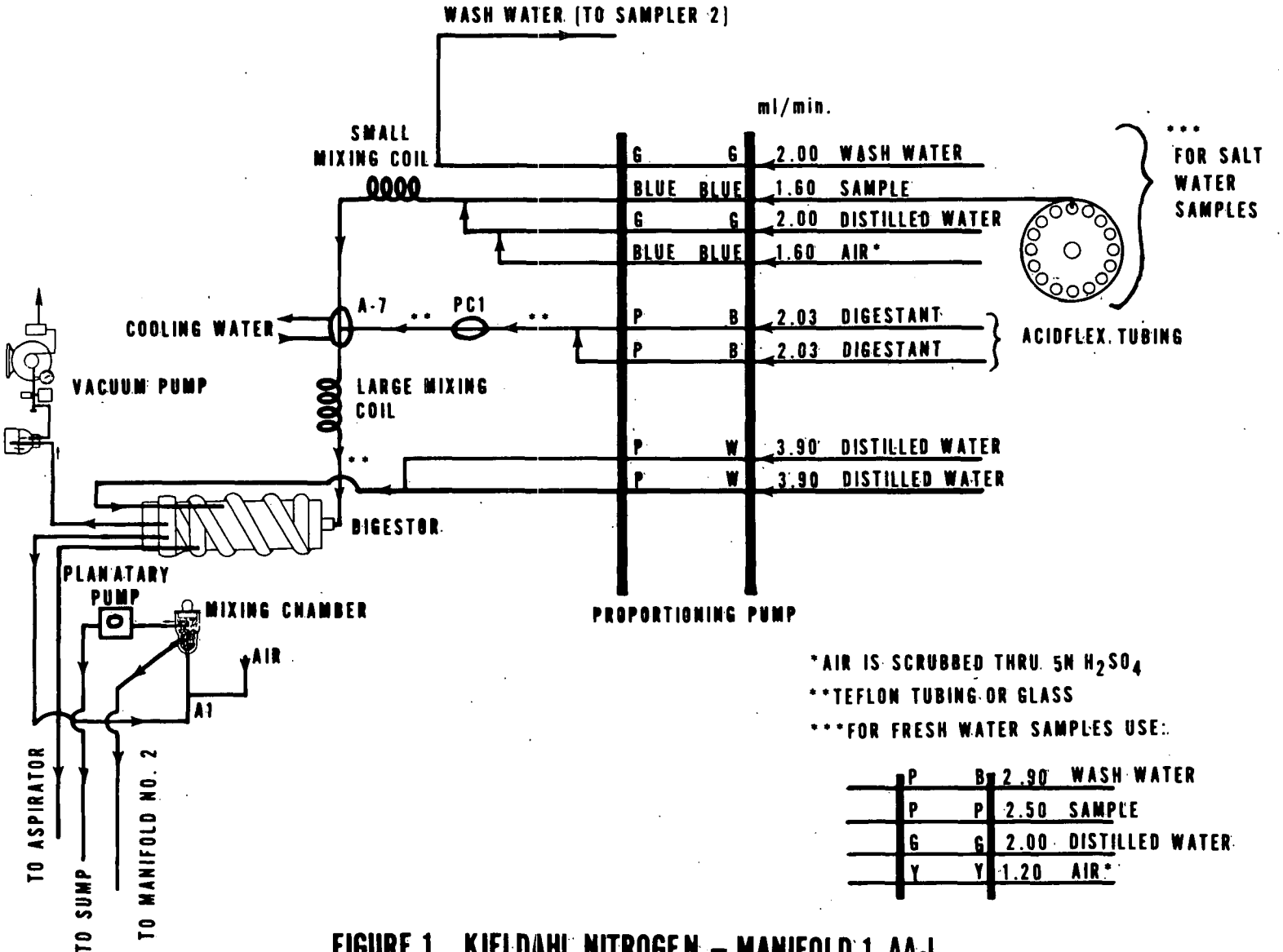


**FIGURE 1. KJELDAHL NITROGEN – MANIFOLD 1 AA-I**

## CONTINUOUS DIGESTER & MIXING CHAMBER ASSEMBLY

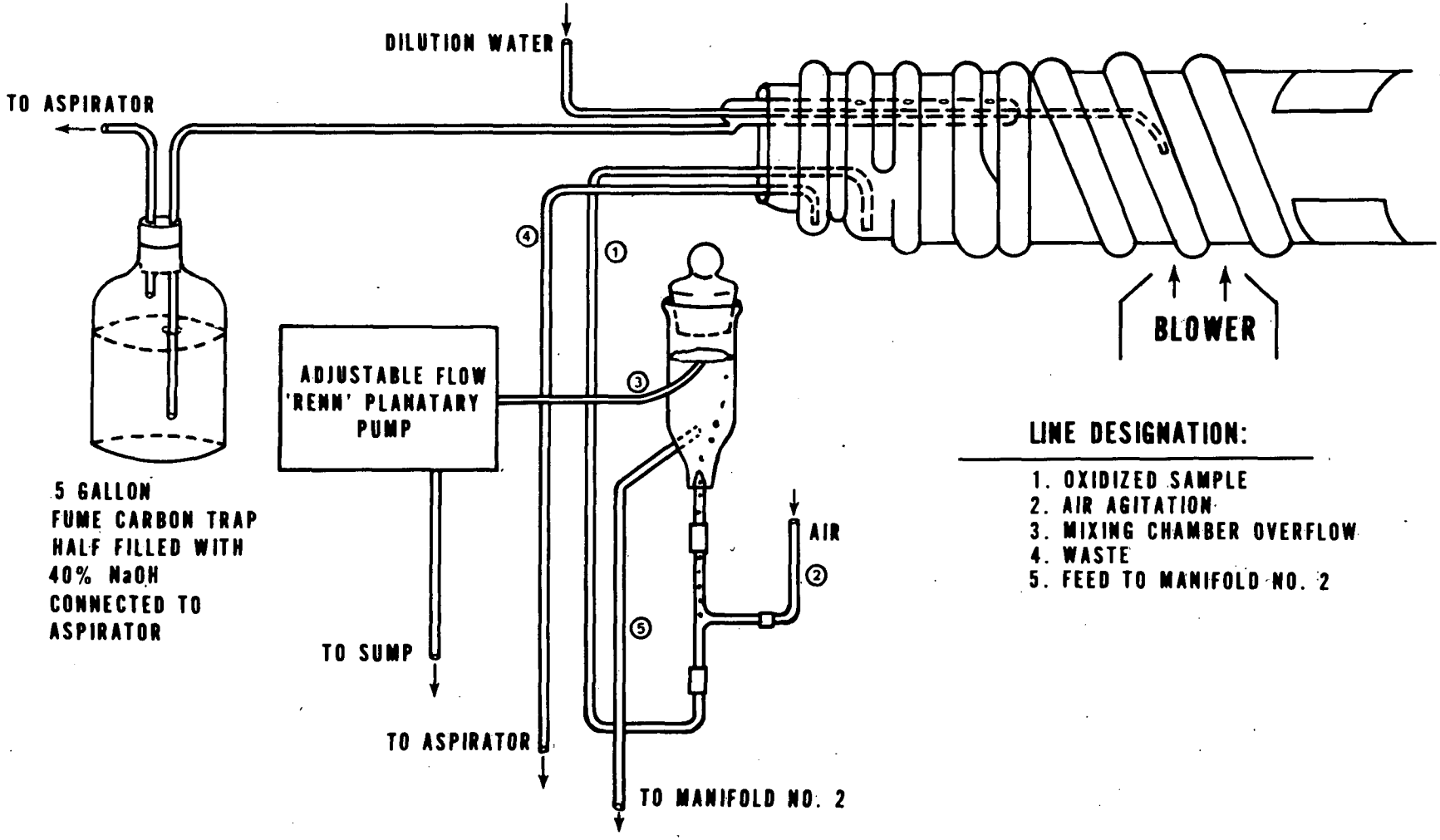


FIGURE 2. KJELDAHL NITROGEN AA-I

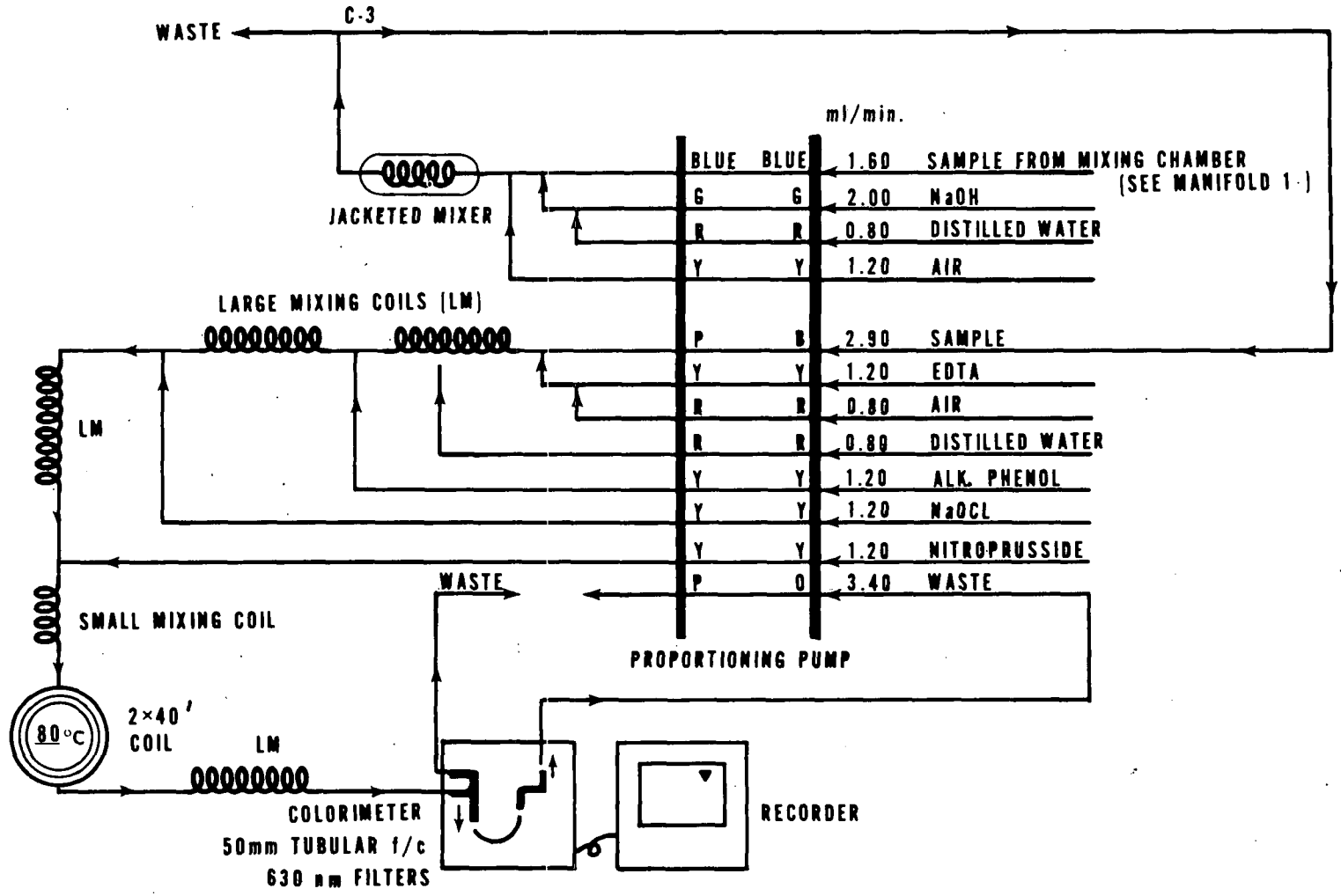


FIGURE 3. KJELDAHL NITROGEN MANIFOLD 2. AA-I

# NITROGEN, KJELDAHL, TOTAL
## (Automated Selenium Method)

STORET NO. 00625

1. Scope and Application
   1.1 This automated method may be used to determine total Kjeldahl nitrogen in drinking and surface waters, domestic and industrial wastes. This method cannot be used on saline waters. The applicable range is 0.1 to 10.0 mg/l. Approximately 15 samples per hour can be analyzed.
2. Summary of Method
   2.1 The sample is automatically digested with a sulfuric acid solution containing selenium dioxide and perchloric acid to convert organic nitrogen to ammonium sulfate. The solution is then treated with sodium hydroxide, alkaline phenol and sodium hypochlorite to form a blue color designated as indophenol. Sodium nitroprusside, which increases the intensity of the color, is added to obtain necessary sensitivity and eliminate interference of iron and manganese.
3. Definitions
   3.1 Total Kjeldahl nitrogen is defined as the sum of free ammonia and of organic compounds which are converted to $(NH_4)_2SO_4$ under the conditions of digestion which are specified below.
   3.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free ammonia from the total Kjeldahl nitrogen value.
4. Sample Handling and Preservation
   4.1 Samples may be preserved by addition of 2 ml of conc. $H_2SO_4$ or 40 mg $HgCl_2$ per liter and refrigeration at 4°C.
5. Apparatus
   5.1 Technicon AutoAnalyzer consisting of:
      5.1.1 Sampler.
      5.1.2 Two manifolds (See Figures 1 and 2 or 3).
      5.1.3 Two proportioning pumps.
      5.1.4 Continuous digestor (speed 6.7 rpm).
      5.1.5 Vacuum pump.
      5.1.6 Two five gallon glass carboys.
      5.1.7 Colorimeter equipped with a 15 or 50 mm flow cell and a 630 or 650 nm filter.
      5.1.8 Recorder.

6. Reagents for AAI

6.1 Ammonia-free water: Ammonia-free water is prepared by passage of distilled water through an ion exchange column comprised of a mixture of both strongly acidic cation and strongly basic anion exchange resins. Dowex XE-75 or equivalent is advised.

NOTE 1: All solutions must be made using ammonia-free water.

6.2 Sulfuric acid: As it readily absorbs ammonia, special precaution must also be taken with respect to its use. Do not store bottles reserved for this determination in areas of potential ammonia contamination.

6.3 Digestion mixture: Dissolve 3 g selenium dioxide in 50 ml of distilled water. Add 20 ml of perchloric acid (67-70%). Dilute slowly to 1 liter with conc. sulfuric acid (6.2).

6.4 Sodium hydroxide-tartrate: Dissolve 350 g NaOH and 50 g of $KNaC_4H_4O_6 \cdot 4H_2O$ in 700 ml of distilled water. Allow to cool and dilute to 1 liter.

6.5 Alkaline phenol: Dissolve 120 g of phenol in 500 ml of distilled water. Add 31 g NaOH. Dilute to 1 liter with distilled water.

6.6 Sodium hypochlorite: Dilute 200 ml of fresh "Clorox" to 1 liter with distilled water. *Caution:* Do not store "Clorox" for extended periods; it is not stable.

6.7 Sodium nitroprusside: Dissolve 0.5 g of sodium nitroprusside in 1 liter of distilled water.

6.8 Stock solution: Dissolve 3.819 g of predried (1 hour at 105°C) ammonium chloride in distilled water and dilute to 1 liter in a volumetric flask. 1.0 ml = 1.0 mg N.

6.9 Standard solution A: Dilute 100.0 ml of stock solution (6.8) to 1 liter in a volumetric flask. 1.0 ml = 0.10 mg N.

6.10 Standard solution B: Dilute 10.0 ml of standard solution A (6.9) to 100.0 ml. 1.0 ml = 0.01 mg N. (See dilution table on p. 192.)

7. Reagents for AAII

*All reagents listed for AAI, Section 6, except the digestion mixture and the sodium hydroxide-tartrate solution.*

7.1 Digestion mixture: Dissolve 3 g selenium dioxide in 100 ml of distilled water. Add 3 ml of perchloric acid (67-70%). Dilute slowly to 1 liter with conc. sulfuric acid (6.2).

7.2 Sodium hydroxide-tartrate: Dissolve 270 g NaOH and 50 g $KNaC_4H_4O_6 \cdot 4H_2O$ in 700 ml of distilled water. Allow to cool and dilute to 1 liter.

| ml of Standard Solution/100 ml | Conc. mg N/l |
|---|---|
| Solution B | |
| 1.0 | 0.1 |
| 2.0 | 0.2 |
| 5.0 | 0.5 |
| 10.0 | 1.0 |
| Solution A | |
| 2.0 | 2.0 |
| 5.0 | 5.0 |
| 8.0 | 8.0 |
| 10.0 | 10.0 |

8. Procedure

8.1 Set up manifolds as shown in Figures 1 and 2 or 3.

8.1.1 In the operation of manifold No. 1, the acidflex tubing should be in good condition at all times. The life of the tubing can be extended by air drying after each run.

8.1.2 The digestor temperature is 390-400°C (4.2 ampere) for the first stage and 370-380°C (7.0 ampere) for the second and third stages.

8.2 Allow digestor, colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line.

8.3 Sampling rate:

8.3.1 Large sampler – use 1 minute, 15 second sample and 2 minute, 30 second wash.

8.3.2 Sampler II or IV – set sampling rate at 20 samples per hour using a sample to wash ratio of 1:2 (1 minute sample, 2 minute wash).

8.4 Arrange series of standards in sampler cups or test tubes in order of decreasing concentration.

8.5 Switch sample line from distilled water sample and begin analysis.

NOTE 2: During sampling, sample must be agitated.

9. Calculations

9.1 Prepare standard curve by plotting peak heights of processed standard against concentration values. Compute concentration of samples by comparing sample peak heights with standard curve.

10. Precision and Accuracy

10.1 In a single laboratory (MDQARL), using surface water samples of concentrations of 0.32, 1.05, 1.26, and 4.30 mg N/l, the precision was ±0.09, ±0.05, ±0.09, and ±0.14 mg N/l, respectively.

10.2 In a single laboratory (MDQARL), using a variety of domestic and industrial wastes ranging from 23 to 68 mg N/l, recoveries were 91 to 102%.

## Bibliography

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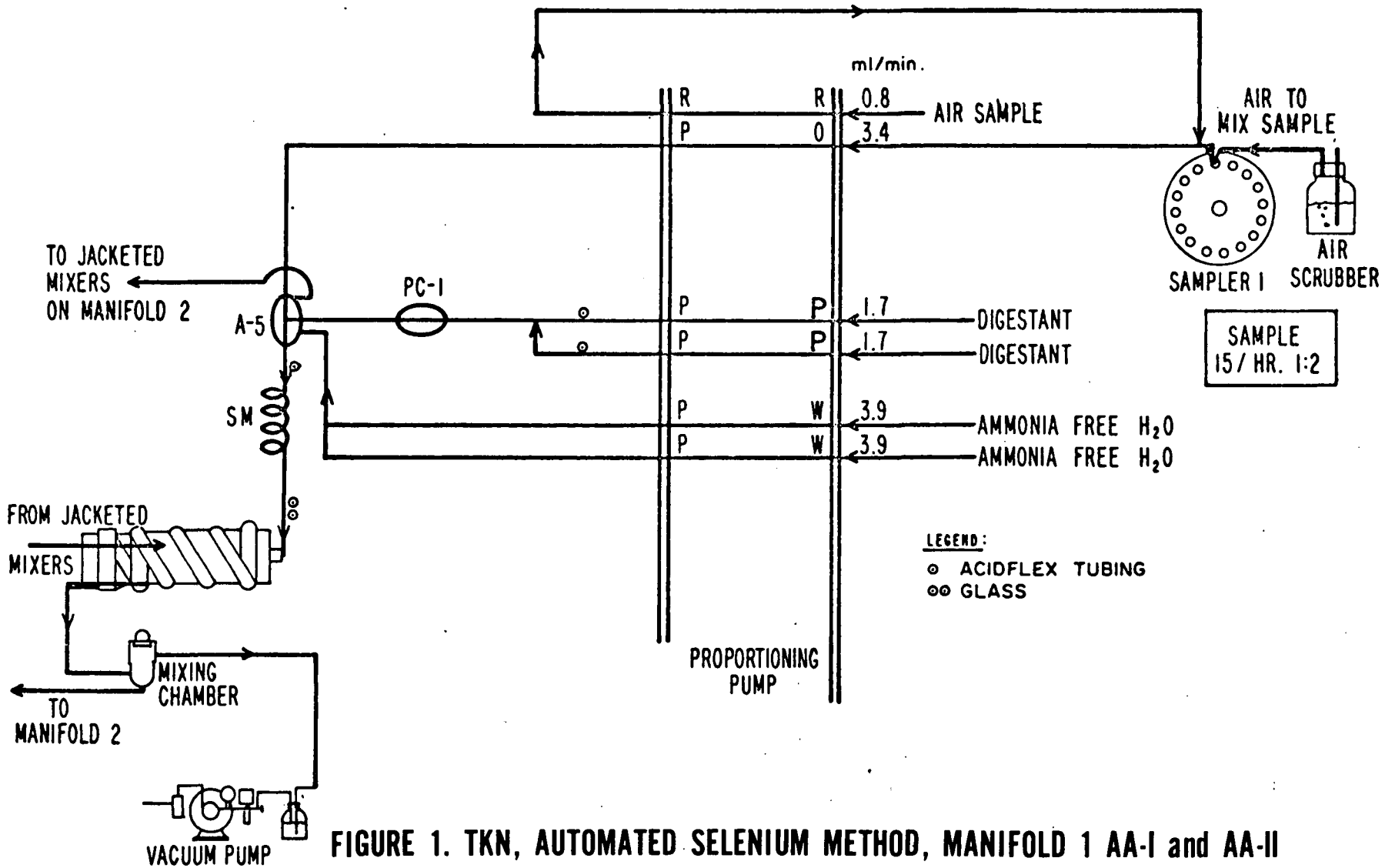


**FIGURE 1. TKN, AUTOMATED SELENIUM METHOD, MANIFOLD 1 AA-I and AA-II**

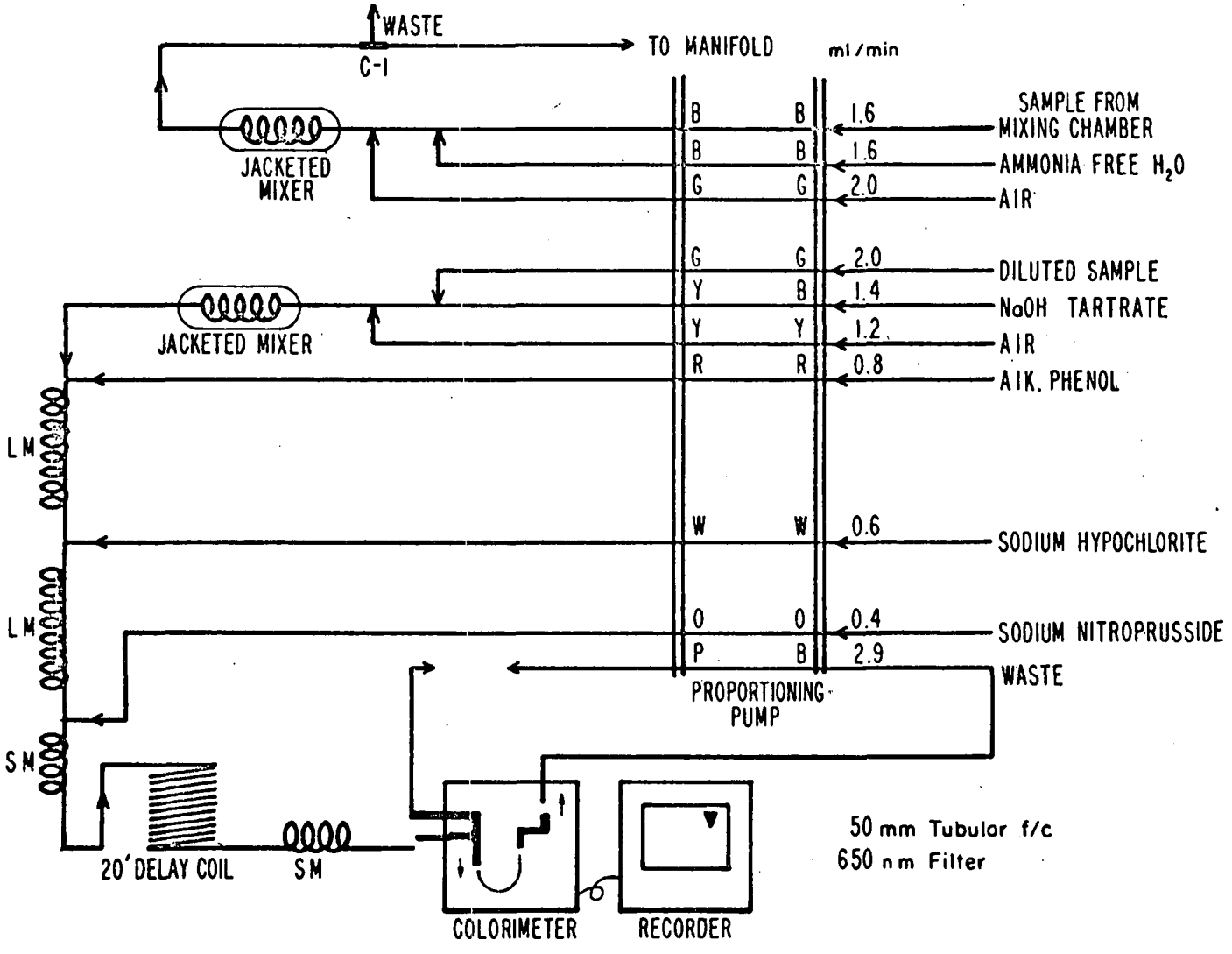


**FIGURE 2. TKN, AUTOMATED SELENIUM METHOD, MANIFOLD 2 AA-I**

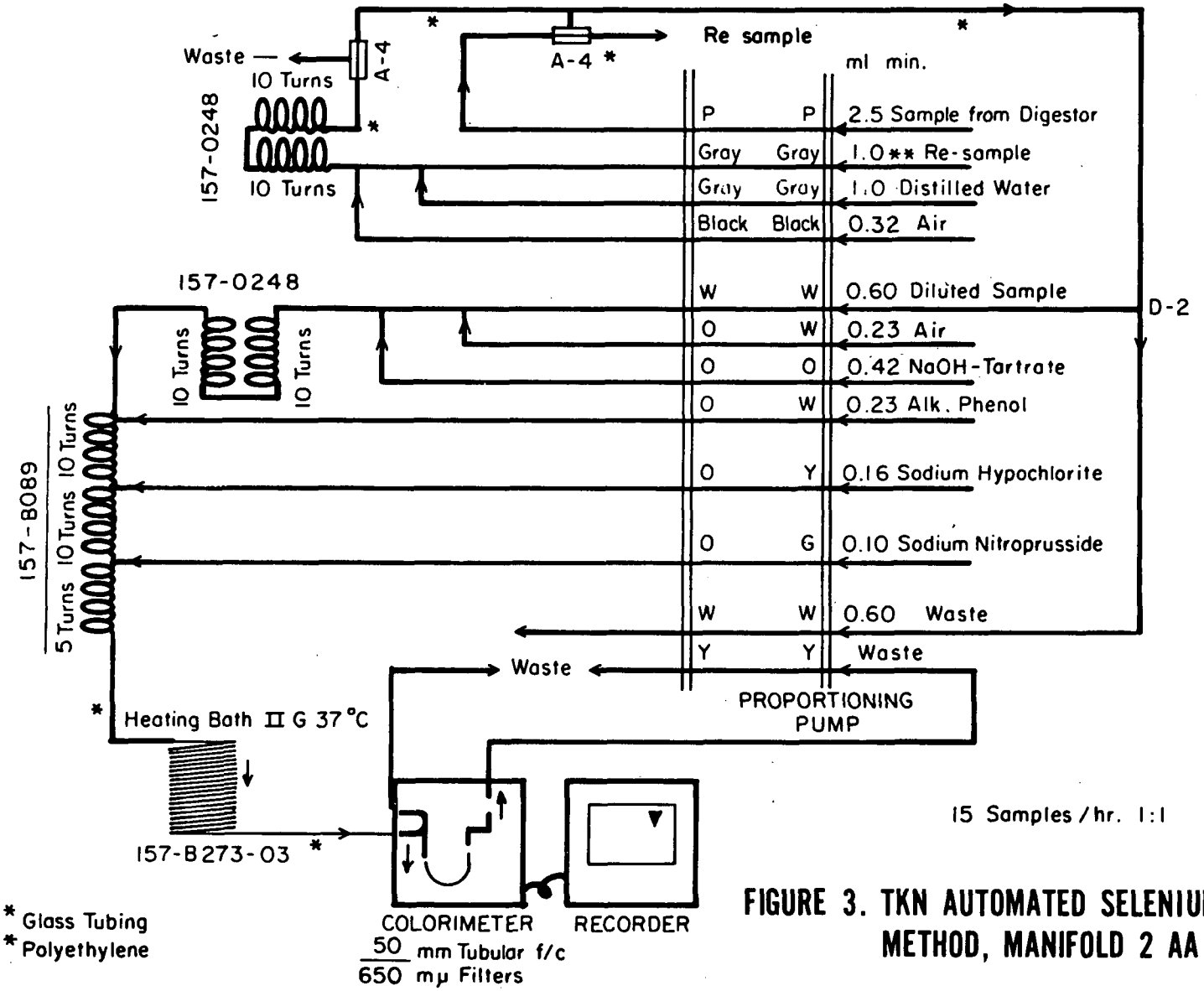


**FIGURE 3. TKN AUTOMATED SELENIUM METHOD, MANIFOLD 2 AA II**

# NITROGEN, NITRATE

## (Brucine)

STORET NO. 00620

1. Scope and Application
   - 1.1 This method is applicable to the analysis of drinking, surface, and saline waters, domestic and industrial wastes. Modification can be made to remove or correct for turbidity, color, salinity, or dissolved organic compounds in the sample.
   - 1.2 The applicable range of concentrations is 0.1 to 2 mg $NO_3$–N/liter.
2. Summary of Method
   - 2.1 This method is based upon the reaction of the nitrate ion with brucine sulfate in a 13 N $H_2SO_4$ solution at a temperature of 100°C. The color of the resulting complex is measured at 410 nm. Temperature control of the color reaction is extremely critical.
3. Sample Handling and Preservation
   - 3.1 Samples may be preserved by addition of 2 ml conc. $H_2SO_4$/liter or by addition of 40 mg $HgCl_2$ per liter and storage at 4°C.
4. Interferences
   - 4.1 Dissolved organic matter will cause an off color in 13 N $H_2SO_4$ and must be compensated for by additions of all reagents except the brucine-sulfanilic acid reagent. This also applies to natural color present not due to dissolved organics.
   - 4.2 The effect of salinity is eliminated by addition of sodium chloride to the blanks, standards and samples.
   - 4.3 All strong oxidizing or reducing agents interfere. The presence of oxidizing agents may be determined by the addition of orthotolidine reagent.
   - 4.4 Residual chlorine interference is eliminated by the addition of sodium arsenite.
   - 4.5 Ferrous and ferric iron and quadrivalent manganese give slight positive interferences, but in concentrations less than 1 mg/l these are negligible.
   - 4.6 Uneven heating of the samples and standards during the reaction time will result in erratic values. The necessity for *absolute control* of temperature during the critical color development period cannot be too strongly emphasized.
5. Apparatus
   - 5.1 Spectrophotometer or filter photometer suitable for measuring optical densities at 410 nm.

5.2 Sufficient number of 40-50 ml glass sample tubes for reagent blanks, standards, and samples.

5.3 Neoprene coated wire racks to hold sample tubes.

5.4 Water bath suitable for use at 100°C. This bath should contain a stirring mechanism so that all tubes are at the same temperature and should be of sufficient capacity to accept the required number of tubes without significant drop in temperature when the tubes are immersed.

5.5 Water bath suitable for use at 10-15°C.

6. Reagents

6.1 Distilled water free of nitrite and nitrate is to be used in preparation of all reagents and standards.

6.2 Sodium chloride solution (300 g/l): Dissolve 300 g NaCl in distilled water and dilute to 1 liter.

6.3 Sulfuric acid solution: Carefully add 500 ml conc. $H_2SO_4$ to 125 ml distilled water. Cool and keep tightly stoppered to prevent absorption of atmospheric moisture.

6.4 Brucine-sulfanilic acid reagent: Dissolve 1 g brucine sulfate [$(C_{23}H_{26}N_2O_4)_2 \cdot H_2SO_4 \cdot 7H_2O$] and 0.1 g sulfanilic acid ($NH_2C_6H_4SO_3H \cdot H_2O$) in 70 ml hot distilled water. Add 3 ml conc. HCl, cool, mix and dilute to 100 ml with distilled water. Store in a dark bottle at 5°C. This solution is stable for several months; the pink color that develops slowly does not effect its usefulness. *Mark bottle with warning; CAUTION: Brucine Sulfate is toxic; take care to avoid ingestion.*

6.5 Potassium nitrate stock solution: 1.0 ml = 0.1 mg $NO_3$–N. Dissolve 0.7218 g anhydrous potassium nitrate ($KNO_3$) in distilled water and dilute to 1 liter in a volumetric flask. Preserve with 2 ml chloroform per liter. This solution is stable for at least 6 months.

6.6 Potassium nitrate standard solution: 1.0 ml = 0.001 mg $NO_3$–N. Dilute 10.0 ml of the stock solution (6.5) to 1 liter in a volumetric flask. This standard solution should be prepared fresh weekly.

6.7 Acetic acid (1 + 3): Dilute 1 volume glacial acetic acid ($CH_3COOH$) with 3 volumes of distilled water.

6.8 Sodium hydroxide: Dissolve 40 g of NaOH in distilled water. Cool and dilute to 1 liter.

7. Procedure

7.1 Adjust the pH of the samples to approximately 7 with acetic acid (6.7) or sodium hydroxide (6.8). If necessary, filter to remove turbidity.

7.2 Set up the required number of sample tubes in the rack to handle reagent blank, standards and samples. Space tubes evenly throughout the rack to allow for even flow of bath water between the tubes. This should assist in achieving uniform heating of all tubes.

7.3 If it is necessary to correct for color or dissolved organic matter which will cause color on heating, a set of duplicate samples must be run to which all reagents except the brucine-sulfanilic acid have been added.

7.4 Pipette 10.0 ml of standards and samples or an aliquot of the samples diluted to 10.0 ml into the sample tubes.

7.5 If the samples are saline, add 2 ml of the 30% sodium chloride solution (6.2) to the reagent blank, standards and samples. For fresh water samples, sodium chloride solution may be omitted. Mix contents of tubes by swirling and place rack in cold water bath (0-10°C).

7.6 Pipette 10.0 ml of sulfuric acid solution (6.3) into each tube and mix by swirling. Allow tubes to come to thermal equilibrium in the cold bath. Be sure that temperatures have equilibrated in all tubes before continuing.

7.7 Add 0.5 ml brucine-sulfanilic acid reagent (6.4) to each tube (except the interference control tubes, 7.3) and carefully mix by swirling, then place the rack of tubes in the 100°C water bath for exactly 25 minutes.

*Caution:* Immersion of the tube rack into the bath should not decrease the temperature of the bath more than 1 to 2°C. In order to keep this temperature decrease to an absolute minimum, flow of bath water between the tubes should not be restricted by crowding too many tubes into the rack. If color development in the standards reveals discrepancies in the procedure, the operator should repeat the procedure after reviewing the temperature control steps.

7.8 Remove rack of tubes from the hot water bath and immerse in the cold water bath and allow to reach thermal equilibrium (20-25°C).

7.9 Read absorbance against the reagent blank at 410 nm using a 1 cm or longer cell.

8. Calculation

8.1 Obtain a standard curve by plotting the absorbance of standards run by the above procedure against mg $NO_3$–N/l. (The color reaction does not always follow Beer's law).

8.2 Subtract the absorbance of the sample without the brucine-sulfanilic reagent from the absorbance of the sample containing brucine-sulfanilic acid and determine mg

$NO_3$–N/l. Multiply by an appropriate dilution factor if less than 10 ml of sample is taken.

9. Precision and Accuracy

9.1 Twenty-seven analysts in fifteen laboratories analyzed natural water samples containing exact increments of inorganic nitrate, with the following results:

| Increment as Nitrogen, Nitrate mg N/liter | Precision as Standard Deviation mg N/liter | Accuracy as Bias, % | Accuracy as Bias, mg N/liter |
|---|---|---|---|
| 0.16 | 0.092 | −6.79 | −0.01 |
| 0.19 | 0.083 | +8.30 | +0.02 |
| 1.08 | 0.245 | +4.12 | +0.04 |
| 1.24 | 0.214 | +2.82 | +0.04 |

(FWPCA Method Study 2, Nutrient Analyses).

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# NITROGEN, NITRATE-NITRITE

(Cadmium Reduction Method)

STORET NO. 00630

1. Scope and Application

   1.1 This method pertains to the determination of nitrite singly, or nitrite and nitrate combined in drinking, surface, and saline waters, domestic and industrial wastes. The applicable range of this method is 0.01 to 1.0 mg/l nitrate-nitrite nitrogen. The range may be extended with sample dilution.

2. Summary of Method

   2.1 A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N–(1-naphthyl)–ethylenediamine dihydrochloride to form a highly colored azo dye which is measured spectrophotometrically. Separate, rather than combined nitrate-nitrite values, are readily obtained by carrying out the procedure first with, and then without, the initial Cu-Cd reduction step.

3. Sample Handling and Preservation

   3.1 Analysis should be made as soon as possible. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 ml $H_2SO_4$ per liter) and refrigeration.

   *Caution:* Samples for reduction column must not be preserved with mercuric chloride.

4. Interferences

   4.1 Build up of suspended matter in the reduction column will restrict sample flow. Since nitrate-nitrogen is found in a soluble state, the sample may be pre-filtered through a glass fiber filter or a 0.45$\mu$ membrane filter. Highly turbid samples may be pretreated with zinc sulfate before filtration to remove the bulk of particulate matter present in the sample.

   4.2 Low results might be obtained for samples that contain high concentrations of iron, copper or other metals. EDTA is added to the samples to eliminate this interference.

   4.3 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

4.4 This procedure determines both nitrate and nitrite. If only nitrate is desired, a separate determination must be made for nitrite and subsequent corrections made. The nitrite may be determined by the procedure below without the reduction step.

5. Apparatus

5.1 Reduction column: The column in Figure I was constructed from a 100 ml pipet by removing the top portion. This column may also be constructed from two pieces of tubing joined end to end. A 10 cm length of 3 cm I.D. tubing is joined to a 25 cm length of 3.5 mm I.D. tubing.

5.2 Spectrophotometer for use at 540 nm, providing a light path of 1 cm or longer.

6. Reagents

6.1 Granulated cadmium: 40-60 mesh (E M Laboratories, Inc., 500 Exec. Blvd., Elmsford, NY 10523, Cat. 2001 Cadmium, Coarse Powder).

6.2 Copper-Cadmium: The cadmium granules (new or used) are cleaned with dilute HCl and copperized with 2% solution of copper sulfate in the following manner:

6.2.1 Wash the cadmium with dilute HCl (6.10) and rinse with distilled water.

6.2.2 Swirl 25 g cadmium in 100 ml portions of a 2% solution of copper sulfate (6.11) for 5 minutes or until blue color partially fades, decant and repeat with fresh copper sulfate until a brown colloidal precipitate forms.

6.2.3 Wash the copper-cadmium with distilled water (at least 10 times) to remove all the precipitated copper.

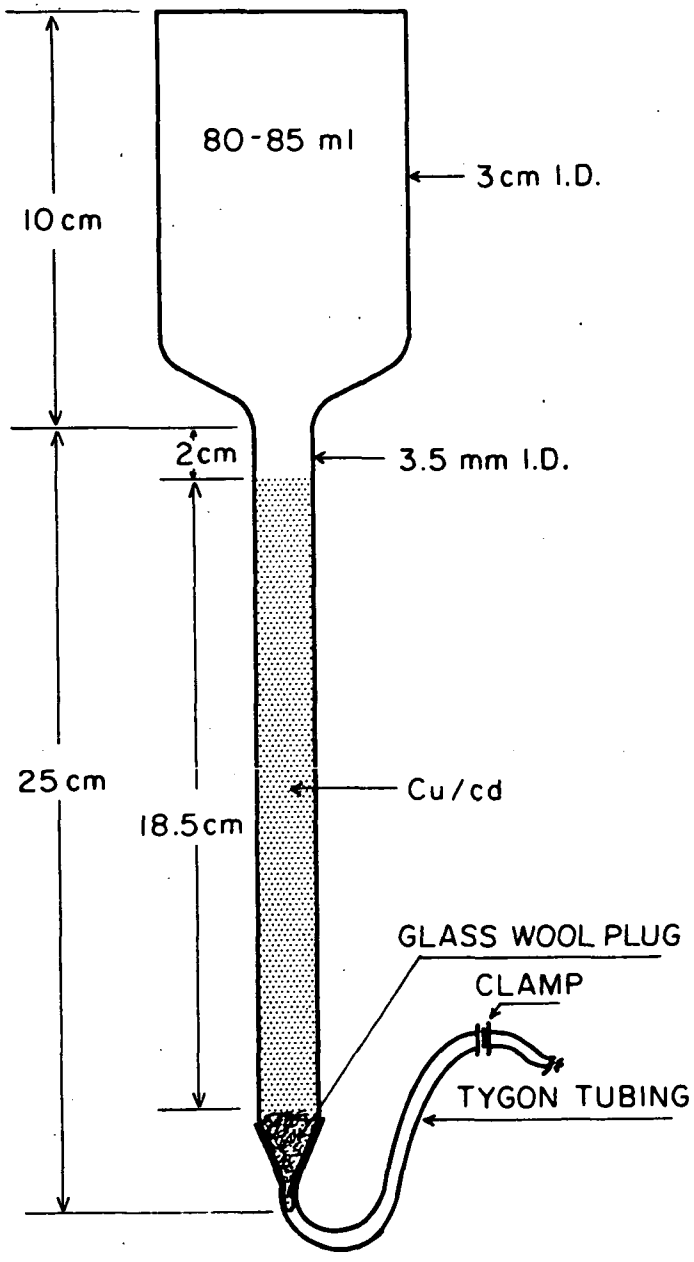


**FIGURE 1. REDUCTION COLUMN**

6.3 Preparation of reaction column: Insert a glass wool plug into the bottom of the reduction column and fill with distilled water. Add sufficient copper-cadmium granules to produce a column 18.5 cm in length. Maintain a level of distilled water above the copper-cadmium granules to eliminate entrapment of air. Wash the column with 200 ml of dilute ammonium chloride solution (6.5). The column is then activated by passing through the column 100 ml of a solution composed of 25 ml of a 1.0 mg/l $NO_3$–N standard and 75 ml of ammonium chloride – EDTA solution (6.4). Use a flow rate between 7 and 10 ml per minute.

6.4 Ammonium chloride – EDTA solution: Dissolve 13 g ammonium chloride and 1.7 g disodium ethylenediamine tetracetate in 900 ml of distilled water. Adjust the pH to 8.5 with conc. ammonium hydroxide (6.9) and dilute to 1 liter.

6.5 Dilute ammonium chloride-EDTA solution: Dilute 300 ml of ammonium chloride-EDTA solution (6.4) to 500 ml with distilled water.

6.6 Color reagent: Dissolve 10 g sulfanilamide and 1 g N(1-naphthyl)–ethylene-diamine dihydrochloride in a mixture of 100 ml conc. phosphoric acid and 800 ml of distilled water and dilute to 1 liter with distilled water.

6.7 Zinc sulfate solution: Dissolve 100 g $ZnSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 liter.

6.8 Sodium hydroxide solution, 6N: Dissolve 240 g NaOH in 500 ml distilled water, cool and dilute to 1 liter.

6.9 Ammonium hydroxide, conc.

6.10 Dilute hydrochloric acid, 6N: Dilute 50 ml of conc. HCl to 100 ml with distilled water.

6.11 Copper sulfate solution, 2%: Dissolve 20 g of $CuSO_4 \cdot 5H_2O$ in 500 ml of distilled water and dilute to 1 liter.

6.12 Stock nitrate solution: Dissolve 7.218 g $KNO_3$ in distilled water and dilute to 1000 ml. Preserve with 2 ml of chloroform per liter. This solution is stable for at least 6 months. 1.0 ml = 1.00 mg $NO_3$–N.

6.13 Standard nitrate solution: Dilute 10.0 ml of nitrate stock solution (6.12) to 1000 ml with distilled water. 1.0 ml = 0.01 mg $NO_3$–N.

6.14 Stock nitrite solution: Dissolve 6.072 g $KNO_2$ in 500 ml of distilled water and dilute to 1000 ml. Preserve with 2 ml of chloroform and keep under refrigeration. Stable for approximately 3 months. 1.0 ml = 1.00 mg $NO_2$–N.

6.15 Standard nitrite solution: Dilute 10.0 ml of stock nitrite solution (6.14) to 1000 ml with distilled water. 1.0 ml = 0.01 mg $NO_2$–N.

6.16 Using standard nitrate solution (6.13) prepare the following standards in 100 ml volumetric flasks:

| Conc., mg-$NO_3$–N/l | ml of Standard Solution/100.0 ml |
|---|---|
| 0.00 | 0.0 |
| 0.05 | 0.5 |
| 0.10 | 1.0 |
| 0.20 | 2.0 |
| 0.50 | 5.0 |
| 1.00 | 10.0 |

7. Procedure

7.1 Turbidity removal: One of the following methods may be used to remove suspended matter.

7.1.1 Filter sample through a glass fiber filter or a 0.45$\mu$ membrane filter.

7.1.2 Add 1 ml zinc sulfate solution (6.7) to 100 ml of sample and mix thoroughly. Add 0.4-0.5 ml sodium hydroxide solution (6.8) to obtain a pH of 10.5 as determined with a pH meter. Let the treated sample stand a few minutes to allow the heavy flocculent precipitate to settle. Clarify by filtering through a glass fiber filter or a 0.45$\mu$ membrane filter.

7.2 Oil and grease removal: Adjust the pH of 100 ml of filtered sample to 2 by addition of conc. HCl. Extract the oil and grease from the aqueous solution with two 25 ml portions of a non-polar solvent (Freon, chloroform or equivalent).

7.3 If the pH of the sample is below 5 or above 9, adjust to between 5 and 9 with either conc. HCl or conc. $NH_4OH$. This is done to insure a sample pH of 8.5 after step (7.4).

7.4 To 25.0 ml of sample or an aliquot diluted to 25.0 ml, add 75 ml of ammonium chloride-EDTA solution (6.4) and mix.

7.5 Pour sample into column and collect sample at a rate of 7-10 ml per minute.

7.6 Discard the first 25 ml, collect the rest of the sample (approximately 70 ml) in the original sample flask.

7.7 Add 2.0 ml of color reagent (6.6) to 50.0 ml of sample. Allow 10 minutes for color development. Within 2 hours measure the absorbance at 540 nm against a reagent blank.

NOTE: If the concentration of sample exceeds 1.0 mg $NO_3$–N/l, the remainder of the reduced sample may be used to make an appropriate dilution before proceeding with step (7.7).

7.8 Standards: Carry out the reduction of standards exactly as described for the samples. At least one nitrite standard should be compared to a reduced nitrate

standard at the same concentration to verify the efficiency of the reduction column.

8. Calculation

8.1 Obtain a standard curve by plotting the absorbance of standards run by the above procedure against $NO_3$–N mg/l. Compute concentration of samples by comparing sample absorbance with standard curve.

8.2 If less than 25 ml of sample is used for the analysis the following equation should be used:

$$\text{mg } NO_2 + NO_3\text{–N/l} = \frac{A \times 25}{\text{ml sample used}}$$

where:

A = Concentration of nitrate from standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (MDQARL), using sewage samples at concentrations of 0.04, 0.24, 0.55 and 1.04 mg $NO_3 + NO_2$–N/l, the standard deviations were ±0.005, ±0.004, ±0.005 and ±0.01, respectively.

9.2 In a single laboratory (MDQARL), using sewage samples at concentrations of 0.24, 0.55, and 1.05 mg $NO_3 + NO_2$–N/l, the recoveries were 100%, 102% and 100%, respectively.

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## NITROGEN, NITRATE-NITRITE

(Automated Cadmium Reduction Method)

STORET NO. 00630

1. Scope and Application

   1.1 This method pertains to the determination of nitrite singly, or nitrite and nitrate combined in surface and saline waters, and domestic and industrial wastes. The applicable range of this method is 0.05 to 10.0 mg/l nitrate-nitrite nitrogen. The range may be extended with sample dilution.

2. Summary of Method

   2.1 A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N–(1-napthyl)–ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically. Separate, rather than combined nitrate-nitrite values, are readily obtained by carrying out the procedure first with, and then without, the initial Cu-Cd reduction step.

3. Sample Handling and Preservation

   3.1 Analysis should be made as soon as possible. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 ml conc. $H_2SO_4$ per liter) and refrigeration.

   *Caution:* Samples for reduction column must not be preserved with mercuric chloride.

4. Interferences

   4.1 Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used for analysis will also interfere.

5. Apparatus

   5.1 Technicon AutoAnalyzer (AAI or AAII) consisting of the following components:

      5.1.1 Sampler.

      5.1.2 Manifold (AAI) or analytical cartridge (AAII).

      5.1.3 Colorimeter equipped with a 15 mm or 50 mm tubular flow cell and 540 nm filters.

      5.1.4 Recorder.

5.1.5 Digital printer for AAII (Optional).

6. Reagents

6.1 Granulated cadmium: 40-60 mesh (E M Laboratories, Inc., 500 Exec. Blvd., Elmsford, NY 10523, Cat. 2001 Cadmium, Coarse Powder).

6.2 Copper-cadmium: The cadmium granules (new or used) are cleaned with dilute HCl (6.7) and copperized with 2% solution of copper sulfate (6.8) in the following manner:

6.2.1 Wash the cadmium with HCl (6.7) and rinse with distilled water.

6.2.2 Swirl 10 g cadmium in 100 ml portions of 2% solution of copper sulfate (6.8) for five minutes or until blue color partially fades, decant and repeat with fresh copper sulfate until a brown colloidal precipitate forms.

6.2.3 Wash the cadmium-copper with distilled water (at least 10 times) to remove all the precipitated copper.

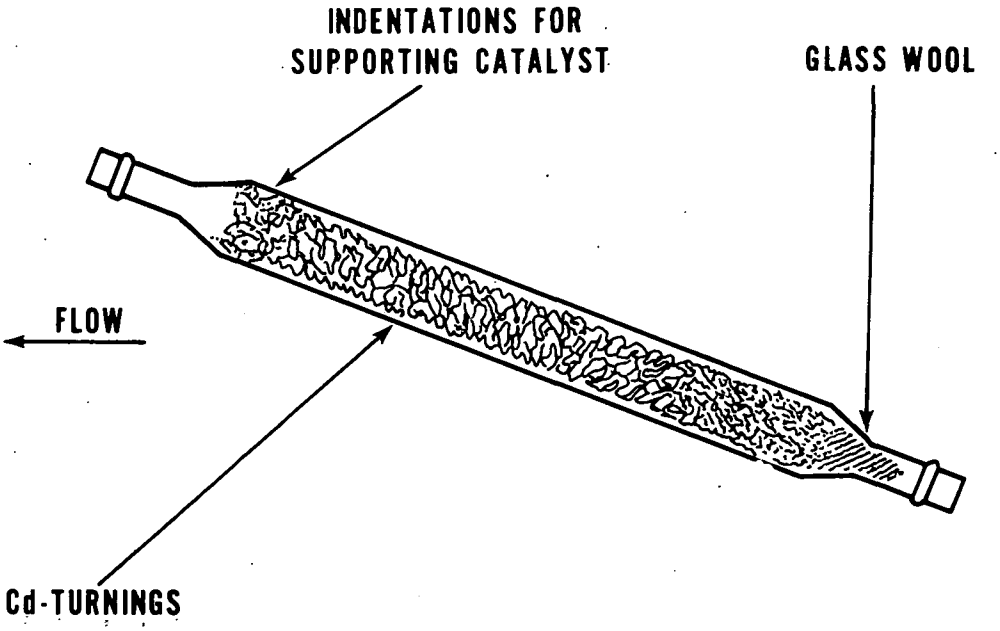
6.3 Preparation of reduction column AAI: The reduction column is an 8 by 50 mm glass tube with the ends reduced in diameter to permit insertion into the system. Copper-cadmium granules (6.2) are placed in the column between glass wool plugs. The packed reduction column is placed in an up-flow 20° incline to minimize channeling. See Figure 1.

6.4 Preparation of reduction column AAII: The reduction column is a U-shaped, 35 cm length, of 2 mm I.D. glass tubing (Note 1). Fill the reduction column with distilled water to prevent entrapment of air bubbles during the filling operations. Transfer the copper-cadmium granules (6.2) to the reduction column and place a glass wool plug in each end. To prevent entrapment of air bubbles in the reduction column be sure that all pump tubes are filled with reagents before putting the column into the analytical system.

NOTE 1: A 0.081 I.D. pump tube (purple) can be used in place of the 2 mm glass tube.

6.5 Distilled water: Because of possible contamination, this should be prepared by passage through an ion exchange column comprised of a mixture of both strongly acidic-cation and strongly basic-anion exchange resins. The regeneration of the ion exchange column should be carried out according to the manufacturer's instructions.

6.6 Color reagent: To approximately 800 ml of distilled water, add, while stirring, 100 ml conc. phosphoric acid, 40 g sulfanilamide, and 2 g N-1 naphthylethylene-diamine dihydrochloride. Stir until dissolved and dilute to 1 liter. Store in brown




TILT COLUMN TO 20° POSTION

FIGURE 1. COPPER-CADMIUM REDUCTION COLUMN
(1 1/2 ACTUAL SIZE)

bottle and keep in the dark when not in use. This solution is stable for several months.

6.7 Dilute hydrochloric acid, 6N: Dilute 50 ml of conc. HCl to 100 ml with distilled water.

6.8 Copper sulfate solution, 2%: Dissolve 20 g of $CuSO_4 \cdot 5H_2O$ in 500 ml of distilled water and dilute to 1 liter.

6.9 Wash solution: Use distilled water for unpreserved samples; samples preserved with $H_2SO_4$, use 2 ml $H_2SO_4$ per liter of wash water.

6.10 Ammonium chloride solution (8.5% $NH_4Cl$): Dissolve 85 g of reagent grade ammonium chloride in distilled water and dilute to 1 liter with distilled water. Add 1/2 ml Brij-35 (Available from Technicon Corporation).

6.11 Stock nitrate solution: Dissolve 7.218 g $KNO_3$ and dilute to 1 liter in a volumetric flask with distilled water. Preserve with 2 ml of chloroform per liter. Solution is stable for 6 months. 1 ml = 1.0 mg $NO_3$–N.

6.12 Stock nitrite solution: Dissolve 6.072 g $KNO_2$ in 500 ml of distilled water and dilute to 1 liter in a volumetric flask. Preserve with 2 ml of chloroform and keep under refrigeration. 1.0 ml = 1.0 mg $NO_2$–N.

6.13 Standard nitrate solution: Dilute 10.0 ml of stock nitrate solution (6.11) to 1000 ml. 1.0 ml = 0.01 mg $NO_3$–N. Preserve with 2 ml of chloroform per liter. Solution is stable for 6 months.

6.14 Standard nitrite solution: Dilute 10.0 ml of stock nitrite (6.12) solution to 1000 ml. 1.0 ml = 0.01 mg $NO_2$–N. Solution is unstable; prepare as required.

6.15 Using standard nitrate solution (6.13), prepare the following standards in 100.0 ml volumetric flasks. At least one nitrite standard should be compared to a nitrate standard at the same concentration to verify the efficiency of the reduction column.

| Conc., mg $NO_2$–N or $NO_3$–N/l | ml Standard Solution/100 ml |
|---|---|
| 0.0 | 0 |
| 0.05 | 0.5 |
| 0.10 | 1.0 |
| 0.20 | 2.0 |
| 0.50 | 5.0 |
| 1.00 | 10.0 |
| 2.00 | 20.0 |
| 4.00 | 40.0 |
| 6.00 | 60.0 |

NOTE 2: When the samples to be analyzed are saline waters, Substitute Ocean Water (SOW) should be used for preparing the standards; otherwise, distilled water is used. A tabulation of SOW composition follows:

| | | |
|---|---|---|
| NaCl – 24.53 g/l | $MgCl_2$ – 5.20 g/l | $Na_2SO_4$ – 4.09 g/l |
| $CaCl_2$ – 1.16 g/l | KCL – 0.70 g/l | $NaHCO_3$ – 0.20 g/l |
| KBr – 0.10 g/l | $H_3BO_3$ – 0.03 g/l | $SrCl_2$ – 0.03 g/l |
| NaF – 0.003 g/l | | |

7. Procedure

7.1 If the pH of the sample is below 5 or above 9, adjust to between 5 and 9 with either conc. HCl or conc. $NH_4OH$.

7.2 Set up the manifold as shown in Figure 1 (AAI) or Figure 2 (AAII). Note that reductant column should be in 20° incline position (AAI). Care should be taken not to introduce air into reduction column on the AAII.

7.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through the sample line.

7.4 Place appropriate nitrate and/or nitrite standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

7.5 For the AAI system, sample at a rate of 30/hr, 1:1. For the AAII, use a 40/hr, 4:1 cam and a common wash.

7.6 Switch sample line to sampler and start analysis.

8. Calculations

8.1 Prepare appropriate standard curve or curves derived from processing $NO_2$ and/or $NO_3$ standards through manifold. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 Three laboratories analyzed four natural water samples containing exact increments of inorganic nitrate, with the following results:

| Increment as Nitrate Nitrogen mg N/liter | Precision as Standard Deviation mg N/liter | Accuracy as | |
|---|---|---|---|
| | | Bias, % | Bias, mg N/liter |
| 0.29 | 0.012 | + 5.75 | +0.017 |
| 0.35 | 0.092 | +18.10 | +0.063 |
| 2.31 | 0.318 | + 4.47 | +0.103 |
| 2.48 | 0.176 | − 2.69 | −0.067 |

(FWQA Method Study 4, Automated Methods – In preparation)

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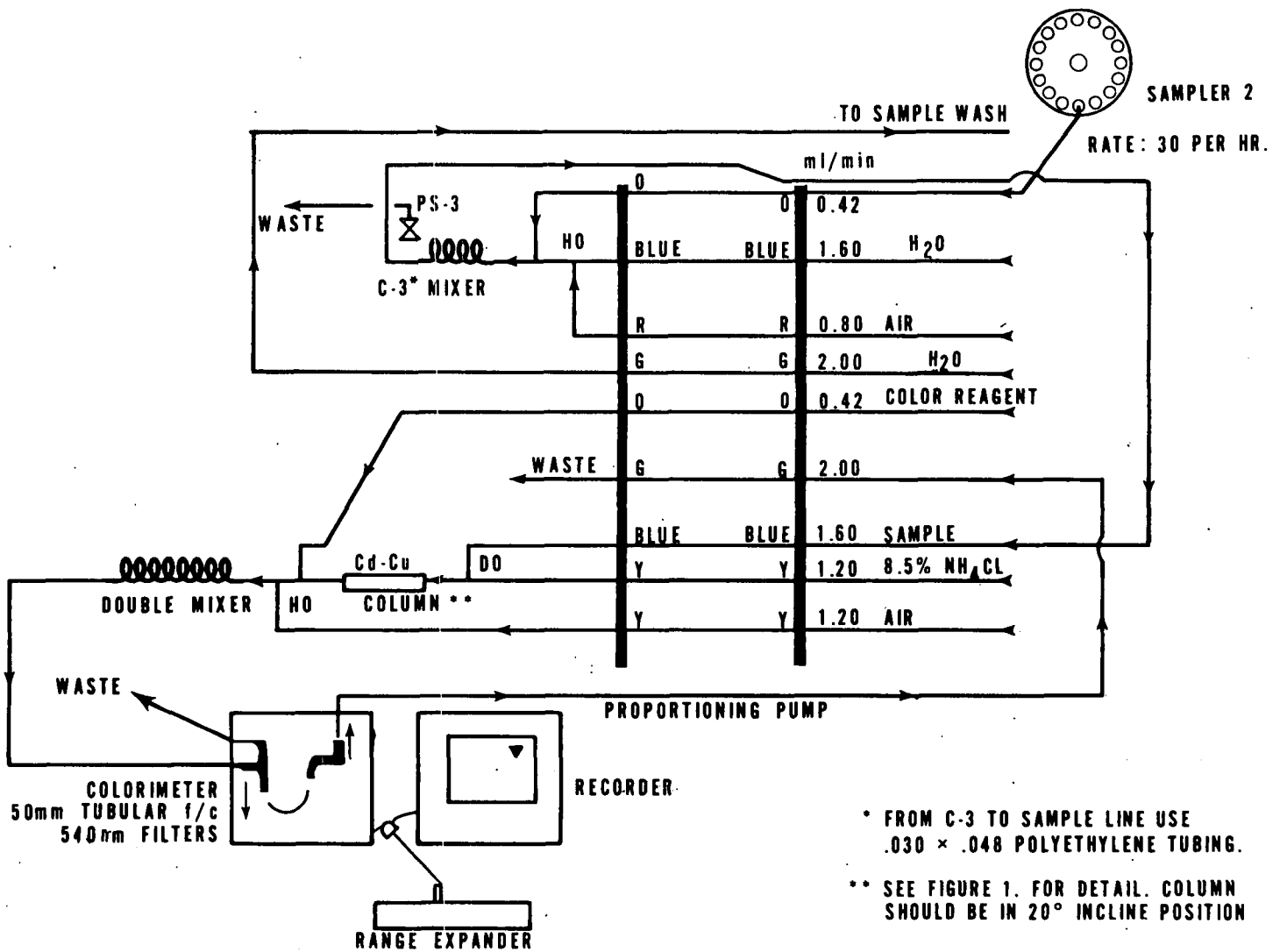



FIGURE 2. NITRATE - NITRITE MANIFOLD AA-I

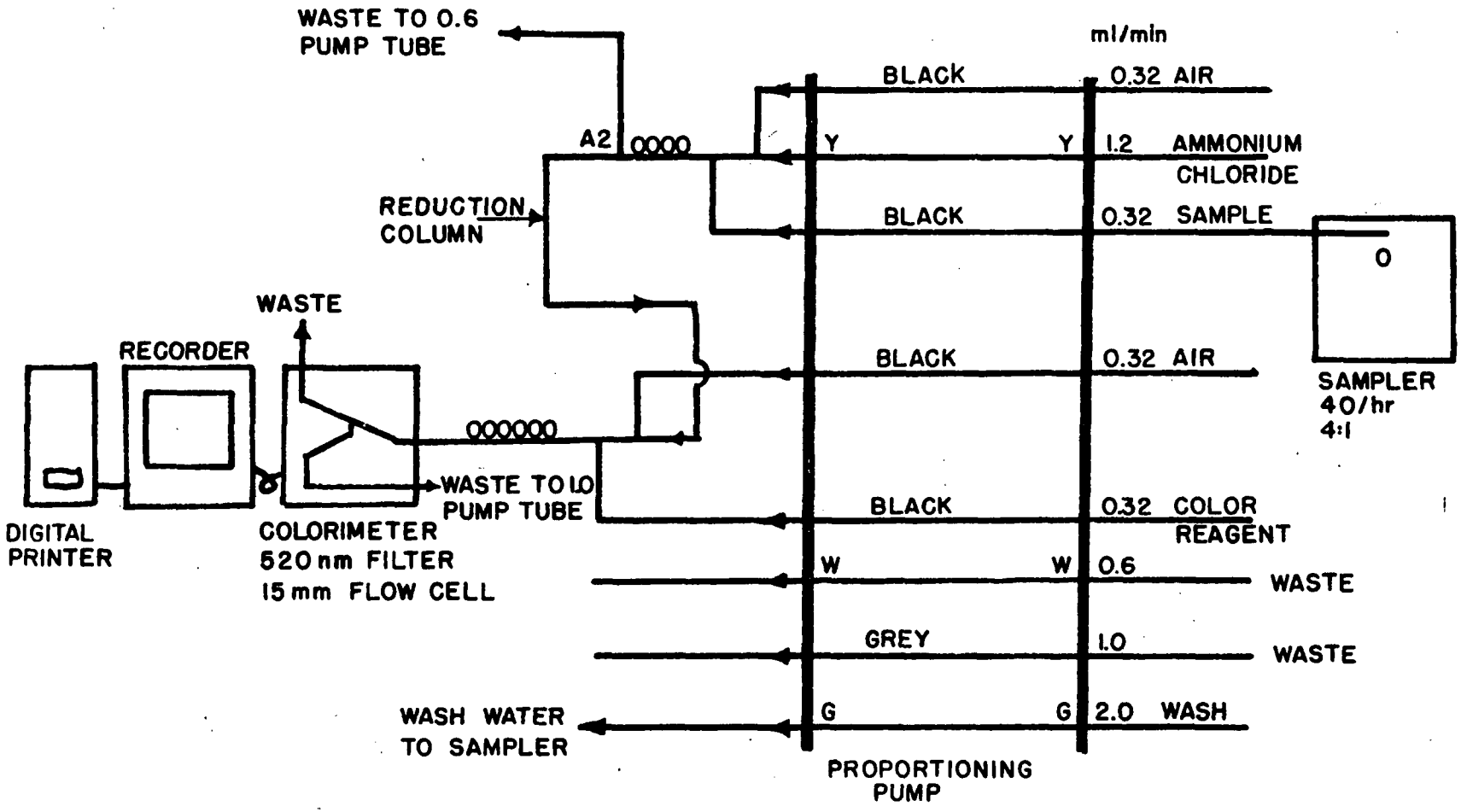



FIGURE 3 NITRATE-NITRITE MANIFOLD AA II

# NITROGEN, NITRITE

STORET NO. 00615

1. Scope and Application
   1.1 This method is applicable to the determination of nitrite in drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The method is applicable in the range from 0.01 to 1.0 mg $NO_2$–N/l.
2. Summary of Method
   2.1 The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acid conditions is coupled with N–(1-naphthyl)–ethylenediamine to produce a reddish-purple color which is read in a spectrophotometer at 540 nm.
3. Sample Handling and Preservation
   3.1 Samples should be analyzed as soon as possible. They may be stored for 24 to 48 hours at 4°C.
4. Interferences
   4.1 There are very few known interferences at concentrations less than 1,000 times that of the nitrite; however, the presence of strong oxidants or reductants to the samples will readily affect the nitrite concentrations. High alkalinity (>600 mg/l) will give low results due to a shift in pH.
5. Apparatus
   5.1 Spectrophotometer equipped with 1 cm or larger cells for use at 540 nm.
   5.2 Nessler tubes, 50 ml or volumetric flasks, 50 ml.
6. Reagents
   6.1 Distilled water free of nitrite and nitrate is to be used in preparation of all reagents and standards.
   6.2 Buffer-color reagent: To 250 ml of distilled water, add 105 ml conc. hydrochloric acid, 5.0 g sulfanilamide and 0.5 g N–(1-naphthyl) ethylenediamine dihydrochloride. Stir until dissolved. Add 136 g of sodium acetate ($CH_3COONa \cdot 3H_2O$) and again stir until dissolved. Dilute to 500 ml with distilled water. This solution is stable for several weeks if stored in the dark.
   6.3 Nitrite stock solution: 1.0 ml = 0.10 mg $NO_2$–N. Dissolve 0.4926 g of dried anhydrous sodium nitrite (24 hours in desiccator) in distilled water and dilute to 1000 ml. Preserve with 2 ml chloroform per liter.
   6.4 Nitrite standard solution: 1.0 ml = 0.001 mg $NO_2$–N. Dilute 10.0 ml of the stock solution (6.3) to 1000 ml.

7. Procedure

7.1 If the sample has a pH greater than 10 or a total alkalinity in excess of 600 mg/l, adjust to approximately pH 6 with 1:3 HCl.

7.2 If necessary, filter the sample through a 0.45 $\mu$ pore size filter using the first portion of filtrate to rinse the filter flask.

7.3 Place 50 ml of sample, or an aliquot diluted to 50 ml, in 50 ml Nessler tube; hold until preparation of standards is completed.

7.4 At the same time prepare a series of standards in 50 ml Nessler tubes as follows:

| ml of Standard Solution 1.0 ml = 0.001 mg $NO_2-N$ | Conc., When Diluted to 50 ml, mg/l of $NO_2-N$ |
|---|---|
| 0.0 (Blank) | 0.0 |
| 0.5 | 0.01 |
| 1.0 | 0.02 |
| 1.5 | 0.03 |
| 2.0 | 0.04 |
| 3.0 | 0.06 |
| 4.0 | 0.08 |
| 5.0 | 0.10 |
| 10.0 | 0.20 |

7.5 Add 2 ml of buffer-color reagent (6.2) to each standard and sample, mix and allow color to develop for at least 15 minutes. The color reaction medium should be between pH 1.5 and 2.0.

7.6 Read the color in the spectrophotometer at 540 nm against the blank and plot concentration of $NO_2-N$ against absorbance.

8. Calculation

8.1 Read the concentration of $NO_2-N$ directly from the curve.

8.2 If less than 50.0 ml of sample is taken, calculate mg/l as follows:

$$NO_2-N,\ mg/l = \frac{\text{mg/l from standard curve} \times 50}{\text{ml sample used}}$$

9. Precision and Accuracy

9.1 Precision and accuracy data are not available at this time.

# NTA

## (Zinc-Zincon Method)

STORET NO. 00695

1. Scope and Application
   1.1 In this method, NTA refers to the tri-sodium salt of nitrilotriacetic acid, $N(CH_2COONa)_3$.
   1.2 This method is applicable to surface waters in the range of 0.5–10.0 mg/l NTA.
2. Summary of Method[1]
   2.1 Zinc forms a blue-colored complex with 2 carboxy-2′-hydroxy-5′-sulfoformazyl-benzene (Zincon) in a solution buffered to pH 9.2. When NTA is added, the Zn-Zincon complex is broken which reduces the optical density in proportion to the amount of NTA present.
3. Sample Handling and Preservation
   3.1 Samples should be analyzed as soon as possible, as NTA has been shown to be biodegradable[2].
4. Interferences
   4.1 Cations, such as calcium, magnesium, zinc, copper, iron, and manganese, complex with NTA and give a negative interference. These ions are removed by batch treating samples with ion-exchange resin. At concentrations higher than expected in typical river waters[3], only zinc, copper, and iron were not completely removed with ion-exchange treatment. Results are summarized in Table 1.

TABLE 1

Interference of Common Metals

| | | 1.0 mg/l NTA | 5.0 mg/l NTA |
|---|---|---|---|
| Metal | mg/l added | Recoveries | |
| Blank | 0.0 | 1.1 | 5.5 |
| Zinc | 2.0 | <0.5 | 0.6 |

TABLE 1-(Cont'd)

| Metal | mg/l added | 1.0 mg/l NTA Recoveries | 5.0 mg/l NTA Recoveries |
|---|---|---|---|
| Boron | 5.0 | 1.1 | 5.5 |
| Iron | 5.0 | 0.95 | 4.6 |
| Molybdenum | 2.0 | 1.0 | 5.5 |
| Manganese | 4.0 | 1.1 | 5.6 |
| Aluminum | 3.0 | 0.85 | 5.2 |
| Copper | 0.5 | <0.5 | 3.4 |
| Strontium | 5.0 | 1.0 | 5.4 |

4.2 This method has not been found applicable to salt waters.

5. Apparatus

5.1 Shaking machine, tray type, for stirring sample-resin mixtures in 125 ml Erlenmeyer flasks.

5.2 Photometer, suitable for measurements at 620 nm.

6. Reagents

6.1 Sodium hydroxide, 6N: Dissolve 120 g NaOH in distilled water and dilute to 500 ml.

6.2 Buffer: Dissolve 31 g boric acid and 37 g potassium chloride in 800 ml distilled water. Adjust pH to 9.2 with 6N NaOH (6.1). Dilute to 1 liter.

6.3 Hydrochloric acid, 2N: Dilute 83 ml conc. HCl to 500 ml with distilled water.

6.4 Zinc: Dissolve 0.44 g $ZnSO_4 \cdot 7H_2O$ in 100 ml 2N HCl (6.3) and dilute to 1 liter with distilled water.

6.5 Sodium hydroxide, 1N: Dissolve 4 g NaOH in distilled water and dilute to 100 ml.

6.6 Zinc-Zincon: Dissolve 0.13 g Zincon (2-carboxy-2′-hydroxy-5′-sulfoformazyl benzene) in 2 ml 1N NaOH (6.5). Add 300 ml buffer (6.2). While stirring, add 15 ml Zinc solution (6.4) and dilute to 1 liter with distilled water.

6.7 Ion-exchange resin: Dowex 50W-X8, 50-100 mesh, Na+ form (or equivalent).

6.8 Stock NTA solution: Dissolve 1.0700 g $N(CH_2COONa)_3 \cdot H_2O$ in distilled water and dilute to 1000 ml. 1.0 ml = 0.01 mg NTA.

7. Procedure

7.1 Filter approximately 50 ml of well-mixed sample through a 0.45$\mu$ membrane filter.

7.2 Prepare a series of standards from 0.5 to 10 mg/l NTA, including a blank of distilled water. Treat standards and blank in same manner as filtered samples.

7.3 To a 25 ml sample in a 125 ml Erlenmeyer flask add approximately 2.5 g ion-exchange resin. Agitate sample for at least 15 minutes.

7.4 Filter through coarse filter paper to remove resin. Pipette 15.0 ml of filtrate into a 50 ml beaker. Add 25.0 ml Zinc-Zincon (6.6) by pipette.

7.5 Read absorbance against distilled water at 620 nm in a 1 cm or 2 cm cell.

8. Calculation

8.1 Prepare standard curve by plotting absorbance of standards vs. NTA concentrations. Calculate concentrations of NTA, mg/l, directly from this curve.

9. Precision and Accuracy

9.1 In a single laboratory (MDQARL), using spiked surface water samples at concentrations of 0.5, 2, 6, and 10 mg/l NTA, standard deviations were ±0.17, ±0.14, ±0.1, and ±0.16, respectively.

9.2 In a single laboratory (MDQARL), using spiked surface water samples at concentrations of 1.0 and 7.5 mg/l NTA, recoveries were 120% and 103%, respectively.

## Bibliography


1. Thompson, J. E., and Duthie, J. R., "The Biodegradability and Treatment of NTA". Jour. WPCF, *40*, No. 2, 306 (1968).
2. Shumate, K. S. et al, "NTA Removal by Activated Sludge – Field Study". ibid., *42*, No. 4, 631 (1970).
3. Kopp, J. F., and Kroner, R. C., "Trace Metals in Waters of the United States", USDI, FWPCA, DPS, 1014 Broadway, Cincinnati, Ohio 45202.

# NTA

## (Automated Zinc-Zincon Method)

STORET NO. 00695

1. Scope and Application

   1.1 In this method, NTA refers to the tri-sodium salt of nitrilotriacetic acid, $N(CH_2COONa)_3$.

   1.2 This method is applicable to surface waters in the range of 0.04 to 1.0 mg/l and 0.5 to 10.0 mg/l NTA, depending on which manifold system is used. It does not apply to saline waters; a positive interference of 0.5 to 1.0 mg/l is present in sewage-type samples.

   1.3 Approximately 13 samples per hour can be analyzed.

2. Summary of Method[1]

   2.1 Zinc forms a blue-colored complex with 2-carboxy-2′-hydroxy-5′-sulfoformazyl-benzene (Zincon) in a solution buffered to pH 9.2. When NTA is added, the Zn-Zincon complex is broken which reduces the optical density in proportion to the amount of NTA present.

3. Sample Handling and Preservation

   3.1 Samples should be analyzed as soon as possible, as NTA has been shown to be biodegradable.[2]

4. Interferences

   4.1 Cations, such as calcium, magnesium, zinc, copper, iron, and manganese, complex with NTA and give a negative interference. These ions are removed automatically by passing the sample through an ion-exchange column. At concentrations higher than expected in typical river waters,[3] only iron was not completely removed by this column treatment. Results, summarized in Tables 1 and 2, show that iron gives a negative interference in concentrations above 3.0 mg/l NTA.

TABLE 1

Interference of Common Metals

| Metal | mg/l added | 1.0 mg/l NTA Recoveries | 5.0 mg/l NTA Recoveries |
|---|---|---|---|
| Blank | 0.0 | 1.0 | 5.0 |
| Zinc | 2.0 | 0.9 | 4.9 |
| Iron | 5.0 | 0.8 | 3.8 |
| Manganese | 4.0 | 1.0 | 4.9 |
| Copper | 0.5 | 1.2 | 4.9 |

TABLE 2

Effect of Iron on NTA Recovery in River Water

| Iron Added mg/l | NTA Recovered, mg/l (0.5 mg/l added) |
|---|---|
| 0.0 | 0.52 |
| 0.5 | 0.52 |
| 1.0 | 0.52 |
| 2.0 | 0.52 |
| 3.0 | 0.48 |
| 4.0 | 0.45 |
| 5.0 | 0.39 |

4.2 At concentration levels below 0.05 mg/l NTA, negative peaking may occur during analyses.

5. Apparatus

5.1 Technicon AutoAnalyzer consisting of:

5.1.1 Sampler I or II.

5.1.2 Manifold.

5.1.3 Proportioning pump.

5.1.4 Colorimeter equipped with 15 mm tubular flow cell and 600 or 625 nm filter.

5.1.5 Recorder.

6. Reagents

6.1 Sodium hydroxide, 6N: Dissolve 120 g NaOH in distilled water and dilute to 500 ml.

6.2 Buffer: Dissolve 31 g boric acid and 37 g potassium chloride in 800 ml distilled water. Adjust pH of solution to 9.2 with 6N NaOH (6.1). Dilute to 1 liter.

6.3 Hydrochloric acid, 2N: Dilute 83 ml conc. HCl to 500 ml with distilled water.

6.4 Zinc: Dissolve 0.44 g $ZnSO_4 \cdot 7H_2O$ in 100 ml 2N HCl (6.3). Dilute to 1 liter with distilled water.

6.5 Sodium hydroxide, 1N: Dissolve 4 g NaOH in distilled water and dilute to 100 ml.

6.6 Zinc-Zincon reagent A (0.04-1.0 mg/l NTA): Dissolve 0.065 g Zincon powder (2-carboxy-2′-hydroxy-5′-sulfoformazyl benzene) in 2 ml of 1 N NaOH (6.5). Add 300 ml buffer (6.2). Stir on a magnetic stirrer and add 7.5 ml zinc solution (6.4). Dilute to 1 liter with distilled water. *This solution is stable for 12 hours.*

6.7 Zinc-Zincon reagent B (0.5-10 mg/l NTA): Dissolve 0.13 g Zincon in 2 ml 1N NaOH (6.5). Stir on magnetic stirrer and add 300 ml buffer (6.2) and 15 ml zinc solution (6.4). Dilute to 1 liter with distilled water. *Stable for 1 week.*

6.8 Ion-exchange resin, $H^+$ form: 20-50 mesh or 30-80 mesh, Dowex 50W-XB or equivalent.

NOTE: Column is prepared by sucking a water slurry of the resin into 12 inches of 3/16-inch OD sleeving. This may be conveniently done by using a pipette and a loose-fitting glass wool plug in the sleeve.

6.9 Stock NTA solution: Dissolve 1.0700 g of $N(CH_2COONa)_3 \cdot H_2O$ in 500 ml of distilled water and dilute to 1000 ml. 1.0 ml = 1.0 mg NTA.

6.10 Working solution A: Dilute 10.0 ml of stock NTA solution to 100.0 ml with distilled water. 1.0 ml = 0.1 mg NTA. Prepare daily.

6.11 Working solution B: Dilute 10.0 ml of Solution A to 100.0 ml with distilled water. 1.0 ml = 0.01 mg NTA. Prepare daily.

6.12 Working solution C: Dilute 10.0 ml of Solution B to 100.0 ml with distilled water. 1.0 ml = 0.001 mg NTA. Prepare daily.

6.13 Prepare a series of standards by diluting suitable volumes of working solutions to 100.0 ml with distilled water. The following dilutions are suggested:

| ml of Solution C/100 ml | Conc., mg NTA/l |
|---|---|
| 2 | 0.02 |
| 4 | 0.04 |
| 6 | 0.06 |
| 8 | 0.08 |
| 10 | 0.10 |
| ml of Solution B/100 ml | |
| 2 | 0.20 |
| 4 | 0.40 |
| 6 | 0.60 |
| 8 | 0.80 |
| 10 | 1.00 |
| ml of Solution A/100 ml | |
| 2 | 2.0 |
| 4 | 4.0 |
| 6 | 6.0 |
| 8 | 8.0 |
| 10 | 10.0 |

7. Procedure

   7.1 Set up manifold as shown in Figure 1.

   7.2 Allow both colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on colorimeter to obtain suitable baseline.

   7.3 Place wash water tubes in sampler in sets of two, leaving every third position vacant. Set sampling time at 1.5 minutes.

   7.4 Place NTA standards in sampler in order of increasing or decreasing concentration. Complete filling of sample tray with unknown samples.

   7.5 Switch sample line from distilled water to sampler and begin analysis.

8. Calculation

   8.1 Prepare standard curve by plotting peak heights of processed NTA standards against known concentrations. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

   9.1 In a single laboratory (MDQARL), using surface water samples at concentrations of 0.1, 0.18, 0.27, and 0.44 mg/l, the standard deviations were ±0.01, ±0.004,

±0.004, and ±0.005, respectively. At concentrations of 1.3, 4.0, 5.8, and 7.4 mg/l, the standard deviations were ±0.05, ±0.05, ±0.07, and ±0.1, respectively.

9.2 In a single laboratory (MDQARL), using surface water samples at concentrations of 0.18 and 0.27 mg/l, recoveries were 101% and 106%, respectively. At concentrations of 4.0 and 5.8 mg/l, the recoveries were 98% and 96%, respectively.

## Bibliography


1. Thompson, J. E., and Duthie, J. R., "The Biodegradability and Treatment of NTA." Jour. WPCF, *40,* No. 2, 306 (1968).
2. Shumate, K. S. et al, "NTA Removal by Activated Sludge – Field Study." ibid, *42,* No. 4, 631 (1970).
3. Kopp, J. F. and Kroner, R. C., "Trace Metals in Waters of the United States." USDI, FWPCA, DPS, 1014 Broadway, Cincinnati, Ohio 45202.

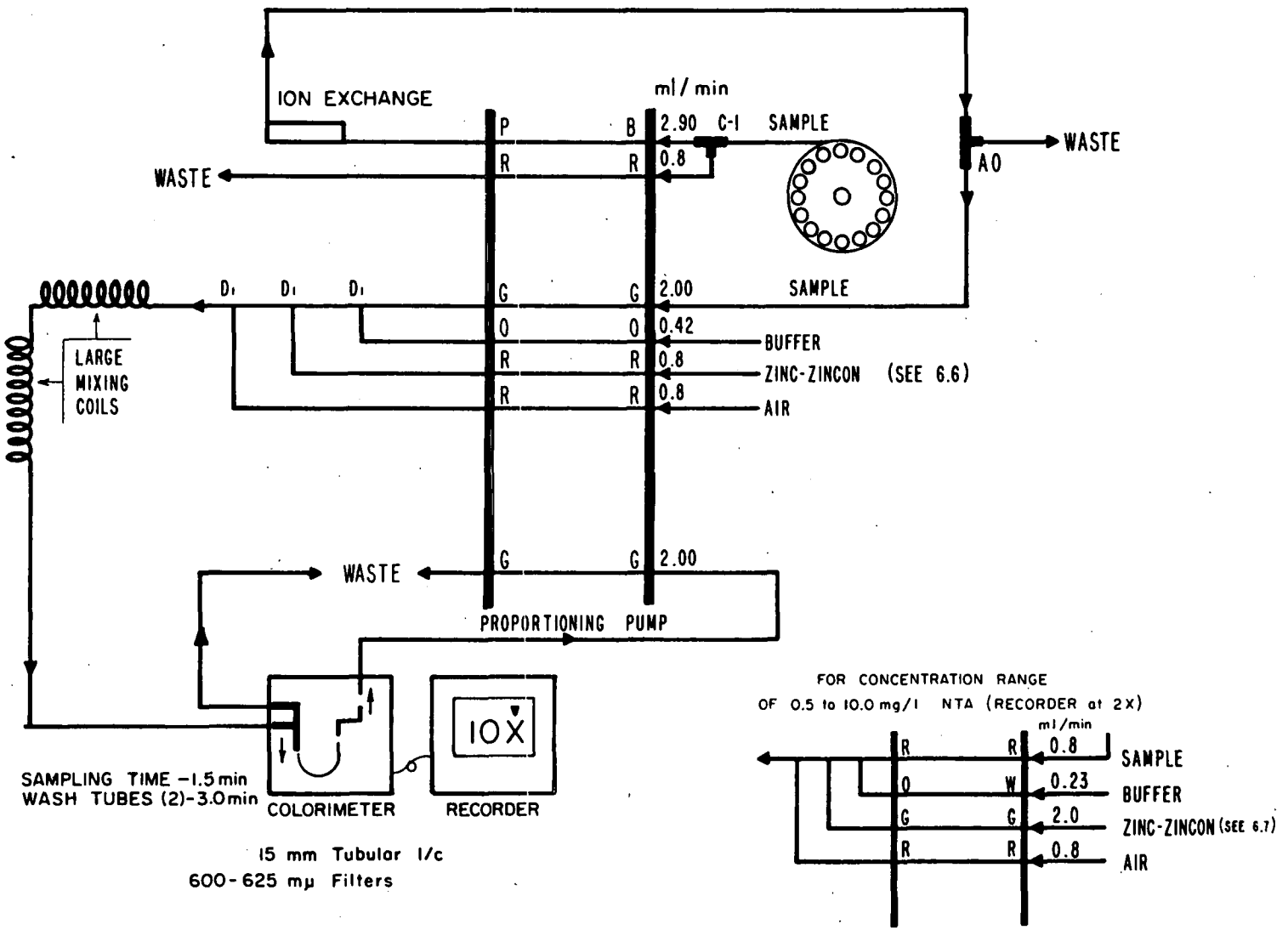



**FIGURE 1. NTA MANIFOLD (0.04-1.0 mg/l NTA) AA-I**

OIL AND GREASE, Total, Recoverable
(Soxhlet Extraction)

STORET NO. 00550

1. Scope and Application
   1.1 This method includes the measurement of Freon extractable matter from surface and saline waters, industrial and domestic wastes. It is applicable to the determination of relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related matters.
   1.2 The method is not applicable to measurement of light hydrocarbons that volatilize at temperatures below 70°C. Petroleum fuels from gasoline through #2 fuel oil are completely or substantially lost in the solvent removal operation.
   1.3 The method covers the range from 5 to 1000 mg/l of extractable material.
2. Summary of Method
   2.1 The sample is acidified to a low pH (<2) to remove the oils and greases from solution. After they are isolated by filtration, they are extracted with Freon using a Soxhlet extraction. The solvent is evaporated from the extract and the residue weighed.
3. Definitions
   3.1 The definition of grease and oil is based on the procedure used. The source of the oil and/or grease, and the presence of extractable non-oily matter will influence the material measured and interpretation of results.
4. Sampling and Storage
   4.1 A representative 1 liter sample should be collected in a wide-mouth glass bottle. If analysis is to be delayed for more than a few hours, the sample is preserved by the addition of 5 ml $H_2SO_4$ or HCl (6.1) at the time of collection.
   4.2 Because losses of grease will occur on sampling equipment, the collection of a composite sample is impractical. Individual portions collected at prescribed time intervals must be analyzed separately to obtain the average concentration over an extended period.
5. Apparatus
   5.1 Extraction apparatus consisting of:
      5.1.1 Soxhlet extractor, medium size (Corning No. 3740 or equivalent).
      5.1.2 Soxhlet thimbles, to fit in Soxhlet extractor, (5.1.1).
      5.1.3 Flask, 125 ml (Corning No. 4100 or equivalent).

5.1.4 Condenser, Allihn (bulb) type, to fit extractor.

5.1.5 Electric heating mantle.

5.2 Vacuum pump, or other source of vacuum.

5.3 Buchner funnel, 12 cm.

5.4 Filter paper, Whatman No. 40, 11 cm.

5.5 Muslin cloth discs, 11 cm (muslin cloth available at sewing centers). The muslin discs are cut to the size of the filter paper and pre-extracted with Freon before use.

6. Reagents

6.1 Sulfuric acid, 1:1. Mix equal volumes of conc. $H_2SO_4$ and distilled water. (Conc. hydrochloric acid may be substituted directly for conc. sulfuric for this reagent.)

6.2 Freon 113, b.p. 48°C, 1,1,2-trichloro-1,2,2-trifluoroethane. At this time, reagent grade Freon is not commercially available. Freon 113 is available from E. I. DuPont de Nemours, Inc., and its distributors in 5-gallon cans. It is best handled by filtering one gallon quantities through paper into glass containers, and maintaining a regular program of solvent blank monitoring.

6.3 Diatomaceous - silica filter aid suspension, 10 g/l in distilled water.

NOTE: Hyflo Super-Cel (Johns-Manville Corp.) or equivalent is used in the preparation of the filter aid suspension.

7. Procedure

7.1 In the following procedure, all steps must be rigidly adhered to if consistent results are to be obtained.

7.2 Mark the sample bottle at the water meniscus for later determination of sample volume. If the sample was not acidified at the time of collection, add 5 ml sulfuric acid or hydrochloric acid (6.1) to the sample bottle. After mixing the sample, check the pH by touching pH-sensitive paper to the cap to insure that the pH is 2 or lower. Add more acid if necessary.

7.3 Prepare a filter consisting of a muslin cloth disc overlaid with filter paper. Place the assembled filter in the Buchner funnel and wet the filter, pressing down the edges to secure a seal. With vacuum on, put 100 ml of the filter aid suspension through the filter and then wash with three 100 ml volumes of distilled water. Continue the vacuum until no more water passes through the filter.

7.4 Filter the acidified sample through the prepared filter pad under vacuum and continue the vacuum until no more water passes through the filter.

7.5 Using forceps, transfer the filter paper and all solid material on the muslin to a watch glass. Wipe the inside and cap of the sample bottle and the inside of the

Buchner funnel with pieces of filter paper soaked in Freon to remove all oil film. Fold the pieces of filter paper and fit them into an extraction thimble. Wipe the watch glass in a similar manner and add the filter paper and all solid matter to the thimble.

7.6 Fill the thimble with small glass beads or glass wool, and dry in an oven at 103°C for *exactly* 30 minutes.

7.7 Weigh the distilling flask (pre-dried in oven at 103°C and stored in desiccator), add the Freon, and connect to the Soxhlet apparatus in which the extraction thimble has been placed. Extract at the rate of 20 cycles per hour for four hours. The four hours is timed from the first cycle.

7.8 Evaporate the solvent from the extraction flask in a water bath at 70°C. Dry by placing the flask on a covered 80°C water bath for 15 minutes. Draw air through the flask by means of an applied vacuum for 1 minute.

7.9 Cool the flask in desiccator for 30 minutes and weigh.

8. Calculation

8.1 $\text{mg/l total grease} = \frac{R-B}{V}$

where:

R = residue, gross weight of extraction flask minus the tare weight, in milligrams.

B = blank determination, residue of equivalent volume of extraction solvent, in milligrams.

V = volume of sample, determined by refilling sample bottle to calibration line and correcting for acid addition if necessary, in liters.

9. Precision and Accuracy

9.1 The three oil and grease methods in this manual were tested by a single laboratory (MDQARL) on a sewage. This method determined the oil and grease level in the sewage to be 14.8 mg/l. When 1 liter portions of the sewage were dosed with 14.0 mg of a mixture of #2 fuel oil and Wesson oil, the recovery was 88% with a standard deviation of 1.1 mg.

## Bibliography


1. Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 409, Method 209A (1971).
2. Hatfield, W. D., and Symons, G. E., "The Determination of Grease in Sewage", Sewage Works J., *17*, 16 (1945).
3. Blum, K. A., and Taras, M. J., "Determination of Emulsifying Oil in Industrial Wastewater", JWPCF Research Suppl. *40*, R404 (1968).

# OIL AND GREASE, Total, Recoverable
## (Separatory Funnel Extraction)

STORET NO. 00556

1. Scope and Application
   - 1.1 This method includes the measurement of Freon extractable matter from surface and saline waters, industrial and domestic wastes. It is applicable to the determination of relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related matter.
   - 1.2 The method is not applicable to measurement of light hydrocarbons that volatilize at temperatures below 70°C. Petroleum fuels from gasoline through #2 fuel oils are completely or substantially lost in the solvent removal operation.
   - 1.3 Some crude oils and heavy fuel oils contain a significant percentage of residue-type materials that are not soluble in Freon. Accordingly, recoveries of these materials will be low.
   - 1.4 The method covers the range from 5 to 1000 mg/l of extractable material.
2. Summary of Method
   - 2.1 The sample is acidified to a low pH (<2) and serially extracted with Freon in a separatory funnel. The solvent is evaporated from the extract and the residue weighed.
3. Definitions
   - 3.1 The definition of grease and oil is based on the procedure used. The source of the oil and/or grease, and the presence of extractable non-oily matter will influence the material measured and interpretation of results.
4. Sampling and Storage
   - 4.1 A representative sample of 1 liter volume should be collected in a glass bottle. If analysis is to be delayed for more than a few hours, the sample is preserved by the addition of 5 ml $H_2SO_4$ or HCl (6.1) at the time of collection.
   - 4.2 Because losses of grease will occur on sampling equipment, the collection of a composite sample is impractical. Individual portions collected at prescribed time intervals must be analyzed separately to obtain the average concentration over an extended period.
5. Apparatus
   - 5.1 Separatory funnel, 2000 ml, with Teflon stopcock.
   - 5.2 Vacuum pump, or other source of vacuum.

5.3 Flask, distilling, 125 ml (Corning No. 4100 or equivalent).

5.4 Filter paper, Whatman No. 40, 11 cm.

6. Reagents

6.1 Sulfuric acid, 1:1. Mix equal volumes of conc. $H_2SO_4$ and distilled water. (Conc. hydrochloric acid may be substituted directly for conc. sulfuric for this reagent).

6.2 Freon 113, b.p. 48°C, 1,1,2-trichloro-1,2,2-trifluoroethane. At this time, reagent grade Freon is not commercially available. Freon 113 is available from E. I. DuPont de Nemours, Inc. and its distributors, in 5-gallon cans. It is best handled by filtering one gallon quantities through paper into glass containers, and maintaining a regular program of solvent blank monitoring.

6.3 Sodium sulfate, anhydrous crystal.

7. Procedure

7.1 Mark the sample bottle at the water meniscus for later determination of sample volume. If the sample was not acidified at time of collection, add 5 ml sulfuric acid or hydrochloric acid (6.1) to the sample bottle. After mixing the sample, check the pH by touching pH-sensitive paper to the cap to insure that the pH is 2 or lower. Add more acid if necessary.

7.2 Pour the sample into a separatory funnel.

7.3 Add 30 ml Freon (6.2) to the sample bottle and rotate the bottle to rinse the sides. Transfer the solvent into the separatory funnel. Extract by shaking vigorously for 2 minutes. Allow the layers to separate.

7.4 Tare a distilling flask (pre-dried in an oven at 103°C and stored in a desiccator), and filter the solvent layer into the flask through a funnel containing solvent moistened filter paper.

NOTE: An emulsion that fails to dissipate can be broken by pouring about 1 g sodium sulfate (6.3) into the filter paper cone and draining the emulsion through the salt. Additional 1 g portions can be added to the cone as required.

7.5 Repeat (7.3 and 7.4) twice more, with additional portions of fresh solvent, combining all solvent in the distilling flask.

7.6 Rinse the tip of the separatory funnel, the filter paper, and then the funnel with a total of 10-20 ml Freon and collect the rinsings in the flask.

7.7 Evaporate the solvent from the extraction flask in a water bath at 70°C. Dry by placing the flask on a covered 80°C water bath for 15 minutes. Draw air through the flask by means of an applied vacuum for 1 minute.

7.8 Cool in desiccator for 30 minutes and weigh.

8. Calculation

8.1 mg/l total oil and grease $= \frac{R-B}{V}$

where:

R = residue, gross weight of extraction flask minus the tare weight, in milligrams.

B = blank determination, residue of equivalent volume of extraction solvent, in milligrams.

V = volume of sample, determined by refilling sample bottle to calibration line and correcting for acid addition if necessary, in liters.

9. Precision and Accuracy

9.1 The three oil and grease methods in this manual were tested by a single laboratory (MDQARL) on a sewage. This method determined the oil and grease level in the sewage to be 12.6 mg/l. When 1 liter portions of the sewage were dosed with 14.0 mg of a mixture of #2 fuel oil and Wesson oil, the recovery was 93% with a standard deviation of 0.9 mg.

## Bibliography


1. Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 254, Method 137 (1971).
2. Blum, K. A., and Taras, M. J., "Determination of Emulsifying Oil in Industrial Wastewater", JWPCF Research Suppl. *40*, R404 (1968).

OIL AND GREASE, Total, Recoverable
(Infrared)

STORET NO. 00560

1. Scope and Application
   1.1 This method includes the measurement of Freon extractable matter from surface and saline waters, industrial and domestic wastes. It is applicable to the determination of hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related matter.
   1.2 The method is applicable to measurement of most light petroleum fuels, although loss of about half of any gasoline present during the extraction manipulations can be expected.
   1.3 The method covers the range from 0.2 to 1000 mg/l of extractable material.
2. Summary of Method
   2.1 The sample is acidified to a low pH (<2) and extracted with Freon. The oil and grease is determined by comparison of the infrared absorbance of the sample extract with standards.
3. Definitions
   3.1 The definition of grease and oil is based on the procedure used. The source of the oil and/or grease, and the presence of extractable non-oily matter will influence the material measured and interpretation of results.
   3.2 An "Unknown Oil" is defined as one for which a representative sample of the oil or grease is not available for preparation of a standard. Examples of unknown oils are the oil and grease in a mixed sewage or an unidentified oil slick on a surface water.
   3.3 A "Known Oil" is defined as a sample of oil and/or grease that represents the only material of that type used or manufactured in the processes represented by a wastewater.
4. Sampling and Storage
   4.1 A representative sample of 1 liter volume should be collected in a glass bottle. If analysis is to be delayed for more than a few hours, the sample is preserved by the addition of 5 ml $H_2SO_4$ or HCl (6.1) at the time of collection.
   4.2 Because losses of grease will occur on sampling equipment, the collection of a composite sample is impractical. Individual portions collected at prescribed time intervals must be analyzed separately to obtain the average concentration over an extended period.

5. Apparatus

5.1 Separatory funnel, 2000 ml, with Teflon stopcock.

5.2 Infrared spectrophotometer, double beam, recording.

5.3 Cells, quartz, 10 mm, 50 mm, and 100 mm path length.

5.4 Syringes, 10, 25, 50, 100 microliter capacity.

5.5 Filter paper, Whatman No. 40, 11 cm.

6. Reagents

6.1 Sulfuric acid, 1:1. Mix equal volumes of conc. $H_2SO_4$ and distilled water. (Conc. hydrochloric acid may be substituted directly for conc. sulfuric for this reagent.)

6.2 Freon 113, b.p. 48°C, 1,1,2-trichloro-1,2,2-trifluoroethane. At this time, reagent grade Freon is not commercially available. Freon 113 is available from E. I. DuPont de Nemours, Inc., and its distributors, in 5-gallon cans. It is best handled by filtering one gallon quantities through paper into glass containers, and maintaining a regular program of solvent blank monitoring.

6.3 Sodium sulfate, anhydrous crystal.

6.4 Known oil reference standard: Accurately weigh about 0.05 g of known oil directly into a 100 ml volumetric flask. Add 80 ml Freon and dissolve the oil. If, as in the case of a heavy fuel oil, all the oil does not go into solution, let stand overnight. The next day filter through paper into another 100 ml volumetric and dilute to mark. Treat calculations as if all oil had gone into solution.

6.5 Unknown oil reference standard (10µl = 7.69 mg oil): Pipette 15.0 ml n-hexadecane, 15.0 ml isooctane, and 10.0 ml benzene into a 50 ml glass stoppered bottle. Assume the specific gravity of this mixture to be 0.769 and maintain the integrity of the mixture by keeping stoppered except when withdrawing aliquots.

7. Procedure

7.1 Mark the sample bottle at the water meniscus for later determination of sample volume. If the sample was not acidified at time of collection, add 5 ml sulfuric or hydrochloric acid (6.1) to the sample bottle. After mixing the sample, check the pH by touching pH-sensitive paper to the cap to insure that the pH is 2 or lower. Add more acid if necessary.

7.2 Pour the sample into a separatory funnel.

7.3 Add 30 ml Freon (6.2) to the sample bottle and rotate the bottle to rinse the sides. Transfer the solvent into the separatory funnel. Extract by shaking vigorously for 2 minutes. Allow the layers to separate.

7.4 Filter the solvent layer into a 100 ml volumetric flask through a funnel containing solvent-moistened filter paper.

NOTE 1: An emulsion that fails to dissipate can be broken by pouring about 1 g sodium sulfate (6.3) into the filter paper cone and draining the emulsion through the salt. Additional 1 g portions can be added to the cone as required.

7.5 Repeat (7.3 and 7.4) twice more with 30 ml portions of fresh solvent, combining all solvent in the volumetric flask.

7.6 Rinse the tip of the separatory funnel, filter paper, and the funnel with a total of 10-20 ml Freon and collect the rinsings in the flask. Dilute the extract to 100 ml, and stopper the flask.

7.7 Select appropriate calibration standards and cell pathlength according to the following table of approximate working ranges:

| Pathlength | Range |
|---|---|
| 1 cm | 4-40 mg |
| 5 cm | 0.5-8 mg |
| 10 cm | 0.1-4 mg |

Prepare calibration standards by pipetting appropriate amounts of the known oil reference standard (6.4) into 100 ml volumetric flasks and diluting to mark with Freon. Alternately, transfer appropriate amounts of the unknown oil reference standard (6.5), using microliter syringes, to 100 ml volumetric flasks and diluting to mark with Freon.

NOTE 2: Ten microliters of the unknown oil is equivalent to 7.69 mg per 100 ml Freon, and 7.69 mg per sample volume.

7.8 Scan standards and samples from 3200 $cm^{-1}$ to 2700 $cm^{-1}$ with Freon in the reference beam and record the results on absorbance paper. The absorbances of samples and standards are measured by constructing a straight baseline over the range of the scan and measuring the absorbance of the peak maximum at 2930 $cm^{-1}$ and subtracting the baseline absorbance at that point. If the absorbance exceeds 0.8 for a sample, select a shorter pathlength or dilute as required.

NOTE 3: Caution must be exercised in the selection of the 2930 $cm^{-1}$ peak, as it may not always be the largest peak in the range of the scan. For an example of a typical oil spectrum and baseline construction, see Gruenfeld[3].

7.9 Use a calibration plot of absorbance vs. mg oil prepared from the standards to determine the mg oil in the sample solution.

8. Calculation

8.1 $$\text{mg/l total oil and grease} = \frac{R \times D}{V}$$

where:

R = oil in solution, determined from calibration plot, in milligrams.

D = extract dilution factor, if used.

V = volume of sample, determined by refilling sample bottle to calibration line and correcting for acid addition if necessary, in liters.

9. Precision and Accuracy

9.1 The three oil and grease methods in this manual were tested by a single laboratory (MDQARL) on a sewage. This method determined the oil and grease level in the sewage to be 17.5 mg/l. When 1 liter portions of the sewage were dosed with 14.0 mg of a mixture of #2 fuel oil and Wesson oil, the recovery was 99% with a standard deviation of 1.4 mg.

## Bibliography


1. Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 254, Method 137 (1971).
2. American Petroleum Institute, "Manual on Disposal of Refinery Wastes", Vol. IV, Method 733-58 (1958).
3. Gruenfeld, M., "Extraction of Dispersed Oils from Water for Quantitative Analysis by Infrared Spectroscopy", Environ. Sci. Technol. *7*, 636 (1973).

# ORGANIC CARBON

(Total and Dissolved)

STORET NO. Total 00680

Dissolved 00681

1. Scope and Application

   1.1 This method includes the measurement of organic carbon in drinking, surface, and saline waters, domestic and industrial wastes. Exclusions are noted under Definitions and Interferences.

   1.2 The method is most applicable to measurement of organic carbon above 1 mg/l.

2. Summary of Method

   2.1 Organic carbon in a sample is converted to carbon dioxide ($CO_2$) by catalytic combustion or wet chemical oxidation. The $CO_2$ formed can be measured directly by an infrared detector or converted to methane ($CH_4$) and measured by a flame ionization detector. The amount of $CO_2$ or $CH_4$ is directly proportional to the concentration of carbonaceous material in the sample.

3. Definitions

   3.1 The carbonaceous analyzer measures all of the carbon in a sample. Because of various properties of carbon-containing compounds in liquid samples, preliminary treatment of the sample prior to analysis dictates the definition of the carbon as it is measured. Forms of carbon that are measured by the method are:

      A) soluble, nonvolatile organic carbon; for instance, natural sugars.

      B) soluble, volatile organic carbon; for instance, mercaptans.

      C) insoluble, partially volatile carbon; for instance, oils.

      D) insoluble, particulate carbonaceous materials, for instance, cellulose fibers.

      E) soluble or insoluble carbonaceous materials adsorbed or entrapped on insoluble inorganic suspended matter; for instance, oily matter adsorbed on silt particles.

   3.2 The final usefulness of the carbon measurement is in assessing the potential oxygen-demanding load of organic material on a receiving stream. This statement applies whether the carbon measurement is made on a sewage plant effluent, industrial waste, or on water taken directly from the stream. In this light, carbonate and bicarbonate carbon are not a part of the oxygen demand in the stream and therefore should be discounted in the final calculation or removed prior to analysis. The manner of preliminary treatment of the sample and

instrument settings defines the types of carbon which are measured. Instrument manufacturer's instructions should be followed.

4. Sample Handling and Preservation

4.1 Sampling and storage of samples in glass bottles is preferable. Sampling and storage in plastic bottles such as conventional polyethylene and cubitainers is permissible if it is established that the containers do not contribute contaminating organics to the samples.

NOTE 1: A brief study performed in the EPA Laboratory indicated that distilled water stored in new, one quart cubitainers did not show any increase in organic carbon after two weeks exposure.

4.2 Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the lapse of time between collection of samples and start of analysis should be kept to a minimum. Also, samples should be kept cool (4°C) and protected from sunlight and atmospheric oxygen.

4.3 In instances where analysis cannot be performed within two hours (2 hours) from time of sampling, it is recommended that the sample is acidified ($pH \leq 2$) with HCl or $H_2SO_4$.

5. Interferences

5.1 Carbonate and bicarbonate carbon represent an interference under the terms of this test and must be removed or accounted for in the final calculation.

5.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a microliter type syringe or pipette. The openings of the syringe or pipette limit the maximum size of particles which may be included in the sample.

6. Apparatus

6.1 Apparatus for blending or homogenizing samples: Generally, a Waring-type blender is satisfactory.

6.2 Apparatus for total and dissolved organic carbon:

6.2.1 A number of companies manufacture systems for measuring carbonaceous material in liquid samples. Considerations should be made as to the types of samples to be analyzed, the expected concentration range, and forms of carbon to be measured.

6.2.2 No specific analyzer is recommended as superior. However, analyzers which have been found to be reliable are the Dow-Beckman Carbonaceous Analyzer Model No. 915, the Dohrmann Envirotech DC-50 Carbon Analyzer and the Oceanography International Total Carbon Analyzer.

7. Reagents

7.1 Distilled water used in preparation of standards and for dilution of samples should be ultra pure to reduce the size of the blank. Carbon dioxide-free, double distilled water is recommended. Ion exchanged waters are not recommended because of the possibilities of contamination with organic materials from the resins.

7.2 Potassium hydrogen phthalate, stock solution, 1000 mg carbon/liter: Dissolve 0.2128 g of potassium hydrogen phthalate (Primary Standard Grade) in distilled water and dilute to 100.0 ml.

NOTE 2: Sodium oxalate and acetic acid are not recommended as stock solutions.

7.3 Potassium hydrogen phthalate, standard solutions: Prepare standard solutions from the stock solution by dilution with distilled water.

7.4 Carbonate-bicarbonate, stock solution, 1000 mg carbon/liter: Weigh 0.3500 g of sodium bicarbonate and 0.4418 g of sodium carbonate and transfer both to the same 100 ml volumetric flask. Dissolve with distilled water.

7.5 Carbonate-bicarbonate, standard solution: Prepare a series of standards similar to step 7.3.

NOTE 3: This standard is not required by some instruments.

7.6 Blank solution: Use the same distilled water (or similar quality water) used for the preparation of the standard solutions.

8. Procedure

8.1 Follow instrument manufacturer's instructions for calibration, procedure, and calculations.

8.2 For calibration of the instrument, it is recommended that a series of standards encompassing the expected concentration range of the samples be used.

9. Precision and Accuracy

9.1 Twenty-eight analysts in twenty-one laboratories analyzed distilled water solutions containing exact increments of oxidizable organic compounds, with the following results:

| Increment as TOC mg/liter | Precision as Standard Deviation TOC, mg/liter | Accuracy as Bias, % | Bias, mg/liter |
|---|---|---|---|
| 4.9 | 3.93 | +15.27 | +0.75 |
| 107 | 8.32 | + 1.01 | +1.08 |

(FWPCA Method Study 3, Demand Analyses)

STORET NO. 00400

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
2. Summary of Method
   2.1 The pH of a sample is an electrometric measurement, using either a glass electrode in combination with a reference potential (saturated calomel electrode) or a combination electrode (glass and reference).
3. Comments
   3.1 The sample must be analyzed as soon as practical; preferably within a few hours. Do not open sample bottle before analyses.
   3.2 Oil and greases, by coating the pH electrode, may interfere by causing sluggish response.
   3.3 At least three buffer solutions must be used to initially standardize the instrument. They should cover the pH range of the samples to be measured.
   3.4 Field pH measurements using comparable instruments are reliable.
4. Precision and Accuracy
   4.1 Forty-four analysts in twenty laboratories analyzed six synthetic water samples containing exact increments of hydrogen-hydroxyl ions, with the following results:

| Increment as pH Units | Precision as Standard Deviation pH Units | Accuracy as Bias, % | Accuracy as Bias, pH Units |
|---|---|---|---|
| 3.5 | 0.10 | −0.29 | −0.01 |
| 3.5 | 0.11 | −0.00 | |
| 7.1 | 0.20 | +1.01 | +0.07 |
| 7.2 | 0.18 | −0.03 | −0.002 |
| 8.0 | 0.13 | −0.12 | −0.01 |
| 8.0 | 0.12 | +0.16 | +0.01 |

(FWPCA Method Study 1, Mineral and Physical Analyses)

4.2 In a single laboratory (MDQARL), using surface water samples at an average pH of 7.7, the standard deviation was ±0.1.

5. Reference

5.1 The procedure to be used for this determination is found in:

Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 276, Method 144 A (1971).

ASTM Standards, Part 23, Water: Atmospheric Analysis, p 186, Method D1293-65 (1973).

# PHENOLICS

## (4-AAP Method With Distillation)

STORET NO. 32730

1. Scope and Application
   - 1.1 This method is applicable to the analysis of drinking, surface, and saline waters, domestic and industrial wastes.
   - 1.2 The method is capable of measuring phenolic materials from about 5 µg/l to about 1000 µg/l when the colored end product is extracted and concentrated in a solvent phase using phenol as a standard.
   - 1.3 The method is capable of measuring phenolic materials that contain more than 50 µg/l in the aqueous phase (without solvent extraction) using phenol as a standard.
   - 1.4 It is not possible to use this method to differentiate between different kinds of phenols.
2. Summary of Method
   - 2.1 Phenolic materials react with 4-aminoantipyrine in the presence of potassium ferricyanide at a pH of 10 to form a stable reddish-brown colored antipyrine dye. The amount of color produced is a function of the concentration of phenolic material.
3. Comments
   - 3.1 For most samples a preliminary distillation is required to remove interfering materials.
   - 3.2 Color response of phenolic materials with 4-amino-antipyrine is not the same for all compounds. Because phenolic type wastes usually contain a variety of phenols, it is not possible to duplicate a mixture of phenols to be used as a standard. For this reason phenol itself has been selected as a standard and any color produced by the reaction of other phenolic compounds is reported as phenol. This value will represent the minimum concentration of phenolic compounds present in the sample.
   - 3.3 Control of the pH of the reaction may be accomplished using the procedure detailed in Standard Methods (p 506, 13th Edition), or ASTM, Part 23, p 535 (Nov. 1973), or by use of the ammonium hydroxide-ammonium chloride buffer used in the water hardness test Standard Methods, 13th Edition, p 181, (1971).
4. Precision and Accuracy
   - 4.1 Using the extraction procedure for concentration of color, six laboratories

analyzed samples at concentrations of 9.6, 48.3, and 93.5 μg/l. Standard deviations were, respectively, ±0.99, ±3.1 and ±4.2 μg/l.

4.2 Using the direct photometric procedure, six laboratories analyzed samples at concentrations of 4.7, 48.2 and 97.0 mg/l. Standard deviations were ±0.18, ±0.48 and ±1.58 mg/l, respectively.

5. References

5.1 The procedure to be used for this determination is found in:

Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 501-510, Method No. 222 through 222E (1971).

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 535, Method D-1783-70 (1973).

# PHENOLICS

## (Automated 4-AAP Method With Distillation)

STORET NO. 32730

1. Scope and Application
   - 1.1 This method is applicable to the analysis of drinking, surface, and saline waters, domestic and industrial wastes.
   - 1.2 The method is capable of measuring phenolic materials from 2 to 500 µg/l in the aqueous phase using phenol as a standard. The working ranges are 2 to 200 µg/l and 10 to 500 µg/l.
2. Summary of Method
   - 2.1 This automated method is based on the distillation of phenol and subsequent reaction of the distillate with alkaline ferricyanide and 4-aminoantipyrine to form a red complex which is measured at 505 or 520 nm. The same manifold is used with the AAI or AAII.
3. Sample Handling and Preservation
   - 3.1 Biological degradation is inhibited by the addition of 1 g/l of copper sulfate to the sample and acidification to a pH of less than 4 with phosphoric acid. The sample should be kept at 5-10°C and analyzed within 24 hours after collection.
4. Interference
   - 4.1 Interferences from sulfur compounds are eliminated by acidifying the sample to a pH of less than 4.0 with $H_3PO_4$ and aerating briefly by stirring and adding $CuSO_4$.
   - 4.2 Oxidizing agents such as chlorine, detected by the liberation of iodine upon acidification in the presence of potassium iodide, are removed immediately after sampling by the addition of an excess of ferrous ammonium sulfate (6.5). If chlorine is not removed, the phenolic compounds may be partially oxidized and the results may be low.
   - 4.3 Background contamination from plastic tubing and sample containers is eliminated by filling the wash receptacle by siphon (using Kel-F tubing) and using glass tubes for the samples and standards.
5. Apparatus
   - 5.1 Technicon AutoAnalyzer (I or II)
     - 5.1.1 Sampler.
     - 5.1.2 Manifold.

5.1.3 Proportioning pump II or III.

5.1.4 Heating bath with distillation coil.

5.1.5 Distillation head.

5.1.6 Colorimeter equipped with a 50 mm flow cell and 505 or 520 nm filter.

5.1.7 Recorder.

6. Reagents

6.1 Distillation reagent: Add 100 ml of conc. phosphoric acid (85% $H_3PO_4$) to 800 ml of distilled water, cool and dilute to 1 liter.

6.2 Buffered potassium ferricyanide: Dissolve 2.0 g potassium ferricyanide, 3.1 g boric acid and 3.75 g potassium chloride in 800 ml of distilled water. Adjust to pH of 10.3 with 1 N sodium hydroxide (6.3) and dilute to 1 liter. Add 0.5 ml of Brij-35. Prepare fresh weekly.

6.3 Sodium hydroxide (1N): Dissolve 40 g NaOH in 500 ml of distilled water, cool and dilute to 1 liter.

6.4 4-Aminoantipyrine: Dissolve 0.65 g of 4-aminoantipyrine in 800 ml of distilled water and dilute to 1 liter. Prepare fresh each day.

6.5 Ferrous ammonium sulfate: Dissolve 1.1 g ferrous ammonium sulfate in 500 ml distilled water containing 1 ml $H_2SO_4$ and dilute to 1 liter with freshly boiled and cooled distilled water.

6.6 Stock phenol: Dissolve 1.00 g phenol in 500 ml of distilled water and dilute to 1000 ml. Add 1 g $CuSO_4$ and 0.5 ml conc. $H_3PO_4$ as preservative. 1.0 ml = 1.0 mg phenol.

6.7 Standard phenol solution A: Dilute 10.0 ml of stock phenol solution (6.6) to 1000 ml. 1.0 ml = 0.01 mg phenol.

6.8 Standard phenol solution B: Dilute 100.0 ml of standard phenol solution A (6.7) to 1000 ml with distilled water. 1.0 ml = 0.001 mg phenol.

6.9 Standard solution C: Dilute 100.0 ml of standard phenol solution B (6.8) to 1000 ml with distilled water. 1.0 ml = 0.0001 mg phenol.

6.10 Using standard solution A, B or C prepare the following standards in 100 ml volumetric flasks. Each standard should be preserved by adding 0.1 g $CuSO_4$ and 2 drops of conc. $H_3PO_4$ to 100.0 ml.

| ml of Standard Solution | Conc. µg/l |
|---|---|
| Solution C | |
| 1.0 | 1.0 |
| 2.0 | 2.0 |
| 3.0 | 3.0 |
| 5.0 | 5.0 |
| Solution B | |
| 1.0 | 10.0 |
| 2.0 | 20.0 |
| 5.0 | 50.0 |
| 10.0 | 100.0 |
| Solution A | |
| 2 | 200 |
| 3 | 300 |
| 5 | 500 |

7. Procedure

7.1 Set up the manifold as shown in Figures 1 or 2.

7.2 Fill the wash receptacle by siphon. Use Kel-F tubing with a fast flow (1 liter/hr).

7.3 Allow colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Use polyethylene tubing for sample line. When new tubing is used, about 2 hours may be required to obtain a stable baseline. This two hour time period may be necessary to remove the residual phenol from the tubing.

7.4 Place appropriate phenol standards in sampler in order of decreasing concentration. Complete loading of sampler tray with unknown samples, using glass tubes. NOTE 1: If samples have not been preserved as instructed in (3.1), add 0.1 g $CuSO_4$ and 2 drops of conc. $H_3PO_4$ to 100 ml of sample.

7.5 Switch sample line from distilled water to sampler and begin analysis.

8. Calculation

8.1 Prepare standard curve by plotting peak heights of standards against concentration values. Compute concentration of samples by comparing sample peak heights with standards.

9. Precision and Accuracy

9.1 In a single laboratory (MDQARL), using sewage samples at concentrations of 3.8, 15, 43 and 89 µg/l, the standard deviations were ±0.5, ±0.6, ±0.6 and ±1.0 µg/l,

respectively. At concentrations of 73, 146, 299 and 447 μg/l, the standard deviations were ±1.0, ±1.8, ±4.2 and ±5.3 μg/l, respectively.

9.2 In a single laboratory (MDQARL), using sewage samples at concentrations of 5.3 and 82 μg/l, the recoveries were 78% and 98%. At concentrations of 168 and 489 μg/l, the recoveries were 97% and 98%, respectively.

## Bibliography


1. Technicon AutoAnalyzer II Methodology, Industrial Method No. 127-71W, AAII.
2. Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 501, American Public Health Association, Inc., New York (1971).

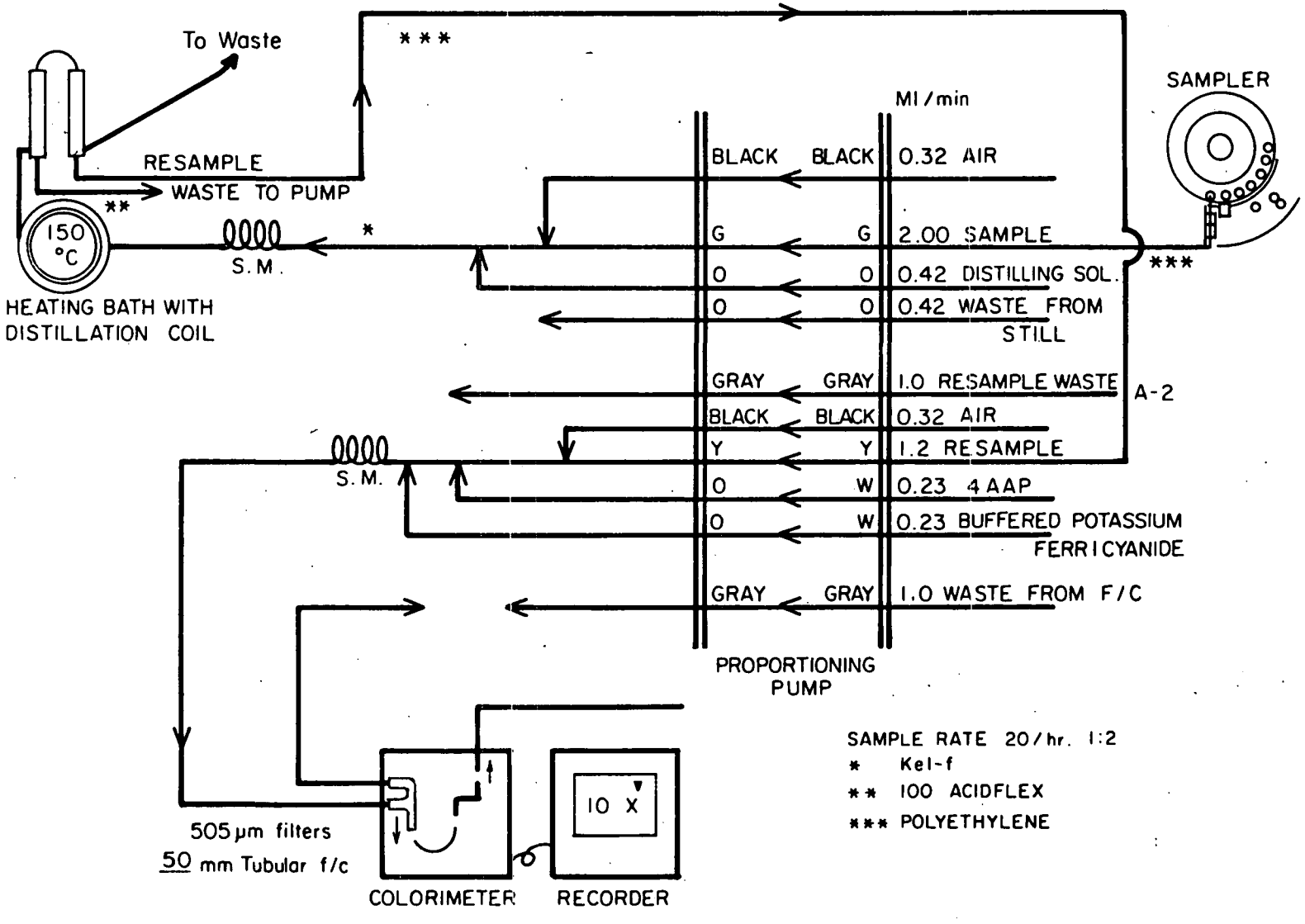



**FIGURE 1. PHENOL AUTO ANALYZER I**

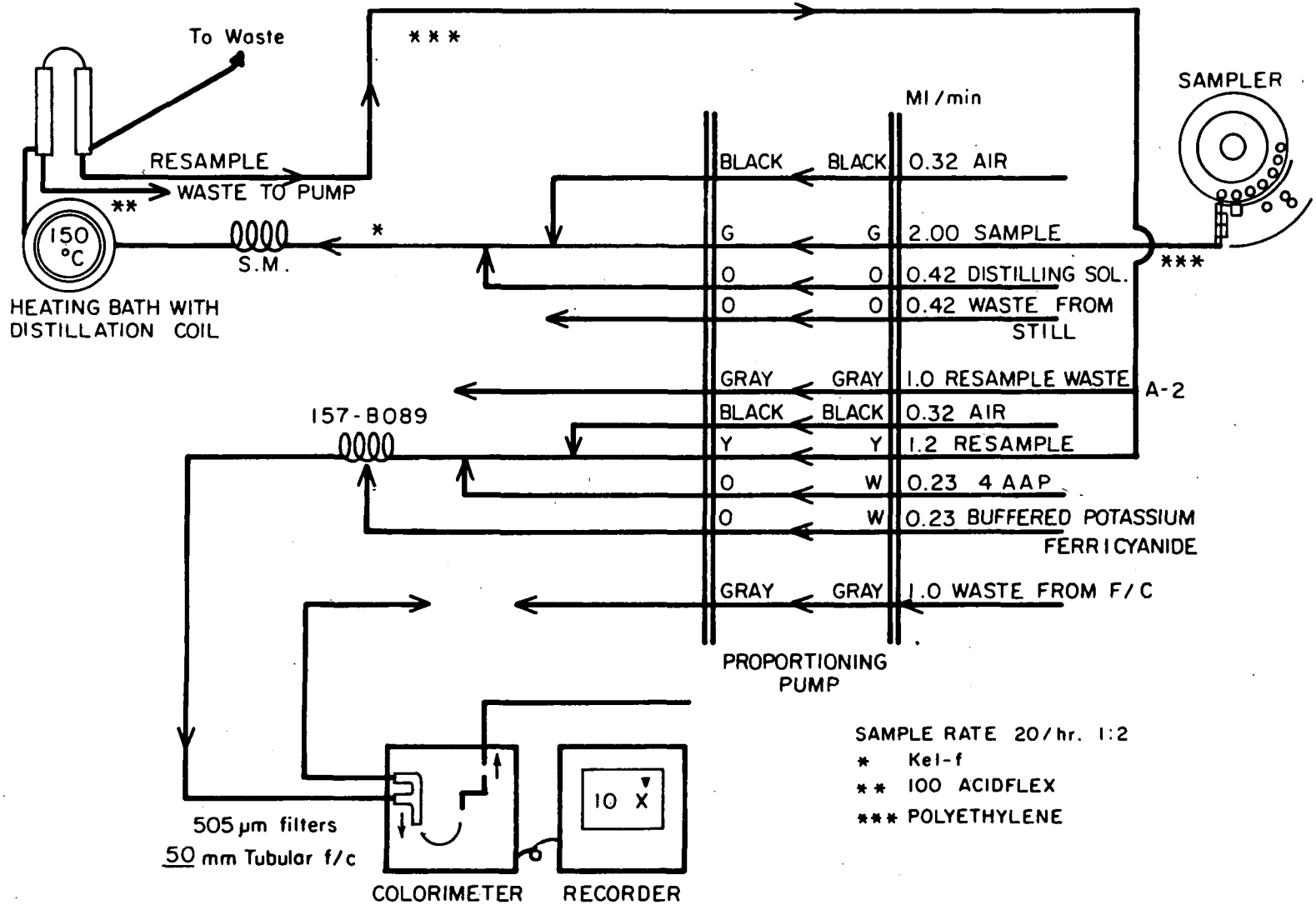



**FIGURE 2. PHENOL AUTO ANALYZER II**

## PHOSPHORUS, ALL FORMS

(Single Reagent Method)

STORET NO. See Section 4

1. Scope and Application

   1.1 These methods cover the determination of specified forms of phosphorus in drinking, surface, and saline waters, domestic and industrial wastes. They may be applicable to sediment-type samples, sludges, algal blooms, etc., but sufficient data is not available at this time to warrant such usage when measurements for phosphorus content are required.

   1.2 The methods are based on reactions that are specific for the orthophosphate ion. Thus, depending on the prescribed pre-treatment of the sample, the various forms of phosphorus given in Figure 1 may be determined. These forms are, in turn, defined in Section 4.

      1.2.1 Except for in-depth and detailed studies, the most commonly measured forms are phosphorus and dissolved phosphorus, and orthophosphate and dissolved orthophosphate. Hydrolyzable phosphorus is normally found only in sewage-type samples and insoluble forms of phosphorus, as noted, are determined by calculation.

   1.3 The methods are usable in the 0.01 to 0.5 mg P/l range.

2. Summary of Method

   2.1 Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

   2.2 Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic phosphorus compounds) may be converted to the orthophosphate form by sulfuric-acid-hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by persulfate digestion([2]).

3. Sample Handling and Preservation

   3.1 If benthic deposits are present in the area being sampled, great care should be taken not to include these deposits.

   3.2 Sample containers may be of plastic material, such as cubitainers, or of Pyrex glass.

   3.3 If the analysis cannot be performed the same day of collection, the sample should

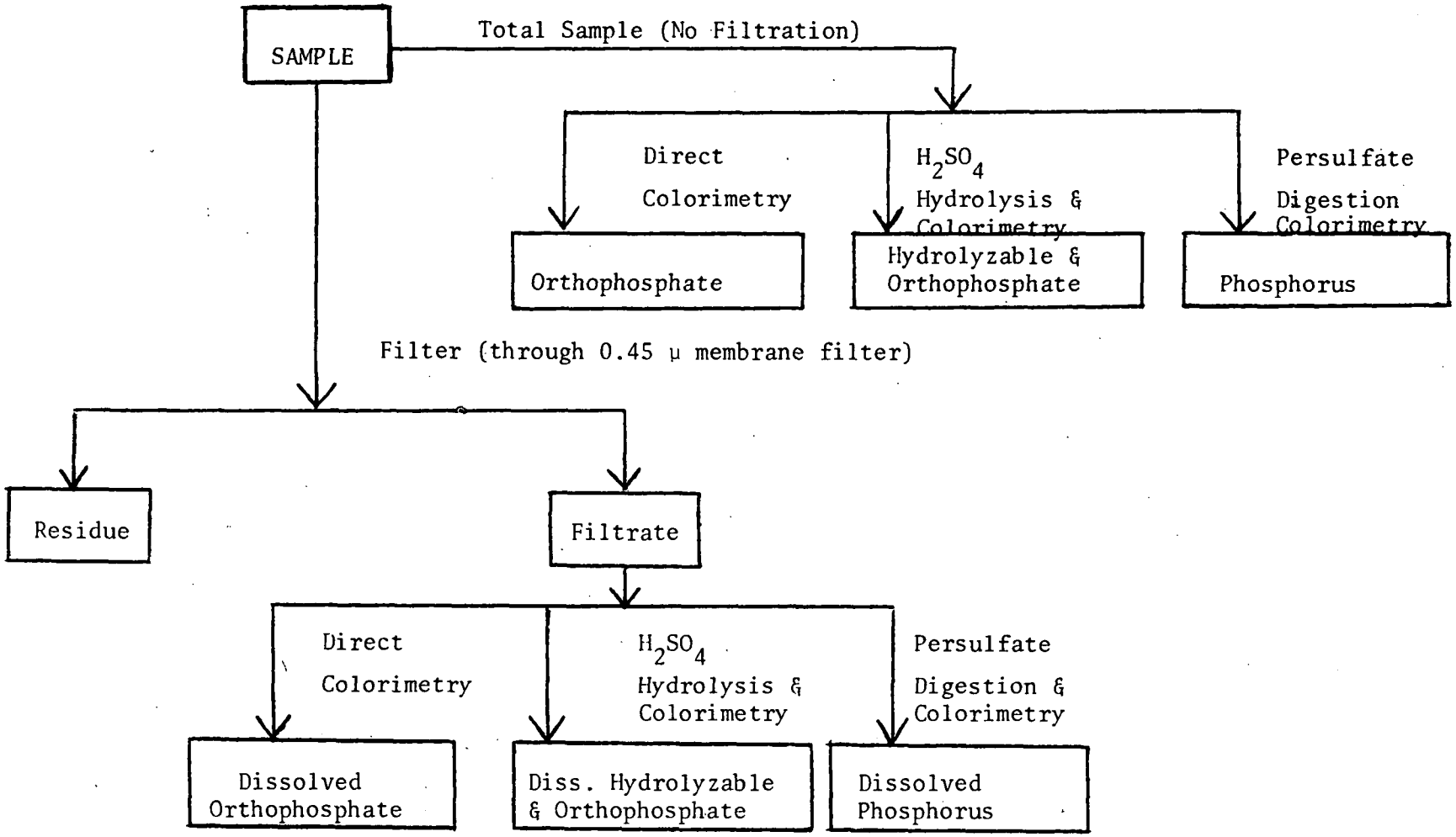



**FIGURE 1. ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS**

be preserved by the addition of 2 ml conc. $H_2SO_4$ or 40 mg $HgCl_2$ per liter and refrigeration at 4°C. Note $HgCl_2$ interference under (5.4).

4. Definitions and Storet Numbers

4.1 Total Phosphorus (P) – all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure. (00665)

4.1.1 Total Orthophosphate (P, ortho) – inorganic phosphorus [$(PO_4)^{-3}$] in the sample as measured by the direct colorimetric analysis procedure. (70507)

4.1.2 Total Hydrolyzable Phosphorus (P, hydro) – phosphorus in the sample as measured by the sulfuric acid hydrolysis procedure, and minus pre-determined orthophosphates. This hydrolyzable phosphorus includes polyphosphorus. [$(P_2O_7)^{-4}$, $(P_3O_{10})^{-5}$, etc.] + some organic phosphorus. (00669)

4.1.3 Total Organic Phosphorus (P, org) – phosphorus (inorganic + oxidizable organic) in the sample measured by the persulfate digestion procedure, and minus hydrolyzable phosphorus and orthophosphate. (00670)

4.2 Dissolved Phosphorus (P–D) – all of the phosphorus present in the filtrate of a sample filtered through a phosphorus-free filter of 0.45 micron pore size and measured by the persulfate digestion procedure. (00666)

4.2.1 Dissolved Orthophosphate (P–D, ortho) – as measured by the direct colorimetric analysis procedure. (00671)

4.2.2 Dissolved Hydrolyzable Phosphorus (P–D, hydro) – as measured by the sulfuric acid hydrolysis procedure and minus pre-determined dissolved orthophosphates. (00672)

4.2.3 Dissolved Organic Phosphorus (P–D, org) – as measured by the persulfate digestion procedure, and minus dissolved hydrolyzable phosphorus and orthophosphate. (00673)

4.3 The following forms, when sufficient amounts of phosphorus are present in the sample to warrant such consideration, may be calculated:

4.3.1 Insoluble Phosphorus (P-I) = (P) - (P-D). (00667)

4.3.1.1 Insoluble orthophosphate (P–I, ortho) = (P, ortho) – (P–D, ortho). (00674)

4.3.1.2 Insoluble Hydrolyzable Phosphorus (P–I, hydro) = (P, hydro) – (P–D, hydro). (00675)

4.3.1.3 Insoluble Organic Phosphorus (P–I, org) = (P, org) – (P–D, org). (00676)

4.4 All phosphorus forms shall be reported as P, mg/l, to the third place.

5. Interferences

5.1 It is reported that no interference is caused by copper, iron, or silicate at concentrations many times greater than their reported concentration in sea water. However, high iron concentrations can cause precipitation of and subsequent loss of phosphorus.

5.2 The salt error for samples ranging from 5 to 20% salt content was found to be less than 1%.

5.3 Arsenate is determined similarily to phosphorus and should be considered when present in concentrations higher than phosphorus. However, at concentrations found in sea water, it does not interfere.

5.4 Mercury chloride, used as a preservative, interferes when the chloride level of the sample is low (<50 mg Cl/l). This interference is overcome by spiking samples with a minimum of 50 mg/l of sodium chloride.

6. Apparatus

6.1 Photometer – A spectrophotometer or filter photometer suitable for measurements at 650 or 880 nm with a light path of 1 cm or longer.

6.2 Acid-washed glassware: All glassware used should be washed with hot 1:1 HCl and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove the last traces of phosphorus that might be adsorbed on the glassware. Preferably, this glassware should be used only for the determination of phosphorus and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with 1:1 HCl and reagents is only required occasionally. *Commercial detergents should never be used.*

7. Reagents

7.1 Sulfuric acid solution, 5N: Dilute 70 ml of conc. $H_2SO_4$ with distilled water to 500 ml.

7.2 Antimony potassium tartrate solution: Weigh 1.3715 g $K(SbO)C_4H_4O_6 \cdot 1/2\ H_2O$, dissolve in 400 ml distilled water in 500 ml volumetric flask, dilute to volume. Store at 4°C in a dark, glass-stoppered bottle.

7.3 Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 ml of distilled water. Store in a plastic bottle at 4°C.

7.4 Ascorbic acid, 0.1 M: Dissolve 1.76 g of ascorbic acid in 100 ml of distilled water. The solution is stable for about a week if stored at 4°C.

7.5 Combined reagent: Mix the above reagents in the following proportions for 100

ml of the mixed reagent: 50 ml of 5N $H_2SO_4$, (7.1), 5 ml of antimony potassium tartrate solution, (7.2), 15 ml of ammonium molybdate solution, (7.3), and 30 ml of ascorbic acid solution (7.4). *Mix after addition of each reagent.* All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the combined reagent, shake and let it stand for a few minutes until the turbidity disappears before proceeding. Since the stability of this solution is limited, it must be freshly prepared for each run.

7.6 Sulfuric acid solution, 11 N: Slowly add 310 ml conc. $H_2SO_4$ to 600 ml distilled water. When cool, dilute to 1 liter.

7.7 Ammonium persulfate.

7.8 Stock phosphorus solution: Dissolve in distilled water 0.2197 g of potassium dihydrogen phosphate, $KH_2PO_4$, which has been dried in an oven at 105°C. Dilute the solution to 1000 ml; 1.0 ml = 0.05 mg P.

7.9 Standard phosphorus solution: Dilute 10.0 ml of stock phosphorus solution (7.8) to 1000 ml with distilled water; 1.0 ml = 0.5 $\mu$g P.

7.9.1 Using standard solution, prepare the following standards in 50.0 ml volumetric flasks:

| ml of Standard Phosphorus Solution (7.9) | Conc., mg/l |
|---|---|
| 0 | 0.00 |
| 1.0 | 0.01 |
| 3.0 | 0.03 |
| 5.0 | 0.05 |
| 10.0 | 0.10 |
| 20.0 | 0.20 |
| 30.0 | 0.30 |
| 40.0 | 0.40 |
| 50.0 | 0.50 |

7.10 Sodium hydroxide, 1 N: Dissolve 40 g NaOH in 600 ml distilled water. Cool and dilute to 1 liter.

7.11 Phenolphthalein: Dissolve 0.5 g of phenolphthalein in a solution of 50 ml of ethyl or isopropyl alcohol and 50 ml of distilled water.

8. Procedure

8.1 Phosphorus

8.1.1 Add 1 ml of $H_2SO_4$ solution (7.6) to a 50 ml sample in a 125 ml Erlenmeyer flask.

8.1.2 Add 0.4 g of ammonium persulfate.

8.1.3 Boil gently on a pre-heated hot plate for approximately 30-40 minutes or until a final volume of about 10 ml is reached. Do not allow sample to go to dryness. Alternatively, heat for 30 minutes in an autoclave at 121°C (15-20 psi).

8.1.4 Adjust the pH of the sample to 7 ±0.2 with 1 N NaOH (7.10) using a pH meter. Cool and dilute the sample to 50 ml. If sample is not clear at this point, filter.

8.1.5 Determine phosphorus as outlined in (8.3.2 Orthophosphate).

8.2 Hydrolyzable Phosphorus

8.2.1 Add 1 ml of $H_2SO_4$ solution (7.6) to a 50 ml sample in a 125 ml Erlenmeyer flask.

8.2.2 Boil gently on a pre-heated hot plate for 30-40 minutes or until a final volume of about 10 ml is reached. Do not allow sample to go to dryness. Alternatively, heat for 30 minutes in an autoclave at 121°C (15-20 psi).

8.2.3 Adjust the pH of the sample to 7 ±0.2 with NaOH (7.10) using a pH meter. Cool and dilute the sample to 50 ml.

8.2.4 The sample is now ready for determination of phosphorus as outlined in (8.3.2 Orthophosphate).

8.3 Orthophosphate

8.3.1 Add 1 drop of phenolphthalein indicator (7.11) to the 50.0 ml sample. If a red color develops, add strong-acid solution drop-wise to just discharge the color.

NOTE: If just orthophosphate is being measured, i.e., and there has been no pretreatment of the sample and no subsequent neutralization as outlined above, the pH of the sample must be adjusted to 7±0.2 using a pH meter.

8.3.2 Add 8.0 ml of combined reagent (7.5) to sample and mix thoroughly. After a minimum of ten minutes, but no longer than thirty minutes, measure the color absorbance of each sample at 650 or 880 nm with a spectrophotometer, using the reagent blank as the reference solution.

9. Calculation

9.1 Prepare a standard curve by plotting the absorbance values of standards versus the corresponding phosphorus concentrations.

9.1.1 Process standards and blank exactly as the samples. Run at least a blank

and two standards with each series of samples. If the standards do not agree within ±2% of the true value, prepare a new calibration curve.

9.2 Obtain concentration value of sample directly from prepared standard curve. Report results as P, mg/l.

10. Precision and Accuracy

10.1 Thirty-three analysts in nineteen laboratories analyzed natural water samples containing exact increments of organic phosphate, with the following results:

| Increment as Total Phosphorus mg P/liter | Precision as Standard Deviation mg P/liter | Accuracy as Bias, % | Accuracy as Bias mg P/liter |
|---|---|---|---|
| 0.110 | 0.033 | + 3.09 | +0.003 |
| 0.132 | 0.051 | +11.99 | +0.016 |
| 0.772 | 0.130 | + 2.96 | +0.023 |
| 0.882 | 0.128 | − 0.92 | −0.008 |

(FWPCA Method Study 2, Nutrient Analyses)

10.2 Twenty-six analysts in sixteen laboratories analyzed natural water samples containing exact increments of *orthophosphate*, with the following results:

| Increment as Orthophosphate mg P/liter | Precision as Standard Deviation mg P/liter | Accuracy as Bias, % | Accuracy as Bias, mg P/liter |
|---|---|---|---|
| 0.029 | 0.010 | −4.95 | −0.001 |
| 0.038 | 0.008 | −6.00 | −0.002 |
| 0.335 | 0.018 | −2.75 | −0.009 |
| 0.383 | 0.023 | −1.76 | −0.007 |

(FWPCA Method Study 2, Nutrient Analyses)

## Bibliography


1. Murphy, J., and Riley, J., "A Modified Single Solution for the Determination of Phosphate in Natural Waters." Anal. Chim. Acta., *27*, 31 (1962).
2. Gales, M., Jr., Julian, E., and Kroner, R., "Method for Quantitative Determination of Total Phosphorus in Water." Jour. AWWA, *58*, No. 10, 1363 (1966).
3. ASTM Standards, Part 23, Water; Atmospheric Analysis, D515-72, p 388 (1973).

# PHOSPHORUS, ALL FORMS

(Automated Colorimetric Ascorbic Acid Reduction Method)

STORET NO. See Section 4

1. Scope and Application

   1.1 These methods cover the determination of specified forms of phosphorus in drinking, surface, and saline waters, domestic and industrial wastes. They may be applicable to sediment-type samples, sludges, algal blooms, etc., but sufficient data is not available at this time to warrant such usage when measurements for phosphorus content are required.

   1.2 The methods are based on reactions that are specific for the orthophosphate ion. Thus, depending on the prescribed pre-treatment of the sample, the various forms of phosphorus given in Figure 1 may be determined. These forms are, in turn, defined in Section 4.

      1.2.1 Except for in-depth and detailed studies, the most commonly measured forms are phosphorus and dissolved phosphorus, and orthophosphate and dissolved orthophosphate. Hydrolyzable phosphorus is normally found only in sewage-type samples and insoluble forms of phosphorus, as noted, are determined by calculation.

   1.3 The methods are usable in the 0.001 to 1.0 mg P/l range. Approximately 20-30 samples per hour can be analyzed.

2. Summary of Method

   2.1 Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

   2.2 Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic phosphorus compounds) may be converted to the orthophosphate form by manual sulfuric-acid-hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by manual persulfate digestion([2]). The developed color is measured automatically on the AutoAnalyzer.

3. Sample Handling and Preservation

   3.1 If benthic deposits are present in the area being sampled, great care should be taken not to include these deposits.

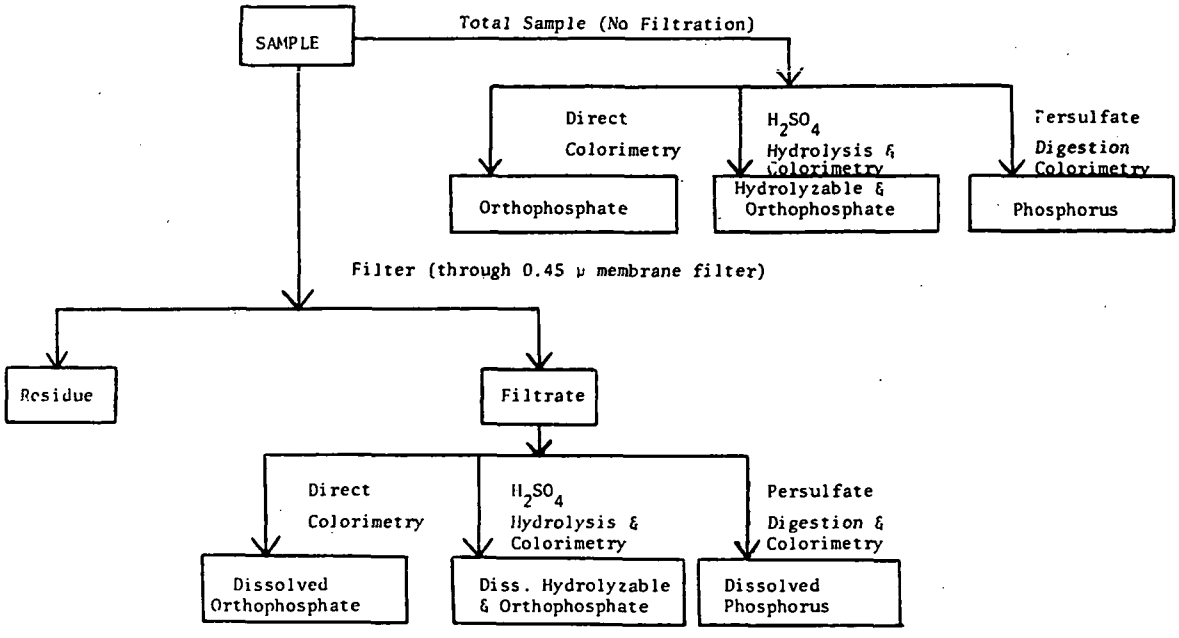



**FIGURE 1. ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS**

3.2 Sample containers may be of plastic material, such as cubitainers, or of Pyrex glass.

3.3 If the analysis cannot be performed the same day of collection, the sample should be preserved by the addition of 2 ml conc. $H_2SO_4$ or 40 mg $HgCl_2$ per liter and refrigeration at 4°C. Note $HgCl_2$ interference under (5.4).

4. Definitions and Storet Numbers

4.1 Total Phosphorus (P) – all of the phosphorus present in the sample regardless of form, as measured by the persulfate digestion procedure. (00665)

4.1.1 Total Orthophosphate (P-ortho)–inorganic phosphorus [$(PO_4)^{-3}$] in the sample as measured by the direct colorimetric analysis procedure. (70507)

4.1.2 Total Hydrolyzable Phosphorus (P-hydro)–phosphorus in the sample as measured by the sulfuric acid hydrolysis procedure, and minus predetermined orthophosphates. This hydrolyzable phosphorus includes polyphosphates [$(P_2O_7)^{-4}$, $(P_3O_{10})^{-5}$, etc.] + some organic phosphorus. (00669)

4.1.3 Total Organic Phosphorus (P-org)–phosphorus (inorganic + oxidizable organic) in the sample as measured by the persulfate digestion procedure, and minus hydrolyzable phosphorus and orthophosphate. (00670)

4.2 Dissolved Phosphorus (P–D) – all of the phosphorus present in the filtrate of a sample filtered through a phosphorus-free filter of 0.45 micron pore size and measured by the persulfate digestion procedure. (00666)

4.2.1 Dissolved Orthophosphate (P–D, ortho) – as measured by the direct colorimetric analysis procedure. (00671)

4.2.2 Dissolved Hydrolyzable Phosphorus (P–D, hydro) – as measured by the sulfuric acid hydrolysis procedure and minus predetermined dissolved orthophosphates. (00672)

4.2.3 Dissolved Organic Phosphorus (P–D, org) – as measured by the persulfate digestion procedure, and minus dissolved hydrolyzable phosphorus and orthophosphate. (00673)

4.3 The following forms, when sufficient amounts of phosphorus are present in the sample to warrant such consideration, may be calculated:

4.3.1 Insoluble Phosphorus (P–I) = (P) – (P–D). (00667)

4.3.1.1 Insoluble orthophosphate (P–I, ortho) = (P, ortho) – (P–D, ortho). (00674)

4.3.1.2 Insoluble Hydrolyzable Phosphorus (P–I, hydro) = (P, hydro) – (P–D, hydro). (00675)

4.3.1.3 Insoluble Organic Phosphorus (P–I, org) = (P, org) – (P–D, org). (00676)

4.4 All phosphorus forms shall be reported as P, mg/l, to the third place.

5. Interferences

5.1 It is reported that no interference is caused by copper, iron, or silicate at concentrations many times greater than their reported concentration in sea water. However, high iron concentrations can cause precipitation of and subsequent loss of phosphorus.

5.2 The salt error for samples ranging from 5 to 20% salt content was found to be less than 1%.

5.3 Arsenate is determined similarly to phosphorus and should be considered when present in concentrations higher than phosphorus. However, at concentrations found in sea water, it does not interfere.

5.4 Mercury chloride, used as a preservative, interferes. This interference is overcome in the AAI method by substituting a solution of sodium chloride (2.5 g/l) in place of the distilled water.

5.5 Sample turbidity must be removed by filtration prior to analysis for orthophosphate. Samples for total or total hydrolyzable phosphorus should be filtered only after digestion. Sample color that absorbs in the photometric range used for analysis will also interfere.

6. Apparatus

6.1 Technicon AutoAnalyzer consisting of:

6.1.1 Sampler.

6.1.2 Manifold (AAI) or Analytical Cartridge (AAII).

6.1.3 Proportioning pump.

6.1.4 Heating bath, 50°C.

6.1.5 Colorimeter equipped with 15 or 50 mm tubular flow cell.

6.1.6 650-660 or 880 nm filter.

6.1.7 Recorder.

6.1.8 Digital printer for AAII (optional).

6.2 Hot plate or autoclave.

6.3 Acid-washed glassware: All glassware used in the determination should be washed with hot 1:1 HCl and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove

the last traces of phosphorus that might be adsorbed on the glassware. Preferably, this glassware should be used only for the determination of phosphorus and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with 1:1 HCl and reagents is only required occasionally. *Commercial detergents should never be used.*

7. Reagents

7.1 Sulfuric acid solution, 5N: Slowly add 70 ml of conc. $H_2SO_4$ to approximately 400 ml of distilled water. Cool to room temperature and dilute to 500 ml with distilled water.

7.2 Antimony potassium tartrate solution: Weigh 0.3 g $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$, dissolve in 50 ml distilled water in 100 ml volumetric flask, dilute to volume. Store at 4°C in a dark, glass-stoppered bottle.

7.3 Ammonium molybdate solution: Dissolve 4 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 100 ml distilled water. Store in a plastic bottle at 4°C.

7.4 Ascorbic acid, 0.1M: Dissolve 1.8 g of ascorbic acid in 100 ml of distilled water. The solution is stable for about a week if prepared with water containing no more than trace amounts of heavy metals and stored at 4°C.

7.5 Combined reagent (AAI): Mix the above reagents in the following proportions for 100 ml of the mixed reagent: 50 ml of 5N $H_2SO_4$ (7.1), 5 ml of antimony potassium tartrate solution (7.2), 15 ml of ammonium molybdate solution (7.3), and 30 ml of ascorbic acid solution (7.4). *Mix after addition of each reagent.* All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the combined reagent, shake and let it stand for a few minutes until the turbidity disappears before processing. This volume is sufficient for 4 hours operation. Since the stability of this solution is limited, it must be freshly prepared for each run.

NOTE 1: A stable solution can be prepared by not including the ascorbic acid in the combined reagent. If this is done, the mixed reagent (molybdate, tartrate, and acid) is pumped through the distilled water line and the ascorbic acid solution (30 ml of 7.4 diluted to 100 ml with distilled water) through the original mixed reagent line.

7.6 Sulfuric acid solution, 11 N: Slowly add 310 ml conc. $H_2SO_4$ to 600 ml distilled water. When cool, dilute to 1 liter.

7.7 Ammonium persulfate.

7.8 Acid wash water: Add 40 ml of sulfuric acid solution (7.6) to 1 liter of distilled

water and dilute to 2 liters. (Not to be used when only orthophosphate is being determined).

7.9 Phenolphthalein indicator solution (5 g/l): Dissolve 0.5 g of phenolphthalein in a solution of 50 ml of ethyl or isopropyl alcohol and 50 ml of distilled water.

7.10 Stock phosphorus solution: Dissolve 0.4393 g of pre-dried (105°C for 1 hour) $KH_2PO_4$ in distilled water and dilute to 1000 ml. 1.0 ml = 0.1 mg P.

7.11 Standard phosphorus solution: Dilute 100.0 ml of stock solution (7.10) to 1000 ml with distilled water. 1.0 ml = 0.01 mg P.

7.12 Standard phosphorus solution: Dilute 100.0 ml of standard solution (7.11) to 1000 ml with distilled water. 1.0 ml = 0.001 mg P.

7.13 Prepare a series of standards by diluting suitable volumes of standard solutions (7.11) and (7.12) to 100.0 ml with distilled water. The following dilutions are suggested:

| ml of Standard Phosphorus Solution (7.12) | Conc., mg P/l |
|---|---|
| 0.0 | 0.00 |
| 2.0 | 0.02 |
| 5.0 | 0.05 |
| 10.0 | 0.10 |

| ml of Standard Phosphorus Solution (7.11) | |
|---|---|
| 2.0 | 0.20 |
| 5.0 | 0.50 |
| 8.0 | 0.80 |
| 10.0 | 1.00 |

8. Procedure

8.1 Phosphorus

8.1.1 Add 1 ml of sulfuric acid solution (7.6) to a 50 ml sample and standards in a 125 ml Erlenmeyer flask.

8.1.2 Add 0.4 g of ammonium persulfate.

8.1.3 Boil gently on a pre-heated hot plate for approximately 30-40 minutes or

until a final volume of about 10 ml is reached. Do not allow sample to go to dryness. Alternately, heat for 30 minutes in an autoclave at 121°C (15-20 psi).

8.1.4 Cool and dilute the sample to 50 ml. If sample is not clear at this point, filter.

8.1.5 Determine phosphorus as outlined in (8.3.2) with acid wash water (7.8) in wash tubes.

8.2 Hydrolyzable Phosphorus

8.2.1 Add 1 ml of sulfuric acid solution (7.6) to a 50 ml sample and standards in a 125 ml Erlenmeyer flask.

8.2.2 Boil gently on a pre-heated hot plate for 30-40 minutes or until a final volume of about 10 ml is reached. Do not allow sample to go to dryness. Alternatively, heat for 30 minutes in an autoclave at 121°C (15-20 psi).

8.2.3 Cool and dilute the sample to 50 ml. If sample is not clear at this point, filter.

8.2.4 Determine phosphorus as outlined in (8.3.2) with acid wash water (7.8) in wash tubes.

8.3 Orthophosphate

8.3.1 Add 1 drop of phenolphthalein indicator solution (7.9) to approximately 50 ml of sample. If a red color develops, add sulfuric acid solution (7.6) drop-wise to just discharge the color. Acid samples must be neutralized with 1 N sodium hydroxide (40 g NaOH/l).

8.3.2 Set up manifold as shown in Figure 2, AAI or Figure 3, AAII.

8.3.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through the sample line.

8.3.4 For the AAI system, sample at a rate of 20/hr, 1 minute sample, 2 minute wash. For the AAII system, use a 30/hr, 2:1 cam, and a common wash.

8.3.5 Place standards in Sampler in order of decreasing concentration. Complete filling of sampler tray with unknown samples.

8.3.6 Switch sample line from distilled water to Sampler and begin analysis.

9. Calculation

9.1 Prepare a standard curve by plotting peak heights of processed standards against known concentrations. Compute concentrations of samples by comparing sample peak heights with standard curve. Any sample whose computed value is less than

5% of its immediate predecessor must be rerun.

10. Precision and Accuracy (AAI system)

10.1 Six laboratories analyzed four natural water samples containing exact increments of orthophosphate, with the following results:

| Increment as Orthophosphate mg P/liter | Precision as Standard Deviation mg P/liter | Accuracy as Bias, % | Accuracy as Bias, mg P/liter |
|---|---|---|---|
| 0.04 | 0.019 | +16.7 | +0.007 |
| 0.04 | 0.014 | − 8.3 | −0.003 |
| 0.29 | 0.087 | −15.5 | −0.05 |
| 0.30 | 0.066 | −12.8 | −0.04 |

(FWPCA Method Study 4, Automated Methods – In preparation).

10.2 In a single laboratory, (MDQARL), using surface water samples at concentrations of 0.04, 0.19, 0.35, and 0.84 mg P/l, standard deviations were ±0.005, ±0.000, ±0.003, and ±0.000, respectively.

10.3 In a single laboratory, (MDQARL), using surface water samples at concentrations of 0.07 and 0.76 mg P/l, recoveries were 99% and 100%, respectively.

## Bibliography


1. Murphy, J. and Riley, J., "A Modified Single Solution for the Determination of Phosphate in Natural Waters." Anal. Chim. Acta., *27*, 31 (1962).
2. Gales, M., Jr., Julian, E., and Kroner, R., "Method for Quantitative Determination of Total Phosphorus in Water." Jour AWWA, *58*, No. 10, 1363 (1966).
3. Lobring, L. B. and Booth, R. L., "Evaluation of the AutoAnalyzer II; A Progress Report." Technicon International Symposium, June, 1972. New York, N.Y.
4. ASTM Standards, Part 23, Water; Atmospheric Analysis, 515-72, p 388 (1973).

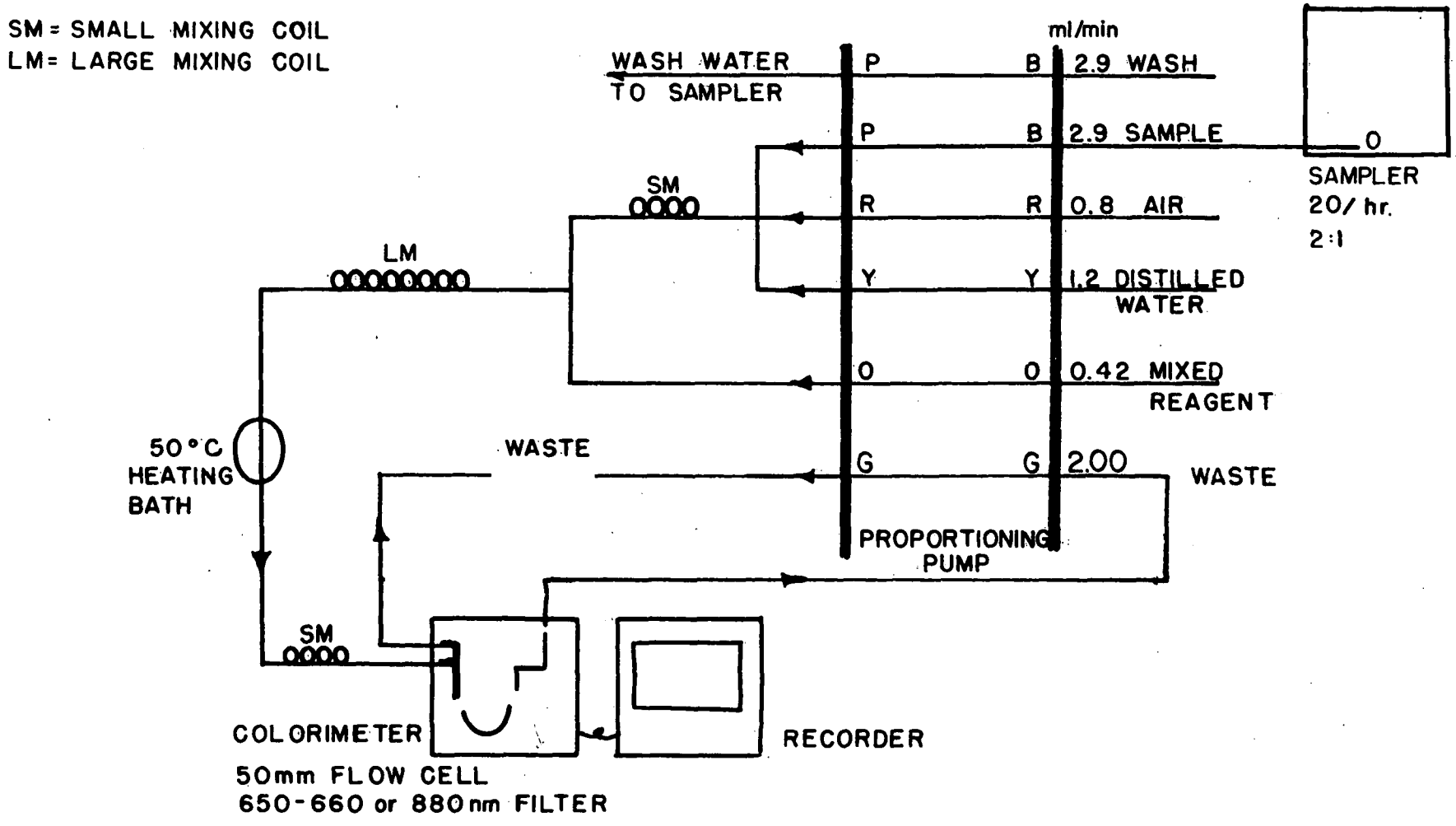



FIGURE 2 PHOSPHORUS MANIFOLD AA I

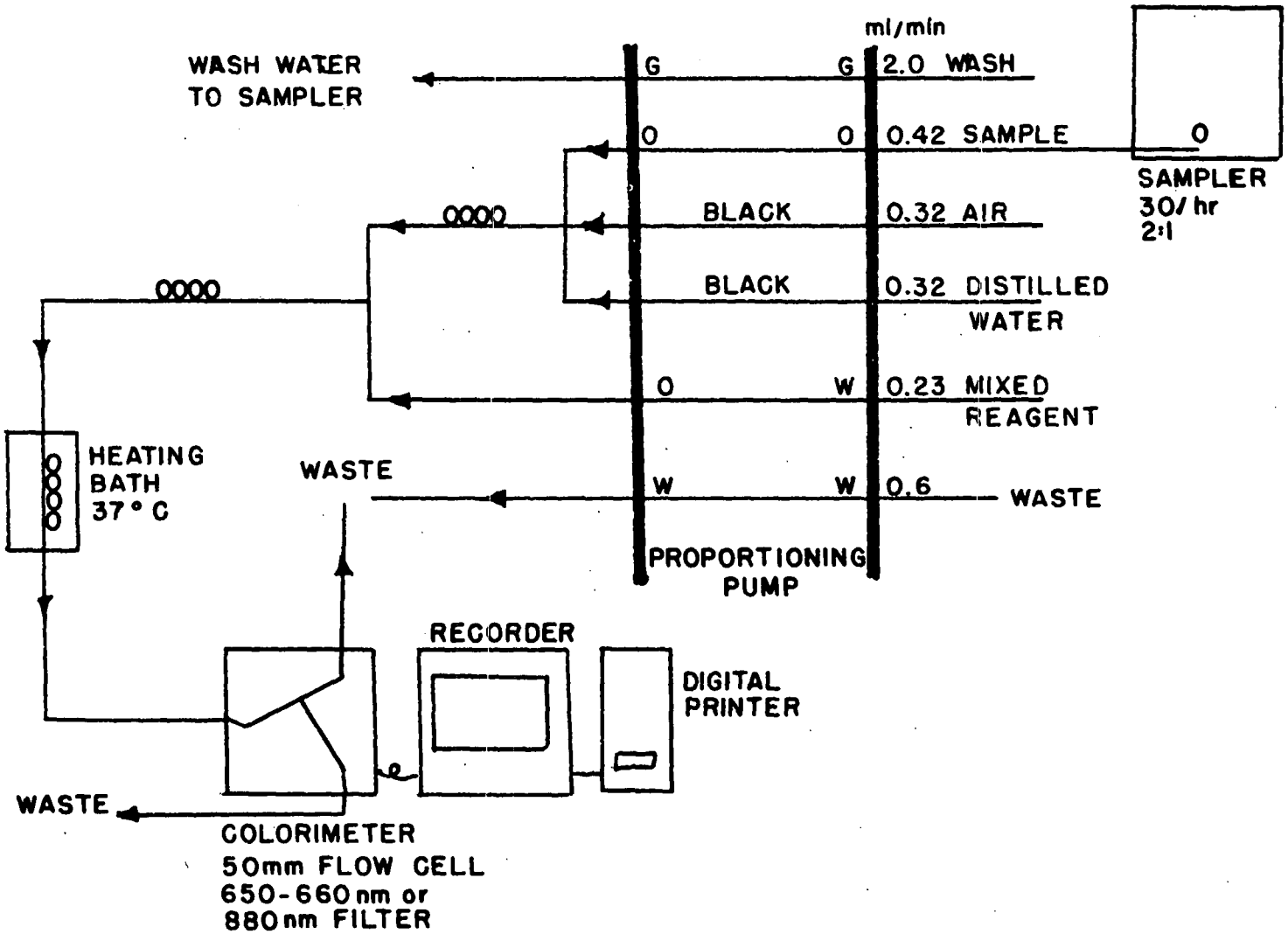



FIGURE 3 PHOSPHORUS MANIFOLD AA II

## RESIDUE, Total Filterable
## (Dried at 180°C)

STORET NO. 70300

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The practical range of the determination is 10 mg/l to 20,000 mg/l.
2. Summary of Method
   2.1 A well-mixed sample is filtered through a standard glass fiber filter. The filtrate is evaporated and dried to constant weight at 180°C.
   2.2 If Residue, Total Non-Filterable is being determined, the filtrate from that method may be used for Residue, Total Filterable.
3. Definitions
   3.1 Filterable solids are defined as those solids capable of passing through a standard glass fiber filter and dried to constant weight at 180°C.
4. Sample Handling and Preservation
   4.1 Preservation of the sample is not practical; analysis should begin as soon as possible.
5. Interferences
   5.1 Highly mineralized waters containing significant concentrations of calcium, magnesium, chloride and/or sulfate may be hygroscopic and will require prolonged drying, desiccation and rapid weighing.
   5.2 Samples containing high concentrations of bicarbonate will require careful and possibly prolonged drying at 180°C to insure that all the bicarbonate is converted to carbonate.
   5.3 Too much residue in the evaporating dish will crust over and entrap water that will not be driven off during drying. Total residue should be limited to about 200 mg.
6. Apparatus
   6.1 Glass fiber filter discs, 4.7 cm or 2.2 cm, without organic binder, Reeve Angel type 934-A, 984-H, Gelman type A, or equivalent.
   6.2 Filter holder, membrane filter funnel or Gooch crucible adapter.
   6.3 Suction flask, 500 ml.
   6.4 Gooch crucibles, 25 ml (if 2.2 cm filter is used).

6.5 Evaporating dishes, porcelain, 100 ml volume. (Vycor or platinum dishes may be substituted).

6.6 Steam bath.

6.7 Drying oven, 180°C±2°C.

6.8 Desiccator.

6.9 Analytical balance, 200 g capacity, capable of weighing to 0.1 mg.

7. Procedure

7.1 Preparation of glass fiber filter disc: Place the disc on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible. While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Discard washings.

7.2 Preparation of evaporating dishes: Heat the clean dish to 550±50°C for one hour in a muffle furnace. Cool in desiccator and store until needed. Weigh immediately before use.

7.3 Assemble the filtering apparatus and begin suction. Shake the sample vigorously and rapidly transfer 100 ml to the funnel by means of a 100 ml graduated cylinder. If total filterable residue is low, a larger volume may be filtered.

7.4 Filter the sample through the glass fiber filter and continue to apply vacuum for about 3 minutes after filtration is complete to remove as much water as possible.

7.5 Transfer 100 ml (or a larger volume) of the filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath.

7.6 Dry the evaporated sample for at least one hour at 180±2°C. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained or until weight loss is less than 0.5 mg.

8. Calculation

8.1 Calculate filterable residue as follows:

$$\text{Filt. residue, mg/l} = \frac{(A-B) \times 1000}{C}$$

where:

A = weight of dried residue + dish

B = weight of dish

C = volume of filtrate used

9. Precision and Accuracy

9.1 Precision data are not available at this time.

9.2 Accuracy data on actual sample cannot be obtained.

# RESIDUE, TOTAL NON-FILTERABLE

STORET NO. 00530

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The practical range of the determination is 10 mg/l to 20,000 mg/l.
2. Summary of Method
   2.1 A well-mixed sample is filtered through a standard glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C.
   2.2 The filtrate from this method may be used for Residue, Total Filterable.
3. Definitions
   3.1 Non-filterable solids are defined as those solids which are retained by a standard glass fiber filter and dried to constant weight at 103-105°C.
4. Sample Handling and Preservation
   4.1 Non-homogeneous particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample.
   4.2 Preservation of the sample is not practical; analysis should begin as soon as possible.
5. Interferences
   5.1 Too much residue on the filter will entrap water and may require prolonged drying.
6. Apparatus
   6.1 Glass fiber filter discs, 4.7 cm or 2.2 cm, without organic binder, Reeve Angel type 934-A or 984-H, Gelman type A, or equivalent.
   6.2 Filter holder, membrane filter funnel or Gooch crucible adapter.
   6.3 Suction flask, 500 ml.
   6.4 Gooch crucibles, 25 ml (if 2.2 cm filter is used).
   6.5 Drying oven, 103-105°C.
   6.6 Desiccator.
   6.7 Analytical balance, 200 g capacity, capable of weighing to 0.1 mg.
7. Procedure
   7.1 Preparation of glass fiber filter disc: Place the disc on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible. While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water.

Remove all traces of water by continuing to apply vacuum after water has passed through. Remove filter from membrane filter apparatus or both crucible and filter if Gooch crucible is used, and dry in an oven at 103-105°C for one hour. Remove to desiccator and store until needed. Weigh immediately before use.

7.2 Assemble the filtering apparatus and begin suction. Shake the sample vigorously and rapidly transfer 100 ml to the funnel by means of a 100 ml volumetric cylinder. If suspended matter is low, a larger volume may be filtered.

7.3 Carefully remove the filter from the membrane filter funnel assembly. Alternatively, remove crucible and filter from crucible adapter. Dry at least one hour at 103-105°C. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained or until weight loss is less than 0.5 mg.

8. Calculations

8.1 Calculate non-filterable residue as follows:

$$\text{Non-filt. residue, mg/l} = \frac{(A-B) \times 1000}{C}$$

where:

A = weight of filter + residue

B = weight of filter

C = ml of sample filtered

9. Precision and Accuracy

9.1 Precision data are not available at this time.

9.2 Accuracy data on actual samples cannot be obtained.

RESIDUE, Total

STORET NO. 00500

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The practical range of the determination is from 10 mg/l to 20,000 mg/l.
2. Summary of Method
   2.1 A well mixed aliquot of the test sample is quantitatively transferred to a pre-weighed evaporating dish and evaporated to dryness at 103-105°C.
3. Definitions
   3.1 Total Residue is defined as the sum of the homogenous suspended and dissolved materials in a sample.
4. Sample Handling and Preservation
   4.1 Samples should be analyzed as soon as practicable.
5. Interferences
   5.1 Large, floating particles or submerged agglomerates (non-homogenous materials) should be excluded from the test sample.
   5.2 Floating oil and grease, if present, should be included in the sample and dispersed by a blender device before aliquoting.
6. Apparatus
   6.1 Evaporating dishes, porcelain, 90 mm, 100 ml capacity. (Vycor or platinum dishes may be substituted and smaller size dishes may be used if required.)
7. Procedure
   7.1 Heat the clean evaporating dish to 550±50°C for 1 hour in a muffle furnace. Cool, desiccate, weigh and store in desiccator until ready for use.
   7.2 Transfer a measured aliquot of sample to the pre-weighed dish and evaporate to dryness on a steam bath or in a drying oven.
      7.2.1 Choose an aliquot of sample sufficient to contain a residue of at least 25 mg. To obtain a weighable residue, successive aliquots of sample may be added to the same dish.
      7.2.2 If evaporation is performed in a drying oven, the temperature should be lowered to approximately 98°C to prevent boiling and splattering of the sample.
   7.3 Dry the evaporated sample for at least 1 hour at 103-105°C. Cool in a desiccator

and weigh. Repeat the cycle of drying at 103-105°C, cooling, desiccating and weighing until a constant weight is obtained or until loss of weight is less than 4% of the previous weight, or 0.5 mg, whichever is less.

8. Calculation

8.1 Calculate total residue as follows:

$$\text{Total residue, mg/l} = \frac{(A-B) \times 1000}{C}$$

where:

A = weight of sample + dish

B = weight of dish

C = volume of sample

9. Precision and Accuracy

9.1 Precision and accuracy data are not available at this time.

# RESIDUE, Volatile

STORET NO. Total 00505
Nonfilterable 00535
Filterable 00520

1. Scope and Application
   1.1 This method determines the weight of solid material combustible at 550°C.
   1.2 The test is useful in obtaining a rough approximation of the amount of organic matter present in the solid fraction of sewage, activated sludge, industrial wastes, or bottom sediments.
2. Summary of Method
   2.1 The residue obtained from the determination of total filterable or non-filterable residue is ignited at 550°C in a muffle furnace. The loss of weight on ignition is reported as mg/l volatile residue.
3. Comments
   3.1 The test is subject to many errors due to loss of water of crystallization, loss of volatile organic matter prior to combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.
   3.2 The results should not be considered an accurate measure of organic carbon in the sample, but may be useful in the control of plant operations.
   3.3 The principal source of error in the determination is failure to obtain a representative sample.
4. Sample Handling and Preservation
   4.1 Preservation of the sample is not practical; analysis should begin as soon as possible.
5. Precision and Accuracy
   5.1 A collaborative study involving three laboratories examining four samples by means of ten replicates showed a standard deviation of ±11 mg/l at 170 mg/l volatile residue concentration.
6. Reference
   6.1 The procedure to be used for this determination is found in:
   Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 536, Method 224B (1971).

# SETTLEABLE MATTER

STORET NO. 50086

1. Scope and Application
   1.1 This method is applicable to surface and saline waters, domestic and industrial wastes.
   1.2 The practical lower limit of the determination is about 1 ml/l/hr.
2. Summary of Method
   2.1 Settleable matter is measured volumetrically with an Imhoff cone.
3. Comments
   3.1 For some samples, a separation of settleable and floating materials will occur; in such cases the floating materials are not measured.
   3.2 Many treatment plants, especially plants equipped to perform gravimetric measurements, determine residue non-filterable (suspended solids), in preference to settleable matter, to insure that floating matter is included in the analysis.
4. Precision and Accuracy
   4.1 Data on this determination is not available at this time.
5. References
   5.1 The procedure to be used for this determination is found in:
   Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 539, Method 224F, Procedure a (1971).

SILICA, Dissolved

STORET NO. 00955

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The working range of the method is approximately 2 to 25 mg silica/l. The upper range can be extended by taking suitable aliquots; the lower range can be extended by the addition of amino-naphthol-sulfonic acid solution, as described in the ASTM reference.
2. Summary of Method
   2.1 A well-mixed sample is filtered through a 0.45 $\mu$ membrane filter. The filtrate, upon the addition of molybdate ion in acidic solution, forms a greenish-yellow color complex proportional to the dissolved silica in the sample. The color complex is then measured spectrophotometrically.
3. Comments
   3.1 Excessive color and/or turbidity interfere. Correct by running blanks prepared without addition of the ammonium molybdate solution.
4. Precision and Accuracy
   4.1 Photometric evaluations by the amino-naphthol-sulfonic acid procedure have an estimated precision of ±0.10 mg/l in the range from 0 to 2 mg/l (ASTM).
   4.2 Photometric evaluations of the silico-molybdate color in the range from 2 to 50 mg/l have an estimated precision of approximately 4% of the quantity of silica measured (ASTM).
5. Reference
   5.1 The procedure to be used for this determination is found in:
   Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 303, Method 151B (1971).
   ASTM Standards, Part 23, Water; Atmospheric Analysis, p 401, Method D859-68 (1973).

## SPECIFIC CONDUCTANCE
(μmhos at 25°C)

STORET NO. 00095

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
2. Summary of Method
   2.1 The specific conductance of a sample is measured by use of a self-contained conductivity meter, Wheatstone bridge-type, or equivalent.
   2.2 Samples are preferably analyzed at 25°C. If not, temperature corrections are made and results reported at 25°C.
3. Comments
   3.1 Instrument must be standardized with KCl solution before daily use.
   3.2 Conductivity cell must be kept clean.
   3.3 Field measurements with comparable instruments are reliable.
4. Precision and Accuracy
   4.1 Forty-one analysts in 17 laboratories analyzed six synthetic water samples containing increments of inorganic salts, with the following results:

| Increment as Specific Conductance μmhos/cm | Precision as Standard Deviation μmhos/cm | Accuracy as Bias, % | Accuracy as Bias, μmhos/cm |
|---|---|---|---|
| 100 | 7.55 | −2.02 | −2.0 |
| 106 | 8.14 | −0.76 | −0.8 |
| 808 | 66.1 | −3.63 | −29.3 |
| 848 | 79.6 | −4.54 | −38.5 |
| 1640 | 106 | −5.36 | −87.9 |
| 1710 | 119 | −5.08 | −86.9 |

(FWPCA Method Study 1, Mineral and Physical Analyses.)

   4.2 In a single laboratory (MDQARL), using surface water samples with an average conductivity of 536 μmhos/cm at 25°C, the standard deviation was ±6.

5. References

5.1 The procedure to be used for this determination is found in:

Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 323, Method 154 (1971).

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 128, Method D1125-64 (1973).

# SULFATE

(Turbidimetric)

STORET NO. 00945

1. Scope and Application
   1.1 This method is applicable to drinking and surface waters, domestic and industrial wastes.
   1.2 The method is suitable for all concentration ranges of sulfate; however, in order to obtain reliable readings, use a sample aliquot containing not more than 40 mg $SO_4$/l.
2. Summary of Method
   2.1 Sulfate ion is converted to a barium sulfate suspension under controlled conditions. The resulting turbidity is determined by a colorimeter or spectrophotometer and compared to a curve prepared from standard sulfate solutions.
   2.2 Suspended matter and color interfere. Correct by running blanks from which the barium chloride has been omitted.
3. Comments
   3.1 Proprietary reagents, such as Hach Sulfaver or equivalent, are acceptable.
4. Precision and Accuracy
   4.1 Thirty-four analysts in 16 laboratories analyzed six synthetic water samples containing exact increments of inorganic sulfate with the following results:

| Increment as Sulfate mg/liter | Precision as Standard Deviation mg/liter | Accuracy as Bias, % | Accuracy as Bias, mg/liter |
|---|---|---|---|
| 8.6 | 2.30 | −3.72 | −0.3 |
| 9.2 | 1.78 | −8.26 | −0.8 |
| 110 | 7.86 | −3.01 | −3.3 |
| 122 | 7.50 | −3.37 | −4.1 |
| 188 | 9.58 | +0.04 | +0.1 |
| 199 | 11.8 | −1.70 | −3.4 |

(FWPCA Method Study 1, Mineral and Physical Analyses).

5. References

5.1 The procedure to be used for this determination is found in:

Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 334, Method 156C (1971).

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 425, Method B, D516-68 (1973).

# SULFATE

## (Automated Chloranilate Method)

STORET NO. 00945

1. Scope and Application
   1.1 This automated method is applicable to drinking and surface waters, domestic and industrial wastes, in the range of 10 to 400 mg $SO_4$/l. Approximately 15 samples per hour can be analyzed.
2. Summary of Method
   2.1 When solid barium chloranilate is added to a solution containing sulfate, barium sulfate is precipitated, releasing the highly colored acid chloranilate ion. The color intensity in the resulting chloranilic acid is proportional to the amount of sulfate present.
3. Sample Handling and Preservation
   3.1 No special requirements.
4. Interferences
   4.1 Cations, such as calcium, aluminum, and iron, interfere by precipitating the chloranilate. These ions are removed automatically by passage through an ion exchange column.
5. Apparatus
   5.1 Technicon AutoAnalyzer consisting of:
      5.1.1 Sampler I.
      5.1.2 Continuous filter.
      5.1.3 Manifold.
      5.1.4 Proportioning pump.
      5.1.5 Colorimeter equipped with 15 mm tubular flow cell and 520 nm filters.
      5.1.6 Recorder.
      5.1.7 Heating bath, 45°C.
   5.2 Magnetic stirrer.
6. Reagents
   6.1 Barium chloranilate: Add 9 g of barium chloranilate ($BaC_6Cl_2O_4$) to 333 ml of ethyl alcohol and dilute to 1 liter with distilled water.
   6.2 Acetate buffer, pH 4.63: Dissolve 13.6 g of sodium acetate in distilled water. Add 6.4 ml of acetic acid and dilute to 1 liter with distilled water. Make fresh weekly.

6.3 NaOH-EDTA solution: Dissolve 65 g of NaOH and 6 g of EDTA in distilled water and dilute to 1 liter.

NOTE 1: This solution is also used to clean out manifold system at end of sampling run.

6.4 Ion exchange resin: Dowex-50 W-X8, ionic form – H+.

NOTE 2: Column is prepared by sucking a slurry of the resin into 12 inches of 3/16-inch OD sleeving. This may be conveniently done by using a pipette and a loose-fitting glass wool plug in the sleeve. The column, upon exhaustion, turns red.

6.5 Stock solution: Dissolve 1.4790 g of oven-dried (105°C) $Na_2SO_4$ in distilled water and dilute to 1 liter in a volumetric flask. 1.0 ml = 1.0 mg.

6.5.1 Prepare a series of standards by diluting suitable volumes of stock solution to 100.0 ml with distilled water. The following dilutions are suggested:

| ml of Stock Solution | Conc., mg/l |
|---|---|
| 1.0 | 10 |
| 2.0 | 20 |
| 4.0 | 40 |
| 6.0 | 60 |
| 8.0 | 80 |
| 10.0 | 100 |
| 15.0 | 150 |
| 20.0 | 200 |
| 30.0 | 300 |
| 40.0 | 400 |

7. Procedure

7.1 Set up manifold as shown in Figure 1. (Note that any precipitated $BaSO_4$ and the unused barium chloranilate are removed by filtration. If any $BaSO_4$ should come through the filter, it is complexed by the NaOH–EDTA reagent).

7.2 Allow both colorimeter and recorder to warm up for 30 minutes. Run a baseline with all reagents, feeding distilled water through the sample line. Adjust dark current and operative opening on colorimeter to obtain suitable baseline.

7.3 Place distilled water wash tubes in alternate openings in sampler and set sample timing at 2.0 minutes.

7.4 Place working standards in sampler in order of decreasing concentration. Complete filling of sampler tray with unknown samples.

7.5 Switch sample line from distilled water to sampler and begin analysis.

8. Calculation

8.1 Prepare standard curve by plotting peak heights of processed standards against known concentrations. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (MDQARL), using surface water samples at concentrations of 39, 111, 188 and 294 mg $SO_4$ /l, the standard deviations were ±0.6, ±1.0, ±2.2 and ±0.8, respectively.

9.2 In a single laboratory (MDQARL) using surface water samples at concentrations of 82 and 295 mg $SO_4$ /l, recoveries were 99% and 102%, respectively.

## Bibliography


1. Barney, J. E., and Bertolocini, R. J., Anal. Chem., *29*, 283 (1957).
2. Gales, M. E., Jr., Kaylor, W. H. and Longbottom, J. E., "Determination of Sulphate by Automatic Colorimetric Analysis". Analyst, *93*, 97 (1968).

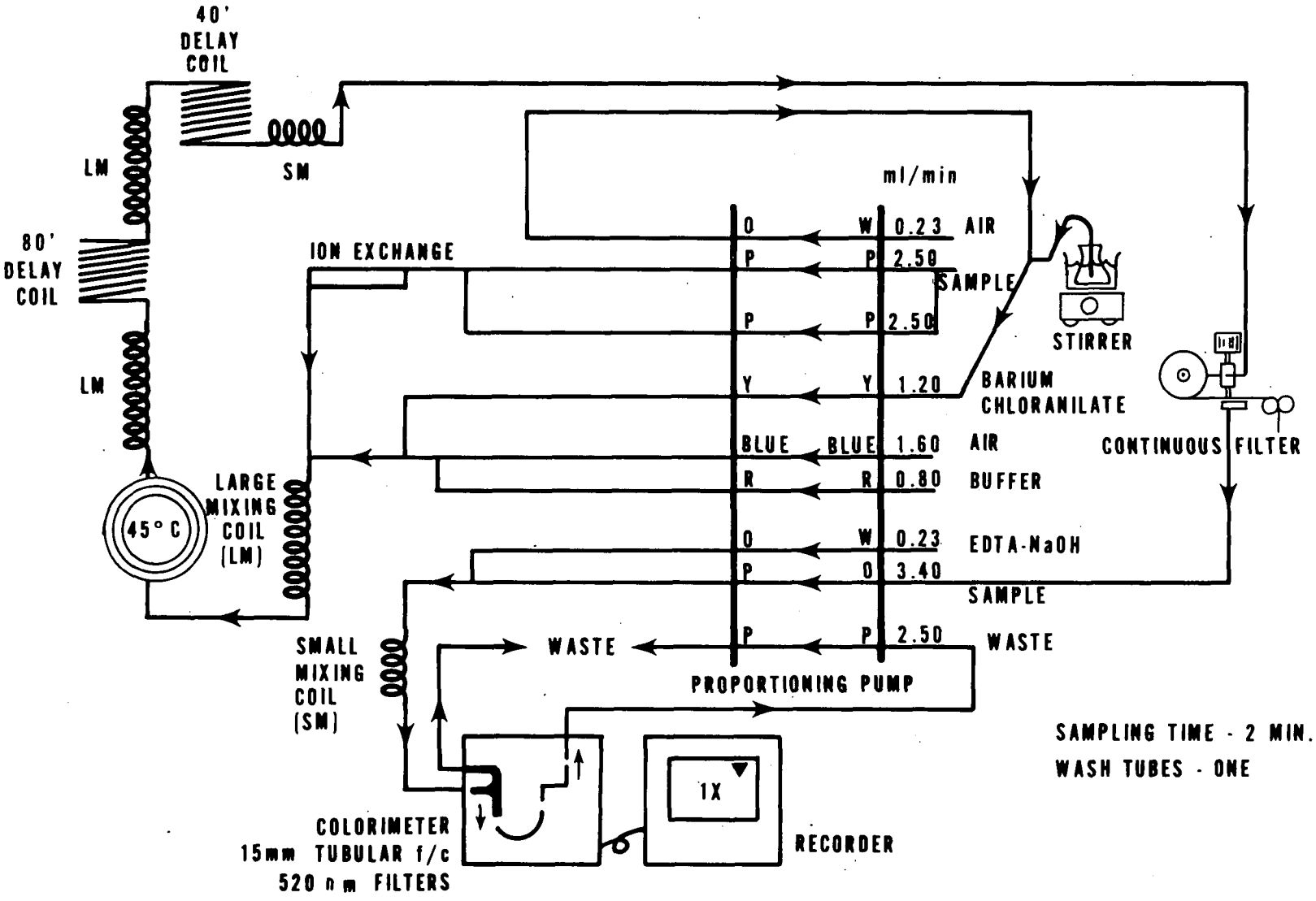



FIGURE 1 - SULFATE MANIFOLD AA-I

# SULFATE

(Gravimetric)

STORET NO. 00945

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 This method is the most accurate method for sulfate concentrations above 10 mg/l. Therefore, it should be used whenever results of the greatest accuracy are required.
2. Summary of Method
   2.1 Sulfate is precipitated as barium sulfate in a hydrochloric acid medium by the addition of barium chloride. After a period of digestion, the precipitate is filtered, washed with hot water until free of chloride, ignited, and weighed as $BaSO_4$.
3. Comments
   3.1 High results may be obtained for samples that contain suspended matter, nitrate, sulfite and silica.
   3.2 Alkali metal sulfates frequently yield low results. This is especially true of alkali hydrogen sulfates. Occlusion of alkali sulfate with barium sulfate causes the substitution of an element of lower atomic weight than barium in the precipitate. Hydrogen sulfate of alkali metal acts similarly and decomposes when heated. Heavy metals such as chromium and iron, cause low results by interfering with complete precipitation and by formation of heavy metal sulfates.
4. Precision and Accuracy
   4.1 A synthetic unknown sample containing 259 mg/l sulfate, 108 mg/l Ca, 82 mg/l Mg, 3.1 mg/l K, 19.9 mg/l Na, 241 mg/l chloride, 250 µg/l nitrite N, 1.1 mg/l nitrate N and 42.5 mg/l total alkalinity (contributed by $NaHCO_3$), was analyzed by the gravimetric method, with a relative standard deviation of 4.7% and a relative error of 1.9% in 32 laboratories.
5. Reference
   5.1 The procedure to be used for this determination is found in:

   Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 331, Method 156 A (1971).

   ASTM Standards, Part 23, Water; Atmospheric Analysis, p 425, Method A, D516-68 (1973).

# SULFIDE

(Titrimetric Iodine Method)

STORET NO. Total 00745
Dissolved 00746

1. Scope and Application
   1.1 This method is applicable to the measurement of total and dissolved sulfides in drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 Acid insoluble sulfides are not measured by the use of this test. (Copper sulfide is the only common sulfide in this class).
   1.3 This method is suitable for the measurement of sulfide in concentrations above 1 mg/l.
2. Summary of Method
   2.1 Sulfides are stripped from the acidified sample with an inert gas and collected in a zinc acetate solution. Excess iodine added to the zinc sulfide suspension reacts with the sulfide under acidic conditions. Thiosulfate is used to measure unreacted iodine to indicate the quantity of iodine consumed by sulfide.
3. Comments
   3.1 Reduced sulfur compounds, such as sulfite, thiosulfate and hydrosulfite, which decompose in acid may yield erratic results. Also, volatile iodine-consuming substances will give high results.
   3.2 Samples must be taken with a minimum of aeration. Sulfide may be volatilized by aeration and any oxygen inadvertently added to the sample may convert the sulfide to an unmeasurable form.
   3.3 If the sample is not preserved with zinc acetate, the analysis must be started immediately. Similarly, the measurement of dissolved sulfides must also be commenced immediately.
4. Precision and Accuracy
   4.1 Precision and accuracy for this method have not been determined.
5. References
   5.1 The procedure to be used for this determination is found in:
   Standard Methods for the Examination of Water and Wastewaters, 13th Edition, p 551-555, Method No. 228A (1971).

# SULFITE

STORET NO. 00740

1. Scope and Application
   1.1 This method is applicable to drinking and surface waters, sewage and industrial wastes.
   1.2 The minimum detectable limit is 2-3 mg/l $SO_3$.
2. Summary of Method
   2.1 An acidified sample containing an indicator is titrated with a standard potassium iodide-iodate titrant to a faint permanent blue end point.
3. Comments
   3.1 The temperature of the sample must be below 50°C.
   3.2 Care must be taken to allow as little contact with air as possible. For example, do not filter the sample and keep the buret tip below the surface of the sample.
   3.3 Other oxidizable substances, such as organic compounds, ferrous iron and sulfide are positive interferences. Nitrite gives a negative interference by oxidizing sulfite when the sample is acidified; this is corrected by either using a proprietary indicator which eliminates nitrite or by adding sulfamic acid. Copper and possibly other heavy metals catalyze the oxidation of sulfite; EDTA is used to complex metals.
   3.4 A blank must be run.
4. Precision and Accuracy
   4.1 Precision and accuracy data are not available at this time.
5. References
   5.1 The procedure to be used for this determination is found in:
   Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 337-338, Method 158 (1971).

   ASTM Standards, Part 23, Water; Atmospheric Analysis, p 436 Method D-1339 Method C (1973).

# TEMPERATURE

STORET NO. 00010

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
2. Summary of Method
   2.1 Temperature measurements may be made with any good grade of mercury-filled or dial type centigrade thermometer, or a thermistor.
3. Comments
   3.1 Measurement device should be checked against a precision thermometer certified by the National Bureau of Standards.
4. Precision and Accuracy
   4.1 Precision and accuracy for this method have not been determined.
5. Reference
   5.1 The procedure to be used for this determination is found in:
   Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 348, Method 162 (1971).

# THRESHOLD ODOR

(Consistent Series Method)

STORET NO. 60°C: 00086
Room Temp: 00085

1. Scope and Application
   1.1 This method is applicable to the determination of threshold odor of drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 Highly odorous samples are reduced in concentration proportionately before being tested. Thus, the method is applicable to samples ranging from nearly odorless natural waters to industrial wastes with threshold odor numbers in the thousands.
2. Summary of Method([1])
   2.1 The sample of water is diluted with odor-free water until a dilution that is of the least definitely perceptible odor to each tester is found. The resulting ratio by which the sample has been diluted is called the "threshold odor number" (T.O.).
   2.2 People vary widely as to odor sensitivity, and even the same person will not be consistent in the concentrations he can detect from day to day. Therefore, panels of not less than five persons, and preferably 10 or more, are recommended to overcome the variability of using one observer.([2])
      2.2.1 As an absolute minimum, two persons are necessary: One to make the sample dilutions and one to determine the threshold odor.
3. Sample Handling and Preservation
   3.1 Water samples must be collected in glass bottles with glass or Teflon-lined closures.
      3.1.1 Plastic containers are not reliable for odor samples and must not be used.
   3.2 Odor tests should be completed as soon as possible after collection of the sample. If storage is necessary, collect at least 1000 ml of sample in a bottle filled to the top. Refrigerate, making sure no extraneous odors can be drawn into the sample as the water cools.
4. Interferences
   4.1 Most tap waters and some waste waters are chlorinated. It is often desirable to determine the odor of the chlorinated sample as well as of the same sample after removal of chlorine. Dechlorination is achieved using sodium thiosulfate in exact stoichiometric quantity.

4.1.1 It is important to check a blank to which a similar amount of dechlorinating agent has been added to determine if any odor has been imparted. Such odor usually disappears upon standing if excess reagent has not been added.

5. Apparatus

5.1 Odor-free glassware: Glassware must be freshly cleaned shortly before use, with non-odorous soap and acid cleaning solution followed by rinsing with odor-free water (6.1). Glassware used in odor testing should be reserved for that purpose only. Rubber, cork, and plastic stoppers must not be used.

5.2 Constant temperature bath: A water bath or electric hotplate capable of maintaining a temperature control of ±1°C for performing the odor test at 60°C. The temperature bath must not contribute any odor to the odor flasks.

5.3 Odor flasks: Glass stoppered 500 ml (ℸ 32) Erlenmeyer flasks, or wide-mouthed 500 ml Erlenmeyer flasks equipped with Petri dishes as cover plates.

NOTE: Narrow-mouth vessels are not suitable for running odor tests. Potential positive bias due to color and/or turbidity of water sample under observation can be eliminated by wrapping odor flasks in aluminum foil, painting flasks with non-odorous paint, or by using red actinic Erlenmeyer flasks.

5.4 Sample bottles: Glass bottles with glass or Teflon-lined closures.

5.5 Pipets, measuring: 10.0 and 1.0 ml graduated in tenths.

5.6 Graduated cylinders: 250, 200, 100, 50, and 25 ml.

5.7 Thermometer: 0-110°C (±1°C), chemical or metal stem dial type.

5.8 Odor-free water generator: See Figure 1.

6. Reagents

6.1 Odor-free water: Odor-free dilution water must be prepared as needed by filtration through a bed of activated carbon. Most tap waters are suitable for preparation of odor-free waters, except that it is necessary to check the filtered water for chlorine residual, unusual salt concentrations, or unusually high or low pH. All these may affect some odorous samples.

Where supplies are adequate, distilled water avoids these problems as a source of odor-free water. A convenient odor-free water generator may be made as shown in Figure 1. Pass tap or distilled water through the odor-free water generator at a rate of 0.1 liter/minute. When the generator is first started, it should be flushed to remove carbon fines before the odor-free water is used.

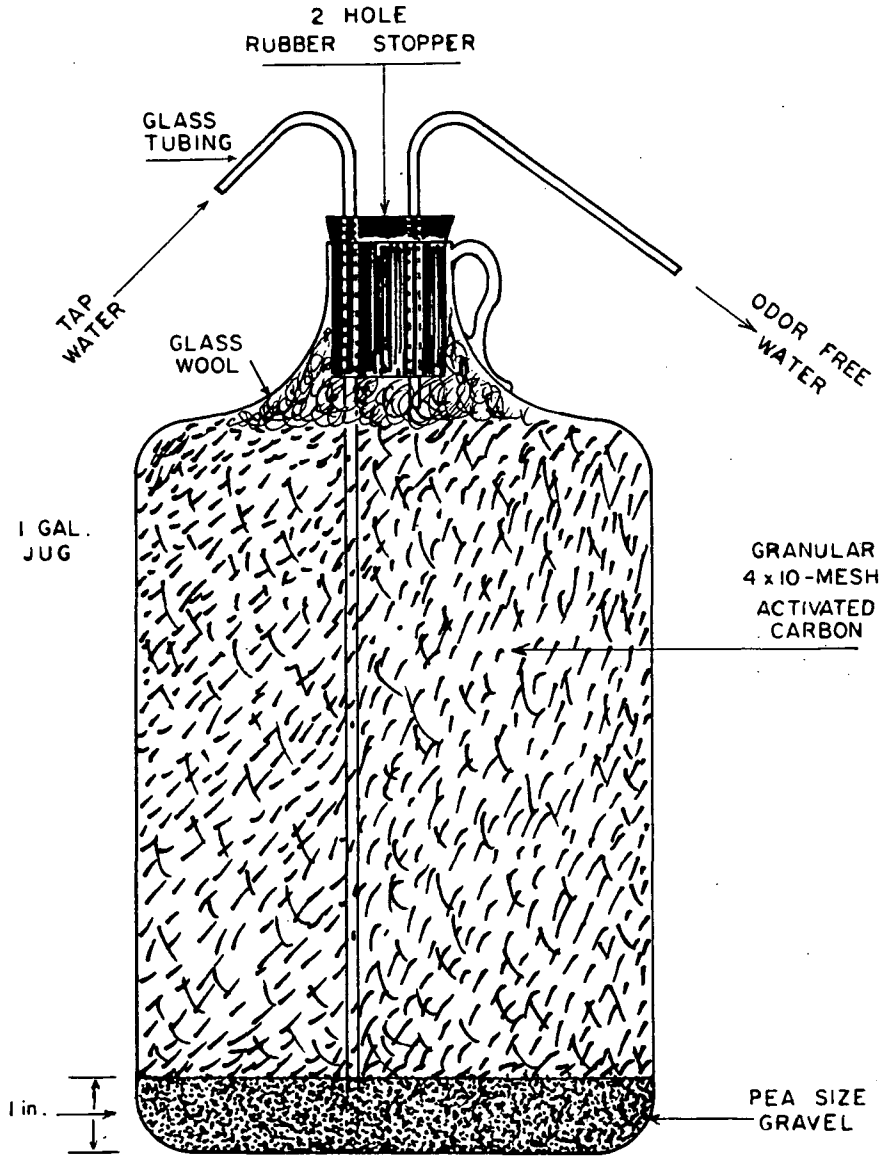
6.1.1 The quality of water obtained from the odor-free water generator should be checked daily at the temperature tests are to be conducted (room

temperature and/or 60°C). The life of the carbon will vary with the condition and amount of water filtered. Subtle odors of biological origin are often found if moist carbon filters are permitted to stand idle between test periods. Detection of odor in the water coming through the carbon indicates a change of carbon is needed.

## 7. Procedure

7.1 *Precautions*: Selection of persons to make odor tests should be carefully made. Extreme sensitivity is not required, but insensitive persons should not be used. A good observer has a sincere interest in the test. Extraneous odor stimuli such as those caused by smoking and eating prior to the test or through the use of scented soaps, perfumes, and shaving lotions must be avoided. The tester should be free from colds or allergies that affect odor-response. Frequent rests in an odor-free atmosphere are recommended. The room in which the tests are to be conducted should be free from distractions, drafts, and other odor. In certain industrial atmospheres, a special odor-free room may be required, ventilated by air filtered through activated carbon and maintained at a constant comfortable temperature and humidity. For precise work a panel of five or more testers should be used. The persons making the odor measurements should not prepare the samples and should not know the dilution concentrations being evaluated. These persons should have been made familiar with the procedure before participating in a panel test. Always start with the most dilute sample to avoid tiring the senses with the concentrated sample. The temperature of the samples during testing should be kept within 1 degree of the specified temperature for the test.

7.2 Threshold measurement: The ratio by which the odor-bearing sample has to be diluted with odor-free water for the odor to be just detectable by the odor test is the "threshold odor number" (T.O.). The total volume of sample and odor-free water used in each test is 200 ml. The proper volume of odor-free water is put into the flask first; the sample is then added to the water. Table 1 gives the dilutions and corresponding threshold numbers.




# FIGURE 1. ODOR-FREE WATER GENERATOR

Table 1

**Threshold Odor Number**
**Corresponding to Various Dilutions**

| Sample Volume (ml) Diluted to 200 ml | Threshold Odor Number |
|---|---|
| 200 | 1 |
| 100 | 2 |
| 50 | 4 |
| 25 | 8 |
| 12.5 | 16 |
| 6.3 | 32 |
| 3.1 | 64 |
| 1.6 | 128 |
| 0.8 | 256 |

7.3 Determine the approximate range of the threshold odor by:

7.3.1 Adding 200 ml, 50 ml, 12.5 ml, and 3.1 ml of the sample to separate 500 ml glass-stoppered Erlenmeyer flasks containing odor-free water to make a total volume of 200 ml. A separate flask containing only odor-free water serves as the reference for comparison. If run at 60°C, heat the dilutions and the reference in the constant temperature bath at 60°C (±1°C).

7.3.2 Shake the flask containing the odor-free water, remove the stopper, and sniff the vapors. Test the sample containing the least amount of odor-bearing water in the same way. If odor can be detected in this dilution, more dilute samples must be prepared as described in (7.3.3). If odor cannot be detected in the first dilution, repeat the above procedure using the sample containing the next higher concentration of the odor-bearing water, and continue this process until odor is clearly detected.

7.3.3 If the sample being tested requires more extensive dilution than is provided by Table 1, an intermediate dilution is prepared from 20 ml of sample diluted to 200 ml with odor-free water. Use this dilution for the

threshold determination. Multiply the T.O. obtained by ten to correct for the intermediate dilution. In rare cases more than one tenfold intermediate dilution step may be required.

7.4 Based on the results obtained in the preliminary test, prepare a set of dilutions using Table 2 as a guide. One or more blanks are inserted in the series, in the vicinity of the expected threshold, but avoiding any repeated pattern. The observer does not know which dilutions are odorous and which are blanks. He smells each flask in sequence, beginning with the least concentrated sample and comparing with a known flask of odor-free water, until odor is detected with utmost certainty.

**Table 2**
**Dilutions for Various Odor Intensities**

| Sample Volume in Which Odor First Noted | | | |
|---|---|---|---|
| 200 ml | 50 ml | 12.5 ml | 3.1 ml |
| Volume (ml) of Sample to be Diluted to 200 ml | | | |
| 200 | 100 | 50 | (Intermediate |
| 100 | 50 | 25 | Dilution |
| 50 | 25 | 12.5 | See 7.3.3) |
| 25 | 12.5 | 6.3 | |
| 12.5 | 6.3 | 3.1 | |

7.5 Record the observations of each tester by indicating whether odor is noted (+ sign) in each test flask.

For example:

| ml sample diluted to 200 ml | 12.5 | 0 | 25 | 0 | 50 | 100 | 200 |
|---|---|---|---|---|---|---|---|
| Response | – | – | + | – | + | + | + |

8. Calculations

8.1 The threshold odor number is the dilution ratio at which odor is just detectable. In the example above (7.5), the first detectable odor occurred when 25 ml sample was diluted to 200 ml. Thus, the threshold is 200 divided by 25, equals 8. Table 1 lists the threshold numbers that correspond to common dilutions.

8.2 Anomalous responses sometimes occur; a low concentration may be called positive and a higher concentration in the series may be called negative. In such a case, the threshold is designated as that point of detection after which no further anomalies occur.

For instance:

| ml sample diluted to 200 ml | 6.3 | 12.5 | 0 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| Response | + | – | – | + | + | + |
| | | | | threshold | | |

8.3 Calculations of panel results to find the most probable average threshold are best accomplished by appropriate statistical methods. For most purposes, the threshold of a group can be expressed as the geometric mean (G.M.) of the individual thresholds. The geometric mean is calculated in the following manner:

8.3.1 Obtain odor response as outlined in Procedure and record results.

For example:

Table 3

**Sample Odor Series**

| ml of Odor-free Water | ml of Sample | Observer Response* 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| 188 | 12.5 | – | – | – | – | – |
| 175 | 25 | – | ⊕ | – | + | ⊕ |
| 200 | 0 | – | – | – | – | – |
| 150 | 50 | ⊕ | + | – | – | + |
| 200 | 0 | – | – | – | – | – |
| 100 | 100 | + | + | ⊕ | ⊕ | + |
| 0 | 200 | + | + | + | + | + |

*Circled plus equals threshold level.

8.3.2 Obtain individual threshold odor numbers from Table 1.

| Observer | T.O. |
|---|---|
| 1 | 4 |
| 2 | 8 |
| 3 | 2 |
| 4 | 2 |
| 5 | 8 |

8.3.3 The geometric mean is equal to the nth root of the product of n numbers. Therefore:

$$4 \times 8 \times 2 \times 2 \times 8 = 1024$$

and $$\sqrt[5]{1024} = \frac{\log 1024}{5} = \frac{3.0103}{5} = 0.6021$$

and anti-log of 0.6021 = 4 = T.O.

9. Precision and Accuracy

9.1 Precision and accuracy data are not available at this time.

9.2 A threshold number is not a precise value. In the case of the single observer, it represents a judgment at the time of testing. Panel results are more meaningful because individual differences have less influence on the result. One or two observers can develop useful data if comparison with larger panels has been made to check their sensitivity. Comparisons of data from time to time or place to place should not be attempted unless all test conditions have been carefully standardized and some basis for comparison of observer intensities exists.

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1. Standard Methods, 13th Edition, Amer. Public Health Asso., New York, N.Y., p 248, Method 136 (1971).
2. ASTM, Comm E-18, STP 433, "Basic Principles of Sensory Evaluation"; STP 434, Manual on Sensory Testing Methods; STP 440, "Correlation of Subjective-Objective Methods in the Study of Odors and Taste"; Phil., Pennsylvania (1968).
3. Baker, R. A., "Critical Evaluation of Olfactory Measurement". Jour. WPCF, *34*, 582 (1962).

# TURBIDITY

STORET NO. 00076

1. Scope and Application

   1.1 This method is applicable to drinking, surface, and saline waters in the range of turbidity from 0 to 40 nephelometric turbidity units (NTU).

   NOTE 1: NTU's are considered comparable to the previously reported Formazin Turbidity Units (FTU) and Jackson Turbidity Units (JTU).

2. Summary of Method

   2.1 The method is based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity. Readings, in NTU's, are made in a nephelometer designed according to specifications outlined in Apparatus 5. A standard suspension of Formazin, prepared under closely defined conditions, is used to calibrate the instrument.

      2.1.1 Formazin polymer is used as the turbidity reference suspension for water because it is more reproducible than other types of standards previously used for turbidity standards.

3. Sample Handling and Preservation

   3.1 Samples taken for turbidity measurements should be analyzed as soon as possible. Preservation of samples is not recommended.

4. Interferences

   4.1 The presence of floating debris and coarse sediments which settle out rapidly will give low readings. Finely divided air bubbles will affect the results in a positive manner.

   4.2 The presence of true color, that is the color of water which is due to dissolved substances which absorb light, will cause turbidities to be low, although this effect is generally not significant with finished waters.

5. Apparatus

   5.1 The turbidimeter shall consist of a nephelometer with light source for illuminating the sample and one or more photo-electric detectors with a readout device to indicate the intensity of light scattered at right angles to the path of the incident light. The turbidimeter should be so designed that little stray light reaches the detector in the absence of turbidity and should be free from significant drift after a short warm-up period.

5.2 The sensitivity of the instrument should permit detection of turbidity differences of 0.02 unit or less in waters having turbidities less than 1 unit. The instrument should measure from 0 to 40 units turbidity. Several ranges will be necessary to obtain both adequate coverage and sufficient sensitivity for low turbidities.

5.3 The sample tubes to be used with the available instrument must be of clear, colorless glass. They should be kept scrupulously clean, both inside and out, and discarded when they become scratched or etched. They must not be handled at all where the light strikes them, but should be provided with sufficient extra length, or with a protective case, so that they may be handled.

5.4 Differences in physical design of turbidimeters will cause differences in measured values for turbidity even though the same suspension is used for calibration. To minimize such differences, the following design criteria should be observed:

5.4.1 Light source: Tungsten lamp operated at not less than 85% of rated voltage or more than rated voltage.

5.4.2 Distance traversed by incident light and scattered light within the sample tube: Total not to exceed 10 cm.

5.4.3 Angle of light acceptance of the detector: Centered at 90° to the incident light path and not to exceed ±30° from 90°.

5.4.4 Maximum turbidity to be measured: 40 units.

5.5 The Hach Turbidimeter, Model 2100 and 2100 A, is in wide use and has been found to be reliable; however, other instruments meeting the above design criteria are acceptable.

6. Reagents

6.1 Turbidity-free water: Pass distilled water through a 0.45$\mu$ pore size membrane filter if such filtered water shows a lower turbidity than the distilled water.

6.2 Stock turbidity suspension:

Solution 1: Dissolve 1.00 g hydrazine sulfate, $(NH_2)_2 \cdot H_2SO_4$, in distilled water and dilute to 100 ml in a volumetric flask.

Solution 2: Dissolve 10.00 g hexamethylenetetramine in distilled water and dilute to 100 ml in a volumetric flask.

In a 100 ml volumetric flask, mix 5.0 ml Solution 1 with 5.0 ml Solution 2. Allow to stand 24 hours at 25 ± 3°C, then dilute to the mark and mix.

6.3 Standard turbidity suspension: Dilute 10.00 ml stock turbidity suspension to 100 ml with turbidity-free water. The turbidity of this suspension is defined as 40 units. Dilute portions of the standard turbidity suspension with turbidity-free water as required.

6.3.1 A new stock turbidity suspension should be prepared each month. The standard turbidity suspension and dilute turbidity standards should be prepared weekly by dilution of the stock turbidity suspension.

7. Procedure

7.1 Turbidimeter calibration: The manufacturer's operating instructions should be followed. Measure standards on the turbidimeter covering the range of interest. If the instrument is already calibrated in standard turbidity units, this procedure will check the accuracy of the calibration scales. At least one standard should be run in each instrument range to be used. Some instruments permit adjustments of sensitivity so that scale values will correspond to turbidities. Reliance on a manufacturer's solid scattering standard for setting overall instrument sensitivity for all ranges is not an acceptable practice unless the turbidimeter has been shown to be free of drift on all ranges. If a pre-calibrated scale is not supplied, then calibration curves should be prepared for each range of the instrument.

7.2 Turbidities less than 40 units: Shake the sample to thoroughly disperse the solids. Wait until air bubbles disappear then pour the sample into the turbidimeter tube. Read the turbidity directly from the instrument scale or from the appropriate calibration curve.

7.3 Turbidities exceeding 40 units: Dilute the sample with one or more volumes of turbidity-free water until the turbidity falls below 40 units. The turbidity of the original sample is then computed from the turbidity of the diluted sample and the dilution factor. For example, if 5 volumes of turbidity-free water were added to 1 volume of sample, and the diluted sample showed a turbidity of 30 units, then the turbidity of the original sample was 180 units.

7.3.1 The Hach Turbidimeters, Models 2100 and 2100A, are equipped with 5 separate scales: 0-0.2, 0-1.0, 0-100, and 0-1000 NTU. The upper scales are to be used only as indicators of required dilution volumes to reduce readings to less than 40 NTU.

NOTE 2: Comparative work performed in the MDQAR Laboratory indicates a progressive error on sample turbidities in excess of 40 units.

8. Calculation

8.1 Multiply sample readings by appropriate dilution to obtain final reading.

8.2 Report results as follows:

| NTU | Record to Nearest: |
|---|---|
| 0.0–1.0 | 0.05 |
| 1–10 | 0.1 |
| 10–40 | 1 |
| 40–100 | 5 |
| 100–400 | 10 |
| 400–1000 | 50 |
| >1000 | 100 |

9. Precision and Accuracy

9.1 In a single laboratory (MDQARL), using surface water samples at levels of 26, 41, 75 and 180 NTU, the standard deviations were ±0.60, ±0.94, ±1.2 and ±4.7 units, respectively.

9.2 Accuracy data is not available at this time.

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APPENDIX G

# HANDBOOK FOR ANALYTICAL QUALITY CONTROL IN WATER AND WASTEWATER LABORATORIES

For
U.S. ENVIRONMENTAL PROTECTION AGENCY
Technology Transfer

By
ANALYTICAL QUALITY CONTROL LABORATORY
National Environmental Research Center
Cincinnati, Ohio

June 1972

## ABSTRACT

One of the fundamental responsibilities of management is the establishment of a continuing program to insure the reliability and validity of analytical laboratory and field data gathered in water treatment and wastewater pollution control activities.

This handbook is addressed to laboratory directors, leaders of field investigations, and other personnel who bear responsibility for water and wastewater data. Subject matter of the handbook is concerned primarily with quality control for chemical and physical tests and measurements. Sufficient information is offered to allow the reader to inaugurate, or to reinforce, a program of analytical quality control which will emphasize early recognition, prevention and correction of factors leading to breakdowns in the validity of data.

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## Chapter 1

# IMPORTANCE OF QUALITY CONTROL

### 1.1 General

The role of the analytical laboratory is to provide qualitative and quantitative data to be used in decision making. To be valuable, the data must accurately describe the characteristics or the concentration of constituents in the sample submitted to the laboratory. In many cases, an approximate answer or incorrect result is worse than no answer at all, because it will lead to faulty interpretations.

Decisions made using water and wastewater data are far-reaching. Water quality standards are set to establish satisfactory conditions for a given water use. The laboratory data define whether that condition is being met, and whether the water can be used for its intended purpose. If the laboratory results indicate a violation of the standard, action is required on the part of pollution control authorities. With the present emphasis on legal action and social pressures to abate pollution, the analyst should be aware of his responsibility to provide laboratory results that are a reliable description of the sample. Furthermore, the analyst must be aware that his professional competence, the procedures he has used, and the reported values may be used and challenged in court. To satisfactorily meet this challenge, the laboratory data must be backed up by an adequate program to document the proper control and application of all of the factors which affect the final result.

In wastewater analyses, the laboratory data define the treatment plant influent, the status of the steps in the treatment process, and the final load imposed upon the water resources. Decisions on process changes, plant modification, or even the construction of a new facility may be based upon the results of laboratory analyses. The financial implications alone are significant reasons for extreme care in analysis.

Research investigations in water pollution control rest upon a firm base of laboratory data. The final result sought can usually be described in numerical terms. The progress of the research and the alternative pathways available are generally evaluated on the basis of laboratory data. The value of the research effort will depend upon the validity of the laboratory results.

### 1.2 Quality Control Program

Because of the importance of laboratory analyses and the resulting actions which they produce, a program to insure the reliability of the data is essential. It is recognized that all analysts practice quality control to varying degrees, depending somewhat upon their training, professional pride, and awareness of the importance of the work they are doing. However, under the pressure of daily workload, analytical quality control may be easily neglected. Therefore, an established, routine control program applied to every analytical test is important in assuring the reliability of the final results.

The quality control program in the laboratory has two primary functions. First, the program should monitor the reliability (truth) of the results reported. It should continually provide an answer to "How good (true) are the results submitted?" This phase may be termed "measurement of quality." The second function is the control of quality in order to meet

the program requirements for reliability. For example, the processing of spiked samples is the measurement of quality, while the use of analytical grade reagents is a control measure. Just as each analytical method has a rigid protocol, so the quality control associated with that test must also involve definite required steps to monitor and assure that the result is correct. The steps in quality control will vary with the type of analysis. For example, in a titration, standardization of the titrant on a frequent basis is an element of quality control. In an instrumental method, the check-out of instrument response and the calibration of the instrument in concentration units is also a quality control function. Ideally, all of the variables which can affect the final answer should be considered, evaluated, and controlled.

This handbook considers the factors which go into creating an analytical result, and provides recommendations for the control of these factors in order to insure that the best possible answer is obtained. A program based upon these recommendations will give the analyst and his supervisor confidence in the reliability and the representative nature of the sample charactieristics being reported.

Without exception, the final responsibility for the reliability of the analytical results submitted rests with the Laboratory Director.

### 1.3 Analytical Methods

In general, the widespread use of an analytical method indicates that it is a reliable means of analysis, and this fact tends to support the validity of the test result reported. Conversely, the use of a little-known technique forces the data user to place faith in the judgement of the analyst. When the analyst uses a "private" method, or one not commonly accepted in the field, he must stand alone in defining both his choice of the method and the result obtained.

The need for standardization of methods within a single laboratory is readily apparent. Uniform methods between cooperating laboratories are also important in order to remove the methodology as a variable in comparison or joint use of data between laboratories. Uniformity of methods is particularly important when laboratories are providing data to a common data bank, such as STORET*, or when several laboratories are cooperating in joint field surveys. A lack of standardization of methods raises doubts as to the validity of the results reported. If the same constituent is measured by different analytical procedures within a single laboratory, or in several laboratories, the question is raised as to which procedure is superior, and why the superior method is not used throughout.

The physical and chemical methods used should be selected by the following criteria:

a. The method should measure the desired constituent with precision and accuracy sufficient to meet the data needs in the presence of the interferences normally encountered in polluted waters.

b. The procedure should utilize the equipment and skills normally available in the average water pollution control laboratory.

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*STORET is the acronym used to identify the computer-oriented U.S. Environmental Protection Agency Water Quality Control Information System for STOrage and RETrieval of data and information.

c. The selected methods should be in use in many laboratories or have been sufficiently tested to establish their validity.

d. The method should be sufficiently rapid to permit routine use for the examination of large numbers of samples.

The use of EPA methods in all EPA laboratories provides a common base for combined data between Agency programs. Uniformity throughout EPA lends considerable support to the validity of the results reported by the Agency.

Regardless of the analytical method used in the laboratory, the specific methodology should be carefully documented. In some water pollution reports it is customary to state that Standard Methods (1) have been used throughout. Close examination indicates, however, that this is not strictly true. In many laboratories, the standard method has been modified because of recent research or personal preferences of the laboratory staff. In other cases the standard method has been replaced with a better one. Statements concerning the methods used in arriving at laboratory data should be clearly and honestly stated. The methods used should be adequately referenced and the procedures applied exactly as directed.

Knowing the specific method which has been used, the reviewer can apply the associated precision and accuracy of the method when interpreting the laboratory results. If the analytical methodology is in doubt, the data user may honestly inquire as to the reliability of the result he is to interpret.

The advantages of strict adherence to accepted methods should not stifle investigations leading to improvements in analytical procedures. In spite of the value of accepted and documented methods, occasions do arise when a procedure must be modified to eliminate unusual interference, or to yield increased sensitivity. When modification is necessary, the revision should be carefully worked out to accomplish the desired result. It is advisable to assemble data using both the regular and the modified procedure to show the superiority of the latter. This useful information can be brought to the attention of the individuals and groups responsible for methods standardization. For maximum benefit, the modified procedure should be rewritten in the standard format so that the substituted procedure may be used throughout the laboratory for routine examination of samples. Responsibility for the use of a non-standard procedure rests with the analyst and his supervisor, since such use represents a departure from accepted practice.

In field operations, the problem of transport of samples to the laboratory, or the need to examine a large number of samples to arrive at gross values will sometimes require the use of rapid field methods yielding approximate answers. Such methods should be used with caution, and with a clear understanding that the results obtained do not compare in reliability with those obtained using standard laboratory methods. The fact that "quick and dirty" methods have been used should be noted, and the results should not be reported along with more reliable laboratory-derived analytical information. The data user is entitled to know that approximate values have been obtained for screening purposes only, and that the results do not represent the customary precision and accuracy obtained in the laboratory.

### 1.4 References

1. Standard Methods for the Examination of Water and Wastewater, 13th Edition, American Public Health Association, New York (1971).

# CHAPTER 2

# LABORATORY SERVICES

## 2.1 General

Quality control of laboratory analyses involves consideration and control of the many variables which affect the production of reliable data. The quality of the laboratory services available to the analyst must be included among these variables. An abundant supply of distilled water, free from interferences and other undesirable contaminants, is an absolute necessity. An adequate source of clean, dry, compressed air is needed. Electrical power for routine laboratory use and voltage-regulated sources for delicate electronic instrumentation must be provided. This chapter, therefore, will be devoted to describing methods of maintaining the quality of these services, as used in laboratory operations.

## 2.2 Distilled Water

Distilled or demineralized water is used in the laboratory for dilution, preparation of reagent solutions, and final rinsing of glassware. Ordinary distilled water is usually not pure. It may be contaminated by dissolved gases and by materials leached from the container in which it has been stored. Volatile organics distilled over from the feed water may be present, and non-volatile impurities may occasionally be carried over by the steam, in the form of a spray. The concentration of these contaminants is usually quite small, and distilled water is used for many analyses without further purification. However, it is highly important that the still, storage tank, and any associated piping be carefully selected, installed, and maintained in such a way as to assure minimum contamination.

Water purity has been defined in many different ways, but one generally accepted definition states that high purity water is water that has been distilled and/or deionized so that it will have a specific resistance of 500,000 ohms (2.0 micromhos conductivity) or greater. This definition is satisfactory as a base to work from, but for more critical requirements, the breakdown shown in Table 2-1 has been suggested to express degrees of purity (1).

Table 2-1

WATER PURITY

| Degree of Purity | Maximum Conductivity (micromhos/cm) | Approximate Concentration of Electrolyte (mg/l) |
|---|---|---|
| Pure | 10 | 2-5 |
| Very Pure | 1 | 0.2-0.5 |
| Ultrapure | 0.1 | 0.01-0.02 |
| Theoretically Pure | 0.055 | 0.00 |

Properly designed metal stills from reputable manufacturers offer a convenient and reliable source of distilled water. These stills are usually constructed of copper, brass, and bronze. All surfaces that contact the distillate should be heavily coated with pure tin to prevent metallic contamination. The metal storage tank should be of sturdy construction, have a tight-fitting cover, and a filter in the air vent to remove airborne dust, gases, and fumes.

For special purposes, an all-glass distillation unit may be preferable to the metal still. These stills are usually smaller, and of more limited capacity than the metal stills. An actual comparison in which the distillates from an all-glass still and a metal still were analyzed spectrographically for certain trace metal contaminants is shown below in Table 2-2.

Table 2-2
COMPARISON OF DISTILLATES FROM GLASS AND METAL STILLS

| | Element and Concentration (Micrograms/1) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Zn | B | Fe | Mn | Al | Cu | Ni | Pb |
| All-Glass Still | <1 | 12 | 1 | <1 | 4 | 5 | <1 | <2 |
| Metal Still | 9 | 13 | 2 | <1 | <5 | 11 | <2 | 26 |

It can be seen that the all-glass still produced a product which had substantially lower contamination from zinc, copper, and lead. Other analyses have indicated the same general relationship, except that a boron concentration of 100 micrograms/liter was found in water from the all-glass still on one occasion. This was probably related to the length of time the distillate had remained in the glass storage reservoir.

All stills require periodic cleaning to remove solids which have been deposited from the feed water. Hard water and high dissolved solids content promote scale formation in the evaporator, and cleaning frequency will thus depend on the quality of the feed water. The boiler of an all-glass still should be drained daily and refilled with clean water. Build-up of scale is easily detected, and the boiler and condenser coils should be cleaned at frequent intervals. Metal stills usually incorporate a constant bleeder device which retards scale formation to some extent. However, these units should still be dismantled and cleaned at regular intervals. Cleaning should always be in accordance with the manufacturer's instructions.

Pre-treatment of the incoming feed water will often improve still performance and raise the quality of the distillate. For example, preliminary softening of hard water removes calcium and magnesium prior to distillation. This reduces scale formation in the boiler and condenser, thereby reducing maintenance service. These softeners employ the ion exchange principle using a sodium chloride cycle, and are relatively inexpensive to operate. A carbon filtration system, installed at the feed water intake, will remove organic materials which might subsequently be carried over in the distillate. If trace concentrations of ions are a major concern, the distillate may be passed through a mixed-bed ion exchanger.

At least two commercially manufactured systems are available for production of high purity water by ion exchange. The Millipore Super-Q System (Millipore Corp., Bedford, Mass.), consists essentially of disposable cartridges for prefiltration, organic absorption, deionization and Millipore filtration. The company claims it can produce 10 megohm water, containing no particulate matter larger than 0.45 micron in size, from tap water, at the rate of 20 gallons per hour. Continental Water Conditioning Corp., El Paso, Texas, advertises a

system which can be tailored to the needs of the customer. Performance specifications include minimum flow rates of 45 gallons per hour and total dissolved solids of less than 0.1 mg/l when required.

Specific conductance is a rapid and simple measurement for determining the inorganic quality of distilled water. Stills of the types previously discussed are capable of producing a distillate with a specific conductance of less than 2.0 micromhos at 25° C. This is equivalent to 0.5-1.0 mg/l of ionized material. Frequent checks should be made to determine that optimum performance is being maintained. A purity meter installed between the still and the storage reservoir will monitor the conductivity of the distillate, in terms of the equivalent in mg/l of sodium chloride. If the reading on the meter begins to rise above the present limit of conductivity, effective action should be taken to eliminate the source of contamination. Organic quality is more difficult to monitor, but the total organic carbon determination is a simple and rapid test for organic contaminants.

A piping system for delivering distilled water to the area of use within the laboratory is a convenient and desirable feature. In this case, special care should be taken that the quality of the water is not degraded between the still and the point of use. Piping may be of tin, tin-lined brass, stainless steel, plastic, or chemically resistant glass, depending on the quality of the water desired and on available funds. Tin is best, but is also very expensive. As a compromise, plastic pipe, or glass pipe with Teflon* O-rings at all connecting joints is satisfactory for most purposes. The glass pipe has an obvious advantage when freedom from trace amounts of organic materials is important.

When there is no piped-in supply, distilled water will probably be transported to the laboratory and stored in polyethylene or glass bottles of about 5-gallon capacity. If stored in glass containers, distilled water will gradually leach the more soluble materials from the glass and cause an increase in dissolved solids. Therefore, only borosilicate-free glass containers should be used. Polyethylene bottles contain organic plasticizers, and traces of these materials may be leached from the container walls. These are of little consequence, except in some organic analyses. Rubber stoppers often used in storage containers contain leachable materials, including significant quantities of zinc. This is usually no problem, since the water is not in direct contact with the stopper. However, the analyst should be aware of the potential for contamination, especially when the supply is not replenished by frequent use.

The delivery tube may consist of a piece of glass tubing which extends almost to the bottom of the bottle, and which is bent downward above the bottle neck, with a three-to-four-foot piece of flexible tubing attached for mobility. Vinyl tubing is preferable to latex rubber, because it is less leachable. However, a short piece of latex tubing may be required at the outlet for better control of the pinchcock. The vent tube in the stopper should be protected against the entrance of dust.

Ordinary distilled water is quite adequate for many analyses, including the determination of major cations and anions. Certain needs may require the use of double- or even triple-distilled water. Redistillation from an alkaline permanganate solution can be used to obtain a water with low organic background. When determining trace organics by solvent extraction and gas chromatography, distilled water with sufficiently low background may be extremely difficult to obtain. In this case, pre-extraction of the water with the solvent used in the respective analysis may be helpful in eliminating undesirable peaks in the blank.

*Trademark of E.I. duPont de Nemours & Co.*

Certain analyses require special treatment or conditioning of the distilled water, and these will now be discussed.

### 2.3 Ammonia-Free Water

Removal of ammonia can be accomplished by shaking ordinary distilled water with a strong cation exchanger, or by passing distilled water through a column of such material. For limited volumes of ammonia-free water, use of the Quikpure (Box 254, Chicago, Ill.) 500-ml bottle is highly recommended. The ion-free water described below is also suitable for use in the determination of ammonia.

### 2.4 Carbon-Dioxide-Free Water

Carbon-dioxide-free water may be prepared by boiling distilled water for 15 minutes and cooling to room temperature. As an alternative, distilled water may be vigorously aerated with a stream of inert gas for a period sufficient to achieve saturation and $CO_2$ removal. Nitrogen is most frequently used. The final pH of the water should lie between 6.2 and 7.2. It is not advisable to store $CO_2$-free water for extended periods.

### 2.5 Ion-Free Water

A multi-purpose high purity water, free from trace amounts of the common ions, may be conveniently prepared by slowly passing distilled water through an ion-exchange column containing one part of a strongly acidic cation-exchange resin in the hydroxyl form. Resins of a quality suitable for analytical work must be used. Ion-exchange cartridges of the research grade, available from scientific supply houses, have been found satisfactory. By using a fresh column and high quality distilled water, a water corresponding to the ASTM designation for referee reagent water (2) (maximum 0.1 mg/l total matter and maximum conductivity of 0.1 micromho) can be obtained. This water is suitable for use in the determination of ammonia, trace metals, and low concentrations of most cations and anions. It is not suited to some organic analyses, however, because this treatment adds organic contaminants to the water by contact with the ion-exchange materials.

### 2.6 Compressed Air

The quality of compressed air required in the laboratory is usually very high, and special attention should be given to producing and maintaining clean air until it reaches the outlet. Oil, water, and dirt are undesirable contaminants in compressed air, and it is important to install equipment which generates dry, oil-free air. When pressures of less than 50 psi are required, a rotary-type compressor, using a water seal and no oil, eliminates any addition of oil which would subsequently have to be removed from the system. Large, horizontal, water-cooled compressors will usually be used when higher pressures are required.

Compression heats air, thus increasing its tendency to retain moisture. An aftercooler is therefore necessary to remove water. Absorption filters should be used at the compressor to prevent moisture from entering the piping system. Galvanized steel pipe with threaded, malleable-iron fittings, or solder-joint copper tubing should be used for piping the air to the laboratory.

When the compressed air entering the laboratory is of low quality, an efficient filter should be installed between the outlet and the point of use to trap oil, moisture, and other contaminants. As an alternative, high quality compressed air of the dry grade is commercially available in cylinders when no other source exists.

## 2.7 Electrical Services

An adequate electrical system is indispensable to the modern laboratory. This involves having both 115- and 230-volt sources in sufficient capacity for the type of work that must be done. Requirements for satisfactory lighting, proper functioning of sensitive instruments, and operation of high current devices must be considered. Any specialized equipment may present unusual demands on the electrical supply.

Due to the special type of work, requirements for a laboratory lighting system are quite different from those in other areas. Accurate readings of glassware graduations, balance verniers, and other measuring lines must be made. Titration endpoints, sometimes involving subtle changes in color or shading, must be observed. Levels of illumination, brightness, glare, and location of light sources should be controlled to facilitate ease in making these measurements and to provide maximum comfort for the employees.

Spectrophotometers, flame photometers, atomic absorption equipment, emission spectrographs, gas chromatographs, etc. have complicated electronic circuits which require relatively constant voltage to maintain stable, drift-free instrument operation. If the voltage to these circuits varies, there is a resulting change in resistance, temperature, current, efficiency, light output, and component life. These characteristics are interrelated, and one cannot be changed without affecting the others. Voltage regulation is therefore necessary to eliminate these conditions.

Many instruments have built-in voltage regulators which perform this function satisfactorily. In the absence of these, a small, portable, constant-voltage transformer should be placed in the circuit between the electrical outlet and the instrument. Such units are available from Sola Basic Industries, Elk Grove Village, Ill., and are capable of supplying a constant output of 118 volts from an input which varies between 95 and 130 volts. When requirements are more stringent, special transformer-regulated circuits can be used to supply constant voltage. Only the instrument receiving the regulated voltage should be operated from such a circuit at any given time. These lines are in addition to, and separate from the ordinary circuits used for operation of equipment with less critical requirements.

Electrical heating devices provide desirable heat sources, and should offer continuously variable temperature control. Hot plates and muffle furnaces wired for 230-volt current will probably give better service than those which operate on 115 volts, especially if the lower voltage circuit is only marginally adequate. Water baths and laboratory ovens with maximum operating temperatures of about 200°C perform well at 115 volts. Care must be taken to ground all equipment which could constitute a shock hazard. The three-pronged plugs which incorporate the ground are best for this purpose.

## 2.8 References


1. Applebaum, S. B., and Crits, G. J., "Producing High Purity Water." Industrial Water Engineering, Sept./Oct. 1964.

2. 1968 Book of ASTM Standards, Part 23, Atmospheric Analysis, pp. 225-6, American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

# CHAPTER 3

# INSTRUMENTAL QUALITY CONTROL

## 3.1 Introduction

The modern analytical laboratory depends very heavily upon instrumentation. This statement may be completely obvious, but it should be remembered that the exceptional emphasis on electronic equipment has really begun since the development of the transistor and the computer. Analytical instrumentation, to a certain extent, is always in the development stage, with manufacturers continually redesigning and upgrading their products, striving for miniaturization, better durability and sensitivity, and improved automation. The net result to laboratory supervisors and staff members is a bewildering stream of advertising brochures, announcements, and catalogues of newly available equipment. Consequently, the selection and purchase of analytical equipment is, at all times, beset with uncertainty.

Table 3-1 lists the instruments most commonly used for water and wastewater analysis. These represent basic equipment used in routine work and should be the subject of careful consideration before purchase. Further, operation and maintenance of these devices ought to be a primary consideration in production of satisfactory data. Obviously, a fundamental understanding of instrument design will assist the analyst in the correct use of the instrument and in some cases aid in detecting instrumental failures.

In the pages that follow an attempt is made to discuss basic instrument design and to offer some remarks whenever possible about desirable instrumental features.

Table 3-1

INSTRUMENTS COMMONLY USED
IN WATER AND WASTEWATER ANALYSIS

- Analytical Balance
- Potentiometer (pH meter)
- Conductivity meter
- Turbidimeter
- Spectrophotometers
  - a. Visual
  - b. Ultraviolet
  - c. Infrared
  - d. Atomic absorption
- Total Carbon Analyzer
- Gas Chromatographs
- Miscellaneous
  - a. Temperature devices (ovens, water baths, etc.)
  - b. Recorders
  - c. Selective Ion Electrodes

### 3.2 Analytical Balances

The most important piece of equipment in any analytical laboratory is the analytical balance (See Figure 3-1). It bears the same relationship to accuracy of measurements produced by a laboratory as the Greenwich standard clock has to international time-keeping. If the balance is not accurate all data related to weight-prepared standards will contain the same degree of error. The balance, therefore, should be the most protected and cared-for instrument in the laboratory. Unfortunately, care of the balance is frequently overlooked.

There are many fine balances on the market designed to meet a variety of needs such as sensitivity, speed weighing, batch weighing, etc. Types of balances include general purpose, micro-, electro-, semi-analytical, analytical and other special purpose instruments. Each type of balance has its own place in the scheme of laboratory operation but the analytical balance is by far the most important in the production of reliable data.

Most analytical balances in use today in well equipped laboratories are of the "single pan" variety. Single-pan capacities range from 80 grams to the popular 200-gram models with sensitivities from 0.01 to 1 mg. Features of single-pan balances include mechanical lifting and substitution of weights, digital readout of weights, and mechanical zeroing of the empty balance. The advantage of the single-pan balance over the old "two-pan" balance is in greatly increased weighing speed and improved weighing accuracy because of mechanical weight handling. With all the design improvements, however, the modern analytical balance is still a fragile instrument, subject to shock, temperature and humidity changes, mishandling and various other insults. Some of the precautions to be observed in maintaining and prolonging the dependable life of a balance are as follows:

a. Analytical balances should be mounted on a heavy shock-proof table, preferably one with adequate working surface and a suitable drawer for storage of balance accessories; balance level should be checked frequently and adjusted when necessary.

b. Balances should be located away from laboratory traffic, protected from sudden drafts and humidity changes.

c. Balance temperatures should be equilibrated with room temperature; this is especially important if building heat is shut off or reduced during non-working hours.

d. When not in use, the beam should be raised from the knife edges, the weights returned to the beam, objects such as weighing dish removed from the pan, and the slide door closed.

e. Special precautions should be taken to avoid spillage of corrosive chemicals on the pan or inside the balance case; the interior of balance housing should be kept scrupulously clean.

f. Balances should be checked and adjusted periodically by a company service man or balance consultant; if service is not available locally, follow the manufacturer's instructions as closely as possible.

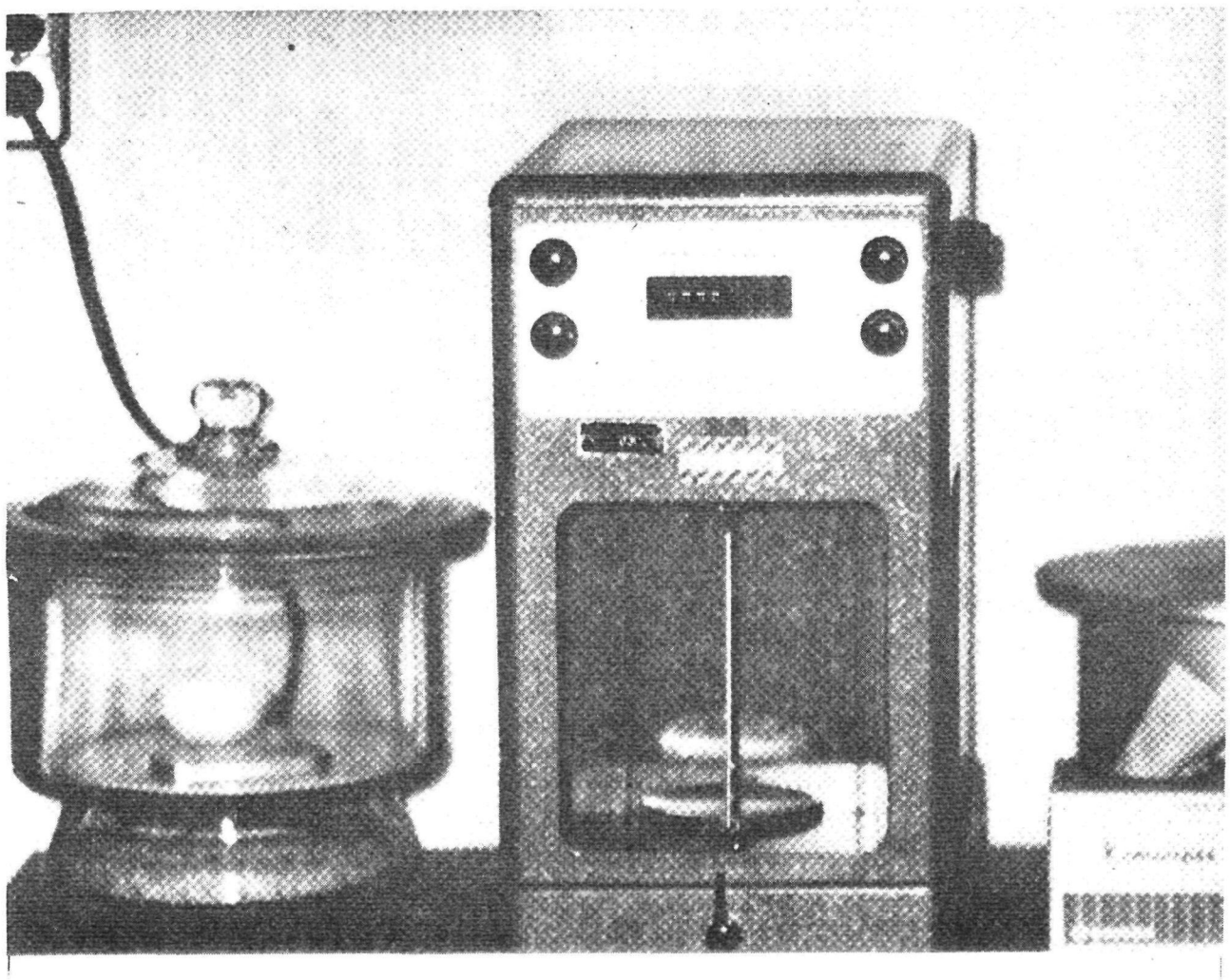

Figure 3-1. ANALYTICAL BALANCE

g. The balance should be operated at all times according to the manufacturer's instructions.

Standardized weights to be used in checking balance accuracy, and that meet U.S. Bureau of Standards specifications, may be purchased from various supply houses. A very complete set of directions for checking the performance of a balance is contained in Part 30 of ASTM Standards (1).

Since all analytical balances of the 200-gram capacity have about the same specifications with reference to sensitivity, precision, convenience, and price, and since these specifications are suitable for normal weighing requirements in water and wastewater laboratories, it is safe to assume that there is no clear preference for a certain model, and selection will probably be made on the basis of service availability.

### 3.3 pH Meters

The concept of pH as a means of expressing the degree of effective acidity or alkalinity as contrasted with total acidity or alkalinity was developed by Sorenson in 1909. It was not until about 1940 that commercial instruments were developed for routine laboratory measurement of pH.

A basic pH meter (See Figure 3-2) consists of a voltage source, amplifier, and readout device, either scale or digital. Certain additional refinements produce varying performance characteristics between models. Some models incorporate expanded scales for increased readability and solid state circuitry for operating stability and extreme accuracy. All instruments of recent design also include temperature adjustment and slope adjustment to correct for asymmetric potential of glass electrodes. Other features are scales that facilitate use of selective ion electrodes, recorder output, and interfacing with complex data handling systems.

In routine analytical work, the glass electrode is used as the indicator and the calomel electrode as the reference. Glass electrodes have a very fast response time in highly buffered solutions. However, accurate readings are obtained slowly in poorly buffered samples, and particularly when changing from buffered to unbuffered samples, as after standardization. Electrodes, both glass and calomel, should be well rinsed with distilled water after each reading, and should be rinsed or dipped several times into the next test sample before the final reading is taken. Weakly buffered samples should be stirred during measurement. Glass electrodes should not be allowed to become dry during periods of inactivity. When not in use they should be immersed in distilled water.

The first step in standardization of the instrument is done by immersing the glass and calomel electrodes into a buffer of known pH, setting the meter scale or needle to the pH of the buffer and adjusting the proper controls to bring the circuit into balance. The temperature compensating dial should be set at the sample temperature. The pH of the standard buffer should be within about two pH units of the sample. For best accuracy, the instrument should be calibrated against two buffers that bracket the pH of the samples.

The presence of a faulty electrode is indicated by failure to obtain a reasonably correct value for the pH of the second reference buffer solution after the meter has been standardized with the first. A cracked glass electrode will often yield pH readings that are essentially the same for both standards. The response of electrodes may also be impaired by

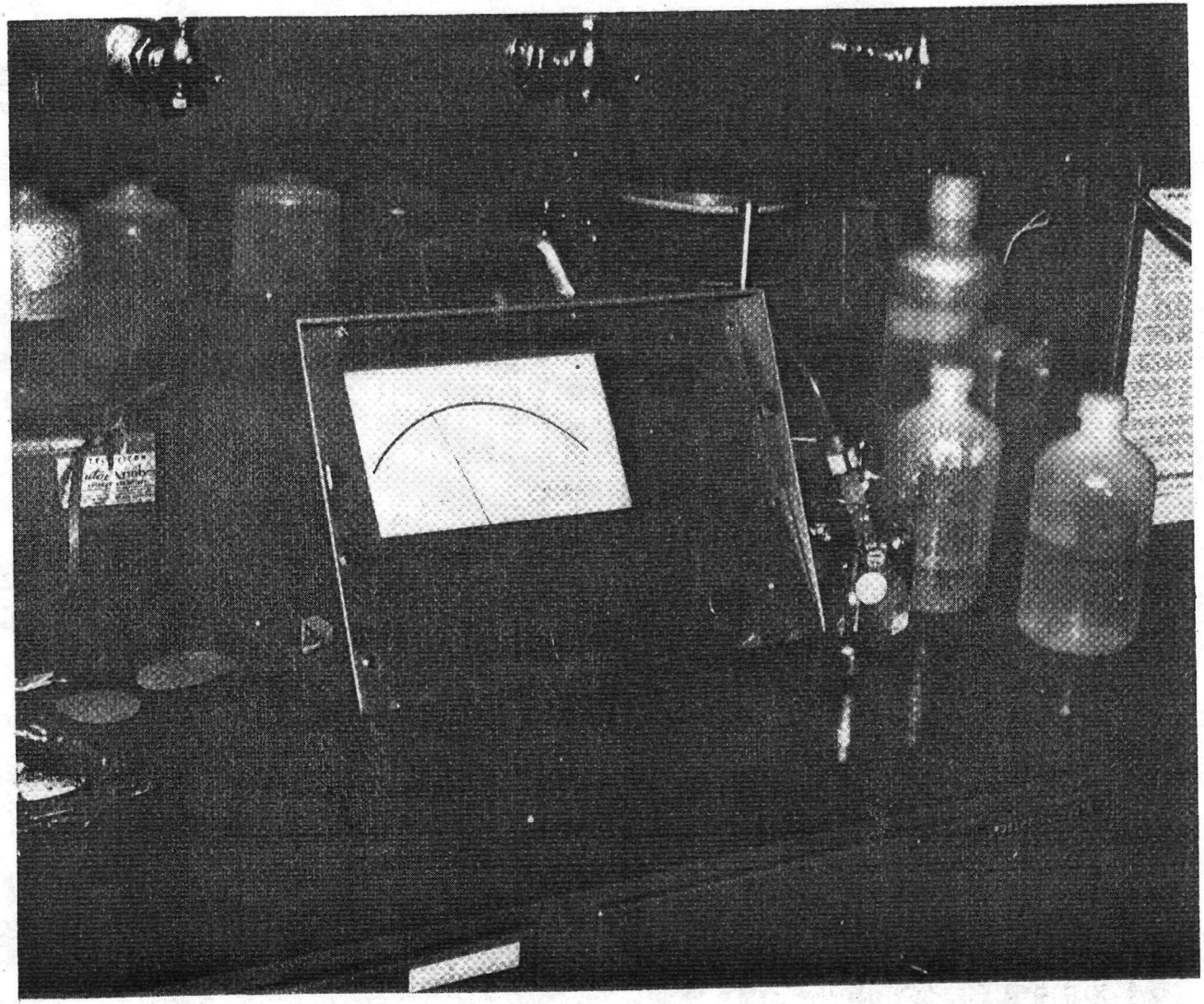

Figure 3-2. pH METER

failure to maintain the KCl level in the calomel electrode, or by certain specific materials such as oily substances and precipitates that may coat the surfaces. A faulty condition can be recognized from the check with the two buffer solutions. If either of these conditions should occur the electrode can probably be restored to normal by an appropriate cleaning procedure. Complete and detailed cleaning methods are given in Part 23 of ASTM Standards (2).

Because of the asymmetric potential of the glass electrode most pH meters are built with a "slope adjustment" which enables the analyst to correct for slight electrode errors that occur when standardization is performed at two different pH levels. Exact details of slope adjustment and slope check may vary with different models of instruments. The slope adjustment must be made whenever electrodes are changed, subjected to vigorous cleaning, or refilled with fresh electrolyte. The slope adjustment feature is highly desirable and recommended for consideration when purchasing a new meter.

Most pH meters now available are built with transistorized circuits rather than vacuum tubes which greatly reduces warm-up time and increases stability of the meter. Also, many instruments are designed with a switching circuit so that the conventional 0-14 scale may be used to read a single pH unit. The "expanded-scale" feature allows for more accurate readings and may be of definite value when the meter is used for potentiometric titrations. It is of dubious value, however, in routine analytical work, since readings more percise than ± 0.1 pH are seldom required.

Solid state circuitry has led to improved design of compact instruments suitable for field work. Field-type instruments are generally battery-powered, and require more maintenance and more frequent standardization than laboratory instruments.

Standard buffer solutions, covering a range of pH, may be purchased from almost any chemical supply house and are completely satisfactory for routine use. Table 3-2 below gives a list of NBS buffers (easily made in the laboratory) and the resulting pH at several different temperatures.

Table 3-2

pH VALUES OF NBS STANDARDS FROM 0 - 30°C

| Temp. °C | 0.05M Potassium Tetroxalate | Potassium Acid Tartrate (Sat. at 25°C) | 0.05 M Potassium Acid Phthalate | 0.025 M Potassium Dihydrogen Phosphate + 0.025 M Sodium Dihydrogen Phosphate | 0.01 M Sodium Tetraborate |
|---|---|---|---|---|---|
| 0 | 1.67 | – | 4.01 | 6.98 | 9.46 |
| 10 | 1.67 | – | 4.00 | 6.92 | 9.33 |
| 15 | 1.67 | – | 4.00 | 6.90 | 9.27 |
| 20 | 1.68 | – | 4.00 | 6.88 | 9.22 |
| 25 | 1.68 | 3.56 | 4.01 | 6.86 | 9.18 |
| 30 | 1.69 | 3.55 | 4.01 | 6.85 | 9.14 |

Some idea of the effect of temperature on pH may be obtained by observing temperature vs pH of various buffers shown in the table. Theoretically, the potential response of the electrode system changes 0.20 mV per pH unit per degree centigrade. Since all pH meters measure potential but read out in pH, a variable compensation is used. A rough rule of thumb is that temperature compensation is about 0.05 pH units per 5 degree increase in temperature.

Typical performance data of a conventional expanded scale pH meter is shown in Table 3-3 below.

Table 3-3

PERFORMANCE CHARACTERISTICS OF TYPICAL pH METER

| | Normal Scale | Expanded Scale |
|---|---|---|
| Range | 0 to 14 pH<br>±1400 mv | 1 pH<br>±100 mv |
| Smallest scale division | 0.1 pH<br>10 mv | 0.005 pH<br>0.5 mv |
| Accuracy | ±0.05 pH<br>±5 mv | ±0.002 pH<br>±2% of reading |
| Reproducibility | ±0.02 pH<br>±2 mv | ±0.002 pH<br>±0.2 mv |
| Temp. compensation | 0 to 100°C (manual or automatic) | |
| Input impedance | $>10^{14}$ | $>10^{13}$ |
| Power requirements | 115/220V, 50/60 Hz | |
| Dimensions | 12½" w x 11" d x 8¼" h | |

### 3.4 Conductivity Meters

Solutions of electrolytes conduct an electric current by the migration of ions under the influence of an electric field. For a constant applied EMF, the current flowing between opposing electrodes immersed in the electrolyte will vary inversely with the resistance of the solution. The reciprocal of the resistance is called conductance and is expressed in reciprocal ohms or mhos. For natural water samples where the resistance is high, the usual reporting unit is in micromhos. Figure 3-3 shows a typical conductivity meter.

The passage of direct current through an electrolyte causes changes in the electrolyte, hence, to prevent polarization, it is necessary to use an alternating current or current pulses of short duration when measuring conductivity. Originally, conductivity meters were built using a rapidly alternating current of low intensity in the audio range, and were equipped

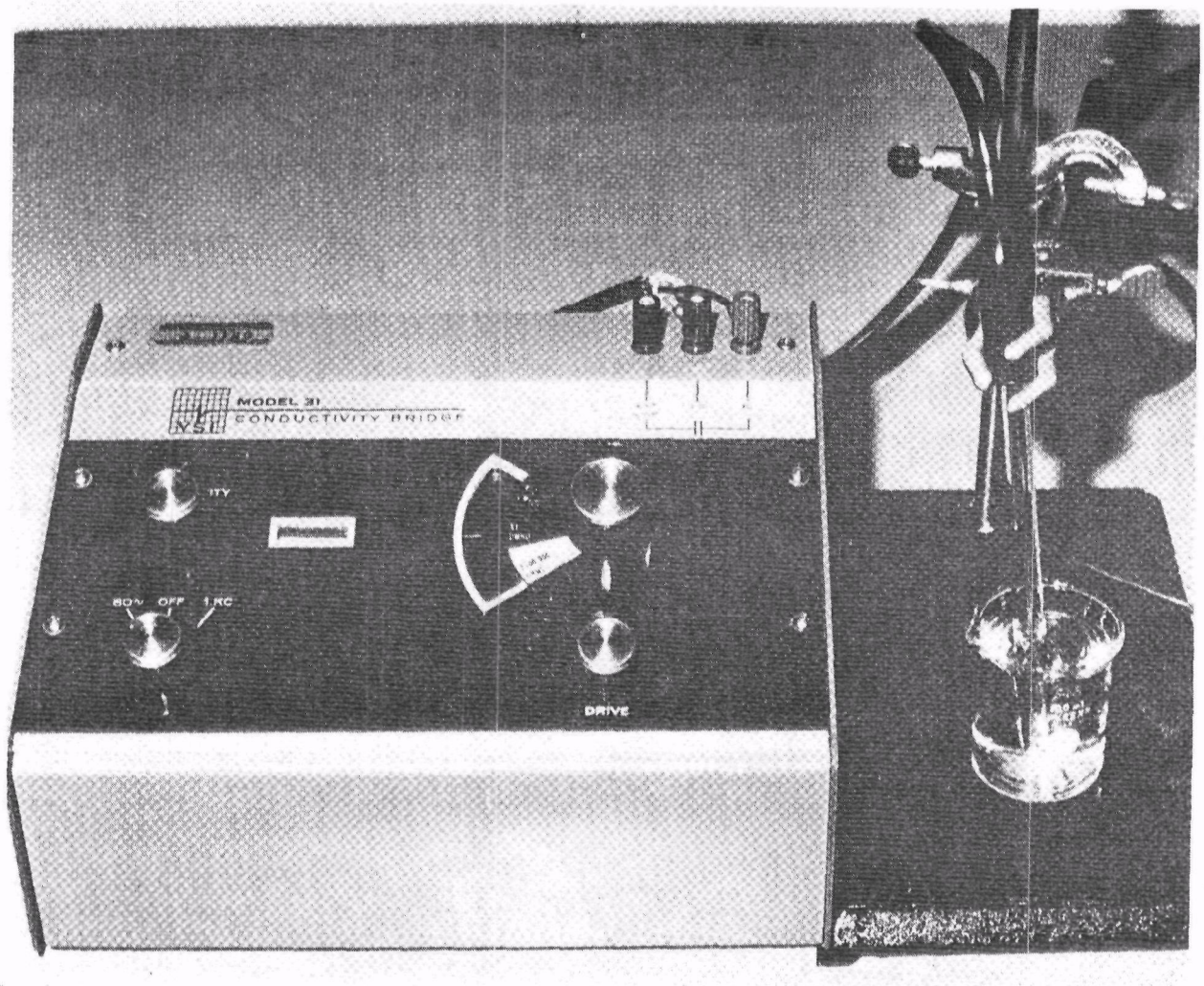


Figure 3-3. CONDUCTIVITY METER

with balance. For obvious reasons, the earpiece has been replaced by other devices, such as the cathode ray tube commonly known as the "magic eye". Practically all conductivity meters on the present market use some variation of the "magic eye" for indicating solution conductivity, and include a stepping switch for varying resistances in steps of 10X. The instruments are therefore capable of reading conductivities from about 0.1 $\mu$ mhos to about 250,000 $\mu$ mhos.

The sensing element for a conductivity measurement is the conductivity cell, which normally consists of two thin plates of platinized metal, rigidly supported with a very precise parallel spacing. For protection, the plates are mounted inside a glass tube, with openings in the side walls and submersible end for access of sample. Variations in designs have included use of hard rubber and plastics for protection of the cell plates. Glass may be preferable, in that the plates may be visually observed for cleanliness and possible damage, but the more durable encasements have the advantage of greater protection and reduced cell breakage. One manufacturer offers a cell containing circular carbon plates embedded in an epoxy-type plastic. Reversing the usual procedures, the sample is poured into the cell. The cell is particularly attractive because of its ruggedness and the fact that it can be cleaned without changing the cell factor.

Special precaution is taken with the arrangement of lead wires from the plates. Since stray electrolytic and capacitive current will pass between them, thereby distorting the bridge balance, the wires must be properly separated, usually by use of a non-conducting bead or collar on one of the lead wires.

In routine use, cells should be frequently examined to insure that (a) platinized coating of plates is intact, (b) plates are not coated with suspended matter, (c) plates are not bent, distorted, or misaligned, and (d) lead wires are properly spaced.

Temperature has a pronounced effect on the conductance of solutions, and must be corrected when results are reported. The specified temperature for reporting data used by most analytical groups (and all EPA laboratories) is 25°C. Data correction may be accomplished by adjusting sample temperatures to 25°C., or by use of mathematical or electronic adjustment. Adjustment of sample temperature is the preferred system, because of the empirical nature of the mathematical correction. However, investigative work in the Analytical Quality Control Laboratory, and at the upper Ohio Basin Office, EPA, has shown that acceptable data is obtained if conductivity readings taken at stream-side are electronically temperature-corrected to 25°C.

Instrumental troubles are seldom encountered with conductivity meters because of the design simplicity. When troubles occur they are usually in the cell, and for most accurate work the following procedures should be used:

a. Standardize the cell and establish a cell factor by measuring the conductivity of a standard potassium chloride solution (standard conductivity tables may be found in various handbooks).

b. Rinse the cell by repeated immersion in distilled water.

c. Again, immerse the cell in the sample several times before obtaining a reading.

d. If the meter is equipped with a "magic eye", determine the maximum width of the

shadow at least twice, by approaching the endpoint from a low reading upward, and from a high reading downward.

Because the cell constants are subject to slow change, even under ideal conditions, and sometimes to more rapid change under adverse conditions, it is recommended that the cell constant be periodically established. Table 3-4, below, can be used for this operation.

Table 3-4

ELECTRICAL CONDUCTIVITY
OF POTASSIUM CHLORIDE REFERENCE SOLUTIONS

| Solution | Normality | Method of Preparation | Temp. (°C) | Conductivity ($\mu$ mhos) |
|---|---|---|---|---|
| A | 0.1 | 7.4365 g KCl/l | 0 | 7,138 |
| | | at 20°C | 18 | 11,167 |
| | | | 25 | 12,856 |
| B | 0.01 | 0.7440 g KCl/l | 0 | 773 |
| | | at 20°C | 18 | 1,220 |
| | | | 25 | 1,408 |
| C | 0.001 | Dilute 100 ml of B | | |
| | | to 1 l at 20°C | 25 | 147 |

For instruments reading in mhos, calculate the cell constant as follows:

$$L = \frac{K_1 + K_2}{1{,}000{,}000 \times K_X}$$

where

$L$ = cell constant
$K_1$ = Conductivity, in $\mu$ mhos/cm, of the KCl solution at the temperature of measurement
$K_2$ = conductivity, in $\mu$ mhos, at the same temperature, of the distilled water used to prepare the reference solution
$K_X$ = measured conductance, in mhos

Conductivity equipment which has generally been found to be reliable for laboratory work includes the YSI #31 (Yellow Springs Instrument Co., Box 279, Yellow Springs, Ohio 45387), the Lab-Line Mark IV #1100 and MC3 #11025 (Lab-Line Instruments Inc., 15th & Bloomingdale Aves., Melrose Park, Ill. 60160), and Industrial Instruments #RC 16B2 and RC-18 (Industrial Instruments Inc., 89 Commerce Road, Cedar Grove, N.J. 07009).

The YSI #31 is particularly suitable for routine lab work because of the brilliance of the magic eye. The Lab-Line meters provide the sturdy cell previously mentioned. The Industrial Instruments #RC-18 is designed for extreme accuracy but the number of dial settings required to obtain a single reading do not recommend it for routine analysis. A recent instrument survey conducted by the Methods and Performance Activity of the Analytical Quality Control Laboratory showed that a preponderance of EPA Laboratories used the Industrial Instruments RC 16B2.

### 3.5 Turbidimeters

Instruments for the measurement of turbidity have traditionally employed principles of design related to transmission or reflectance of light. The lack of a primary standard for turbidity, however, has resulted in a complete absence of uniformity among the available instruments. Further, the Jackson Candle Turbidimeter, which does not depend upon the use of a primary standard, is a primitive instrument, subject to many interferences, and the measurements generally are not reproducible.

Recent investigations (3) have resulted in the design of an instrument which has been adopted by the Environmental Protection Agency as a standard. The specifications for the instrument are described elsewhere (4). Presently, the Hach Turbidimeter, Model 2100, (Box 907, Ames, Iowa 50010) is the only turbidity measuring device manufactured which meets these specifications. Figure 3-4 shows this instrument.

The Hach Turbidimeter Model 2100 employs, for standardization, a suspension of formazin, especially used because of its stability and uniform particle size. For calibration purposes, the formazin is permanently embedded in a cylinder of lucite, the cylinder duplicating the size and shape of the sample cuvette. Although the instrument is designed with a series of scales ranging 0-1, 0-10, 0-100 and 0-1000 JCU, it has been recommended that turbidity readings in excess of 40 JCU be rejected (3). Correct use of the meter therefore requires that samples containing turbidities in excess of 40 JCU be diluted to a value below this level and the results multiplied by the proper dilution factor.

For production of data with maximum accuracy and precision the following precautions should be observed:

a. Protect the lucite standard from nicks, scratches and fingerprints.

b. Use a constant orientation of the lucite standard while calibrating the instrument.

c. Use a well mixed sample in the sample cuvette; do not take readings until finely dispersed bubbles have disappeared.

d. Dilute samples containing excess turbidity to some value below 40 JCU; take reading, and multiply results by correct dilution factor.

### 3.6 Spectrophotometers

Since a large portion of routine quantitative measurements are performed colorimetrically, the spectrophotometer (See Figure 3-5) is usually the workhorse of any analytical laboratory. Indeed, the versatility of the instrument, and the number of demands imposed upon it, have resulted in a large variety of designs and price ranges. A systematic listing and detailed discussion of all instrumental types would be beyond the scope of this chapter. As a matter of convenience and practicality, therefore, spectrophotometers are discussed separately as visible, ultraviolet, infrared, and atomic absorption instruments.

A spectrophotometer is an instrument for measuring an amount of light or radiant energy transmitted through a solution, as a function of wave-length. A spectrophotometer differs

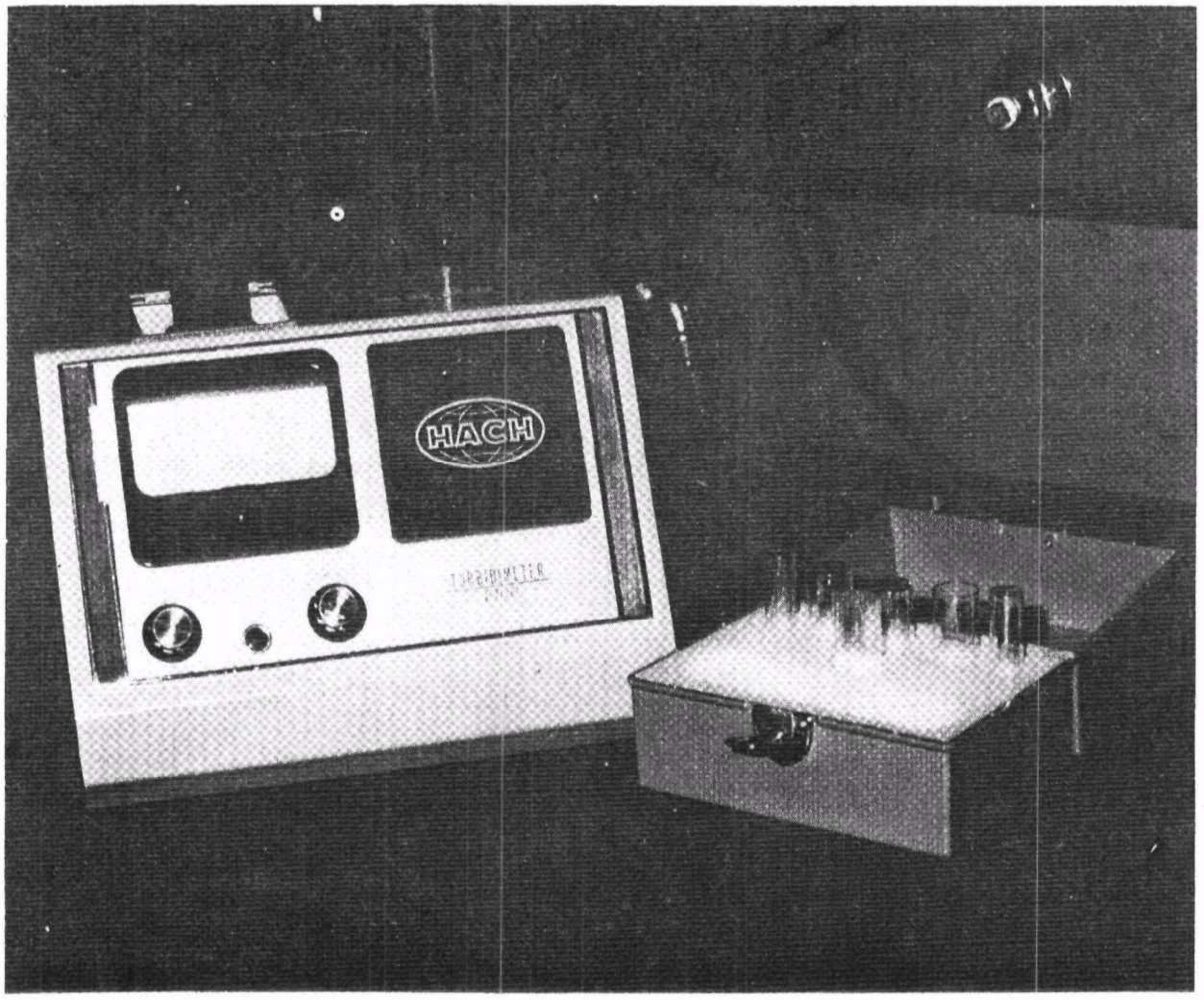


Figure 3-4. TURBIDIMETER

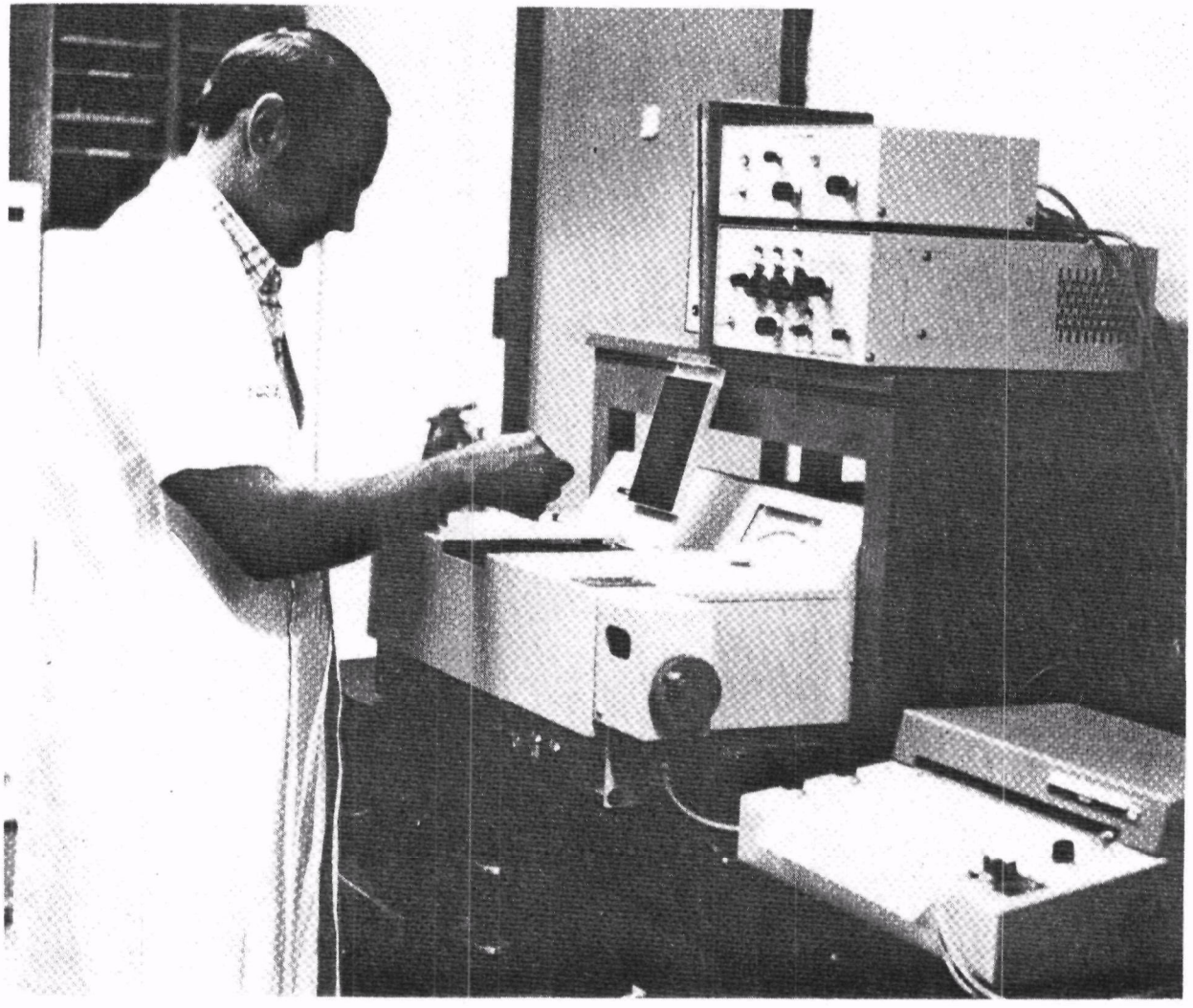

Figure 3-5. SPECTROPHOTOMETER

from a filter photometer in that it uses continuously variable, and more nearly monochromatic, bands of light. Filter photometers are relatively insensitive, and lack the versatility of spectrophotometers. They are used most profitably where a single method can be designed to fit the instrument.

The essential parts of a spectrophotometer are:

a. A source of radiant energy, usually a tungsten filament bulb,

b. The Monochromator, a device for isolating narrow bands of light,

c. Cells (cuvettes), for holding the colored solution under investigation, and

d. The photodetector, a device to detect and measure the radiant energy passing through the sample solution.

Each of the essential features listed, especially the monochromator and the photodetector system, vary in design principles from one instrument to another. Table 3-5 shows some of the features of the more commonly used spectrophotometers.

### 3.6.1. Visible Range

Desirable features on a visible-range spectrophotometer are determined by the anticipated use of the instrument. Simple, limited programs requiring use of only a few parameters at gross concentrations, can probably be supported by an inexpensive, but reliable instrument, such as the B&L Spectronic 20. (See Table 3-5.) On the other hand, if a laboratory program requires a wide variety of measurements on diverse samples at very low concentrations, a more versatile instrument may be needed. One of the prime considerations would be adaptability to various cell sizes, at least from 1.0 to 5.0 cm. Many spectrophotometers now available are satisfactory for water quality analyses.

As shown in Table 3-5, which lists only a few of the available spectrophotometers, higher priced instruments strive for versatility, including interchangeable sources, detectors, and cells. Complete information on instrument specifications can be found in the publication Industrial Research (Beverly Shores, Ind. 46301), Nov. 20, 1969.

### 3.6.2. Ultraviolet Range

An ultraviolet spectrophotometer is similar in design to a visual range instrument, differences being in the light source and the optics. The light source is a hydrogen or deuterium discharge lamp which emits radiations in the UV portion of the spectrum, generally from about 200 m$\mu$ to the low visible region. The optical system, if of the prism type, must be constructed of UV-transparant material, usually quartz. Sample cells must also be constructed of quartz or other UV-transparent material. If a grating system is used in an UV system, the grating may be specially cut (blazed) in the UV region for greater sensitivity. A number of UV spectrophotometers are available: the Beckman DU, the Hitachi, the Bausch and Lomb, the Cary, the Hilger and the Leeds & Northrup.

### 3.6.3. Infrared Range

A number of instrumental changes are required in the construction of spectrophotometers

Table 3-5

DESIGN FEATURES OF SOME COMMON SPECTROPHOTOMETERS

| | Beckman B | Beckman Du | Bausch & Lomb Spectronic 20 | Coleman 101 | Hitachi [1] |
|---|---|---|---|---|---|
| Light Source | Tungsten Bulb from CV Transformer | Tungsten Bulb, CV Transformer Hydrogen Lamp | Tungsten Bulb, Reg. Transformer | Tungsten Bulb, CV Transformer Hydrogen (Deuterium) Lamp | Tungsten Bulb Hydrogen (Deuterium) Lamp |
| Optical System | Glass Prism | Quartz Prism | Grating, 600/mm | Grating, 600/mm | Grating, 1440/mm, Blazed at 200 mμ |
| Slits | Variable | Variable | Fixed, band width 20 mμ | Fixed, band width 10 mμ | Variable |
| Range | 320-1000 mμ | 200-1000 mμ | 325-1000 mμ | 220-900 mμ, using both lamps | 195-950 mμ, using both lamps |
| Detector | Interchangeable Phototubes 320-625 and 625-1000 mμ | Interchangeable Phototubes 200-625 and 625-1000mμ | Interchangeable Phototubes 325-625 and 625-1000 mμ | Hitachi Dual Range Phototube, 220-900 mμ | Dual Range Phototube 195-950 mμ |
| Sample Cells | 1.0 to 5.0 cm | 1.0, 5.0, 10.0 cm | 1.1, 1.6, 2.2 cm | 1.0, 5.0, 20.0 cm | 1.0 to 10.0 cm, micro cells |
| Readout | Meter Scale, %T, O.D. | Null Point Potentiometer, %T, O.D. | Meter Scale %T, O.D. | Meter Scale, %T, O.D. | Meter Scale, %T, O.D. |
| Attachment | Test-tube adapter flame photometry | UV Adapter with $H_2$ lamp flame photometry | Interchangeable test-tube adapters, digital readout, reflectance adapter | Recorder jack or digital readout | Atomic absorption reflectance, fluorescence, emission, flow thru cells |

[1] *Marketed as Perkin-Elmer #139 or Coleman #139-001*

for measurements in the infrared (IR) region. Modifications are needed, because optical materials, such as glass and quartz, absorb radiant energy in the IR region and ordinary photocells do not respond.

Most IR spectrophotometers use front-surfaced mirrors to eliminate the necessity for radiant energy to pass through quartz, glass, or other lens materials. The mirrors are usually parabolic to facilitate gathering the diffuse IR energy. Instruments must be protected from high humidities and water vapor to avoid deterioration of the optical system, and also to avoid extraneous absorption bands in the IR.

The energy or light source for an IR instrument may be a Nernst glower or a Globar. Either source has certain characteristics that recommend it for use, but the Globar is more commonly used because it has a more stable emission and it is more rugged. Receiving or detection units may be a thermocouple, a bolometer, thermistor, or a photoconductor cell.

Of all the IR instruments available, two are in relatively common use in EPA laboratories. The Perkin-Elmer Infracord is a low cost model designed for routine work with simplicity of operation. The Perkin Elmer #621 (723-G Main Ave., Norwalk, Conn., 06852) is a far more sophisticated instrument, designed for basic research.

### 3.6.4 Proper Use of Spectrophotometers

The manufacturer's instructions for proper use should be followed in all cases. Several safeguards against misuse of the instruments, however, are mandatory.

Instruments should be checked for wavelength alignment. If a particular colored solution is to be used at a closely specified wavelength, considerable loss of sensitivity can be encountered if the wavelength control is misaligned. In visual instruments, an excellent reference point is the maximum absorption for a dilute solution of potassium permanganate, which has a dual peak at 526 m$\mu$ and 546m$\mu$. On inexpensive grating instruments, which possess less resolution than the prism instruments, the permanganate peak appears at 525 to 550 m$\mu$ as a single flat-topped spike.

For both UV and IR instruments, standard absorption curves for many organic materials have been published so that reference material for standard peaks is easily available. Standard films of styrene and other transparent plastics are available for IR wavelength checks.

Although most instruments contain built-in transformers for stabilization of the electronic circuits, an exterior, high capacity, constant-voltage transformer is recommended for general laboratory control. A number of controlled-voltage outlets in the laboratory are especially desirable in industrial areas or in buildings containing heavy, electrically operated equipment where voltage surges on adjacent power lines are apt to be frequent. An unstable voltage is frequently indicated by a flickering needle on the meter. The flickering behavior may be intermittent or it may occur at certain times of the day when heavy machinery in the area may be starting or stopping.

Too much emphasis cannot be placed on care of absorption cells. All cells should be kept scrupulously clean, free of scratches, fingerprints, smudges and evaporated film residues. Matched cells should be checked to see that they are equivalent by placing portions of the

same solution in both cells and taking several readings of the %T or OD values. If a cell is mismatched it should be discarded or reserved for rough work. (Directions for cleaning cells are detailed in Chapter 4).

Generally speaking, trained technicians may operate any of the spectrophotometers successfully. However, interpretation of data from both the UV and IR instruments becomes increasingly complex, and requires more training and specialization. IR interpretation requires special training, and because of the special techniques of sample preparation, instrument operation, and interpretation of absorption curves, mere compliance with the operations manual is not sufficient.

### 3.6.5. Atomic Absorption

There are a number of differences in the basic design and accessories for atomic absorption equipment that require consideration before purchase and during subsequent use. These choices concern the light source, nebulizer burners, optical systems, readout devices, and mode conversion. Some of these choices are not readily obvious, and require that the purchaser or user be familiar with the types and numbers of samples to be analyzed and the specific elements to be measured before a choice is made. For a program analyzing a wide variety of samples for a number of elements at varying concentrations, an instrument of maximum versatility would be required. A typical atomic absorption unit is shown in Figure 3-6.

#### 3.6.5.1 Lamp Mounts

A basic design feature of atomic absorption spectrometers is the convenience of the hollow cathode (HC) lamp changeover system. Some instruments provide for as many as six lamps in a rotating turret, all electronically stabilized and ready for use by simply rotating the lamp turret. Other instruments provide for use of only one lamp at a time in the lamp housing, and require manual removal and replacement whenever more than one element is to be measured. A quick changeover system is desirable, especially if a number of lamp changes are needed during a period of operation. Conversely, if lamp changes are infrequent, multi-lamp mounts do not represent a great convenience.

For optimal use of the instrument, certain precautions should be observed. After the proper lamp has been selected the hollow cathode current should be adjusted according to the manufacturer's recommendations and allowed to electronically stabilize (warm up) before use. This requires approximately 15 minutes. During this period, the monochromator may be positioned at the correct wavelength and the proper slit width selected. For those instruments employing a multi-lamp turret, a warm-up current is provided to those lamps not in use, thereby minimizing the warm-up period when the turret is rotated. In a single-lamp instrument, the instability exhibited during warm-up is minimized by the use of a double-beam optical system.

##### 3.6.5.1.1 Single Element and Multi-Element Lamps

As an adjunct to single-lamp mounts, HC lamps using from two to as many as six elements in combination are available, thereby increasing the versatility of the AA spectrometer. For instance, a single-lamp instrument such as the Perkin-Elmer may analyze for elements with only one lamp substitution, whereas a six-lamp turret such as the Jarrell-Ash (590 Lincoln Street, Waltham, Mass. 02154), using multi-element lamps could run 18 or more elements.

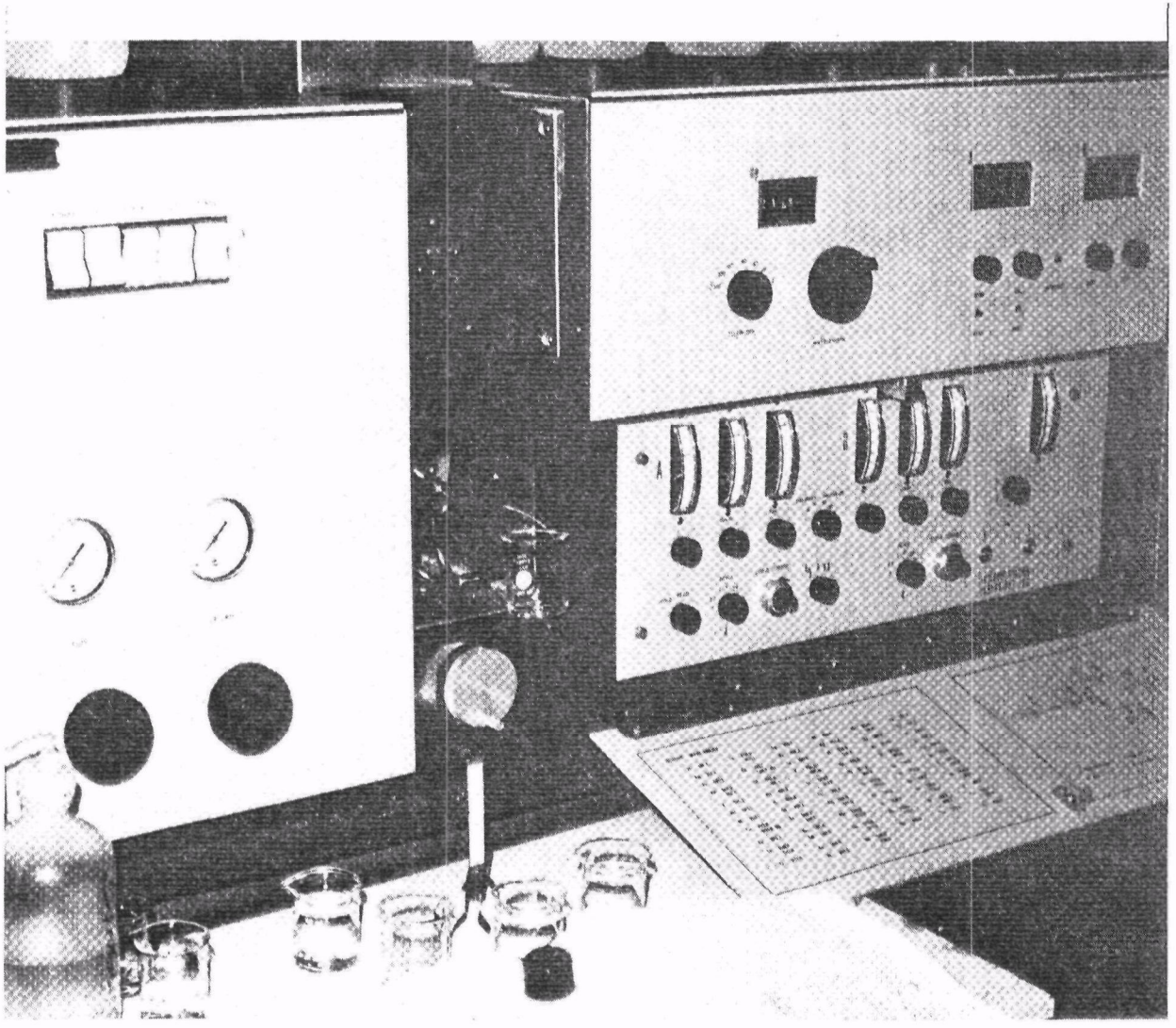

Figure 3-6. ATOMIC ABSORPTION UNIT

Multi-element lamps are considerably cheaper per element than single-element lamps, but the savings may not realized if the lamps are not used strategically, because all the elements in the cathode deteriorate when the lamp is used, regardless of which element is measured. The deterioration phenomena results from the different volatilities of metals used in the cathode. One metal volatilizes (sputters) more rapidly than the others and redeposits upon the cathode causing an increase in surface area of that metal, and decreasing the exposed area of the other cathode metals. Thus, with continual use, a drift in signal will be noted with at least one metal increasing and the other (or others) decreasing. If one can ignore the dubious cost savings of multi-element lamps, use of single-element lamps would result in more precise and accurate data.

The individual line intensities of an element in a multi-element HC lamp will usually be less than that of a lamp containing a pure cathode of the same element. This is because each element must now share the discharge energy with all other elements present. However, this reduction should not affect the output by a factor of more than 1/2 to 1/6, depending on the combination and number of elements combined. The output can be even greater in some multi-element lamps because alloying may permit a higher operating current than the pure cathode itself. All HC lamps have life expectancies which are related to the volatility of the cathode metal, and for this reason, the manufacturer's recommendations for amperage at which the lamp is operated should be closely followed.

A recent advance in HC lamp design, the high-intensity lamp, promises increased sensitivity for some elements. It is also predicted that the newly designed lamps will be used in atomic fluorescence techniques, with significant gains in sensitivity for metals analysis.

Recent improvements in design and manufacture of hollow cathode lamps have resulted in lamps with more constant output and a longer life. Under normal conditions a HC lamp may be expected to operate satisfactorily for several years. At one time, hollow cathode lamps were guaranteed for a minimum amp-hour period. This has been changed, however, to a 90-day warranty. It is good practice to date newly purchased lamps and inspect immediately upon receipt. Operating current and voltage will be indicated on the lamp and should not be exceeded during use. An increase in background noise and/or a loss of sensitivity are signs of lamp deterioration.

#### 3.6.5.2 Burner Types

The most difficult and inefficient step in the AA process is converting the metal in the sample from an ion or a molecule to the neutral atomic state. It is the function of the atomizer and the burner to produce the desired neutral atomic condition of the elements. With minor modifications burners are the same as those used for flame photometry.

Basically there are two different types of burners. They are the total-consumption or surface-mix burner, and the laminar-flow or pre-mix burner. There are many variations of these two basic types, such as the Boling, the high-solids, the turbulent-flow, the tri-flame, nitrous-oxide burner and many others. As one might expect, there are many similarities among the various burners, the different names resulting from the different manufacturers. The element being determined and the type of sample solution dictate the type of burner to be used.

Generally, all types and makes of burners can be adjusted laterally, rotationally and

vertically for selection of the most sensitive absorbing area of the flame for the specific element sought. The vertical adjustment is probably the most important since the position of greatest sensitivity varies from element to element.

Burner height is of utmost importance in changing from one element to another. Certain instruments are provided with a vernier adjustment for reproducing burner-height settings, but many are not. Figure 3-7 shows a simply designed device used in the Analytical Quality Control Laboratory for reproducing exact burner height. The gauge is positioned on the burner and the height determined from the light beam striking the calibrated scale. The point at which the beam strikes the gauge is recorded for future use.

#### 3.6.5.3 Single-Beam and Double- (Split) Beam Instruments

There is a great deal of existing uncertainty among instrument users about the relative merits of single-beam and double-beam instruments. Neither system is the final answer.

With a single-beam instrument the light beam from the source passes directly through the flame to the detector. In a double-beam system the light from the source is divided by a beam splitter into two paths. One path, the reference beam, goes directly to the detector. The second path, the sample beam, goes through the flame to the detector. The chopper alternately reflects and passes each beam, creating two equal beams falling alternately upon the detector. If the beams are equal they cancel the alternate impulses reaching the detector and no signal is generated. If the beams are different, the resulting imbalance causes the detector to generate an a.c. signal which is amplified and measured. Any difference between the reference and sample beam is measured as a direct function of absorbed light. The advantage of the double-beam design, therefore, is that any variations in the source are of reduced importance, and smaller dependence is placed upon the stability of the power supply. However, stabilization of the power supply can eliminate the apparent need for the split-beam system. Further, a beam splitter requires use of additional mirrors or optical accessories that cause some loss of radiant energy. Neither system, however, compensates for variation in flame intensity.

A single-beam system does not monitor source variations but offers certain other advantages. It allows use of low-intensity lamps, smaller slit settings and smaller gain. As a consequence, the single-beam instrument, properly designed, is capable of operating with lower noise, better signal-to-noise ratio and therefore better precision and improved sensitivity. Because the simplified optical system conserves radiant energy, especially in the shorter wavelengths, it facilitates operation in the low wavelength range. With this advantage, it should be possible to obtain better sensitivity for those elements with strong resonance lines below 350 $m\mu$ and even those slightly below 300 $m\mu$.

#### 3.6.5.4. Readout Devices

Early models of the AA instrument offered only a meter, calibrated in percentage absorption. In the surge of competitive design, more sophisticated readout devices were built into or offered as accessories to various models. At the present time any desired readout method may be obtained with almost any instrument. Less expensive designs still provide meters with conventional needle indicators. More costly instruments offer any combination of built-in digital scalers, calibrated in concentration, external digital printout in concentration, typewriter printout or typewriter with punch tape. Even inexpensive instruments are built with recorder interfacing.

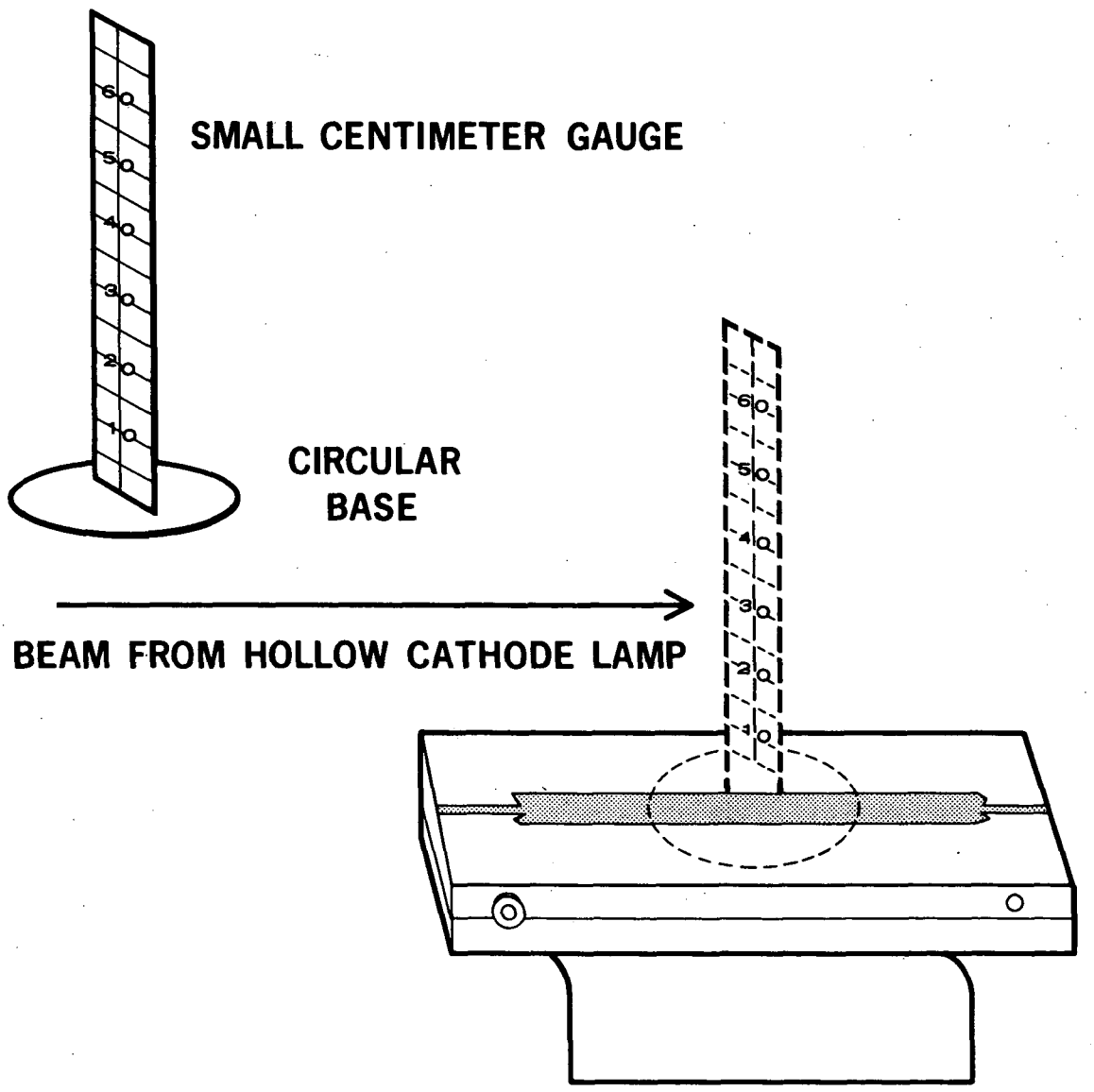


Figure 3-7 DEVICE FOR REPRODUCIBLE POSITIONING OF BURNER HEIGHT

Choice of a readout system is predicated largely upon laboratory needs and availability of budget. In general, any step toward complete automation is desirable but the degree of automation should be compatible with the laboratory program.

#### 3.6.5.5 Miscellaneous Accessories

A number of instruments contain a mode selector, making an instrument usable for either flame absorption or flame emission. The conversion to flame emission is a desirable feature since certain elements are more amenable to analysis by this method.

Automatic sample changers are offered for almost all instruments on the market, and as has been previously stated, any automation feature is desirable. However, unless a laboratory program performs a large number of repetitious measurements daily, an automatic sample changer would not be required. As a practical measure, other commonly used sample-changing devices not expressly designed for AA use, can easily be interfaced with almost any AA instrument.

#### 3.6.5.6 Instrument Choice

Table 3-6 summarizes some of the design features of various commercially available instruments. Since many of the features are common to most models, the basic choice appears to be between double-beam and single-beam instruments. On this basis, at this writing, it is probable that the most widely used instrument in the EPA is the Perkin-Elmer 303, one of two double-beam instruments on the market.

The Instrumentation Laboratories Model 153 (113 Hartwell Ave., Lexington, Mass. 02173) has a number of desirable features. In addition to features listed in the table, it includes push-button ignition, push-button wavelength scan, fail-safe solenoids to prevent improper flame settings, fail-safe flame monitoring, absorption integrator at selected time intervals, visual "peaking" meters, curve correction and several other features. The new Perkin-Elmer Model 403 also offers most of these features in addition to very sophisticated data handling accessories.

### 3.7 Organic Carbon Analyzer

A number of devices designed to measure the organic content of aqueous samples have appeared on the market within the last five years. The oldest, or first of these instruments, is the Dow Beckman Carbonaceous Analyzer (Figure 3-8). The apparatus measures organic carbon as carbon, by oxidizing a very small sample at a temperature of 900°, in a stream of oxygen, converting all organically bound carbon to carbon dioxide, which is then measured by a Golay-type thermal detector. The instrument is able to detect about 20 mg. of carbon. A recent modification of the original Dow Beckman Carbonaceous Analyzer employs a dual-combustion-tube system operating at different temperatures to distinguish between total carbon and inorganic carbon. Organic carbon is found by difference.

Another such instrument, also developed by Dow Chemical Company, is marketed by the Fisher Scientific Company (Instrument Division, 711 Forbes Ave., Pittsburgh, Pa. 15219) as the "Aquarator". The principle of operation is similar to the Dow Beckman Carbonaceous Analyzer, except that carbon dioxide fed through the combustion tube is reduced to carbon monoxide and measured in an infrared detector. The reaction does not measure carbon, per se, but reducing materials. The results can be equated with the COD test, but the instrument

is best suited for industrial wastes and sewage. It lacks the sensitivity needed for COD measurements on relatively clean waters. The lower limit of measurement is about 40 mg/l COD.

A third instrument developed by Dow researchers is franchised to Ionics Corporation (165 Grove Street, Watertown, Mass. 02172) and sold as the "TOD Analyzer". This instrument is also similar in principle to the carbon analyzer, except that the device measures oxygen in and out of the combustion tube. Depletion of oxygen is correlated with oxygen demand of the sample. However, the reaction of oxygen with sulfur and nitrogen is not stoichiometric, and results for some samples may be questionable. A malfunction of the sample injection system appears to be the main problem with the instrument to date. An evaluation study conducted by the Hudson Delaware Basins Office, EPA, indicates a very good potential use for the instrument.

Another device, designed by Union Carbide Corporation (Ionics Inc., 65 Grove St., Watertown, Mass. 02172) is similar to the original Dow Beckman Analyzer except that the combustion tube contains a coil of heated palladium wire. An aqueous sample injected into the combustion tube is decomposed to hydrogen and oxygen through the catalytic action of the palladium. Carbon from organic matter combines with the water-produced oxygen to form carbon dioxide, with a final infrared measurement. Results obtained are theoretically identical to the Dow Beckman Analyzer, but the repeated failure of the sample injection system has prevented evaluation of the apparatus as a laboratory instrument. It is being routinely used, however, as a continuous monitoring system for total carbon.

To date the only instrument which has been successfully demonstrated and which meets the needs of EPA laboratories is the Dow Beckman Carbonaceous Analyzer or its successor, the Model 915. Operating instructions for this device are detailed elsewhere (4).

Precautions to be observed in use of the Dow Beckman Carbonaceous Analyzer or the DB Model 915 are detailed below:

a. All inorganic carbon present in the sample as carbonate or bicarbonate must be removed prior to analysis, or accounted for, when using the Model 915.

b. Particulate matter in the sample should not be larger than the opening of the hypodermic needle used to take the sample. If necessary the sample should be homogenized by some means designed to reduce the particle size.

c. The instrument should be preconditioned with repeated injections of distilled water to obtain stable operating conditions before actual sample injection.

d. Furnace temperature and oxygen flow should be maintained at proper settings during period of operation.

e. Perform periodic maintenance of instrument as follows:

    1. repack combustion tube with fresh asbestos,
    2. clean and dry micro filter,
    3. clean and dry infrared cell,
    4. recharge reference cell with nitrogen.

Need for replacement of asbestos packing is indicated by a loss of sensitivity and wide

Table 3-6.

DESIGN FEATURES OF SOME COMMON ATOMIC ABSORPTION INSTRUMENTS

| Design Feature | Inst. Labs | Jarrell-Ash | Norelco-Unicam | Perkin-Elmer | Varian | Hewlett-Packard | Bausch & Lomb | Beckman |
|---|---|---|---|---|---|---|---|---|
| Model Number | 153 | 82-500 | SP-90 | 403 | AA4 | 5960 A | AC2-20 | 979 |
| Source Arrangement | Turret-6 | Turret-6 | Turret-3 | Single Lamp | Turret-4 | Turret-6 | Turret-3 | Turret-3 |
| Optical System | Dual double-beam, beam, grating, single pass | Single-beam, a.c., grating, multipass | Single-beam a.c., prism | Double-beam, grating | Single-beam, grating | Single-beam, a.c., interference filter | Single-beam, a.c., grating, single-pass | Single-beam, grating, multi-pass |
| Mode | Absorption and emission | Absorption and emission | Absorption and emission | Absorption and emission | Absorption and emission | Absorption only | Absorption and emission plus UV visible spectro-photometry | Absorption, emission, and regular spectro-photometric analysis |
| Detector | Photomultiplier | Photomultiplier | Photomultiplier | Photomultiplier | Photomultiplier | Photomultiplier | Photomultiplier | Photomultiplier |
| Detector Substitution | Yes–Photodiode in Channel B | Yes | Yes | –– | Yes | Not given | Not given | Not given |
| Readout | Direct concentrational readout with curve corrector | Meter readout plug-in recorder | Meter readout or recorder | Digital concentration readout | Meter readout | Meter readout | Meter readout | Meter–plug-in recorder |
| Range | 190-800 mμ | 190-800 mμ | 190-770 mμ 198-850 with special photo tube | 190-800 mμ | –– | 190-800 mμ | 190-800 mμ | 190-770 mμ , 190-852 mμ optional |
| Slit | Adjustable | Adjustable | Adjustable | Adjustable | Adjustable | Fixed | Adjustable and fixed | Adjustable |
| Accessories | Data printer, sample changer, curve correction | Digital concentration readout, sample changer | Plug-in electronics, automatic sample changer with log recorder | Typewriter readout, sample changer | Digital printer with curve correction, sample changer | Recorder readout | Recorder readout, B&L concentrational readout, sample changer | Digital readout, data printer, sample changer |
| Special | 2 elements simultaneously using interference filters, push button operation | 5-pass system | plug-in electronics | Paper punch tape with typewriter to produce a typed report, push button operation | 186-1000 mμ | Push-button operation for one of six preset determinations | Dialable filters for work above 560 mμ | 3-pass system |

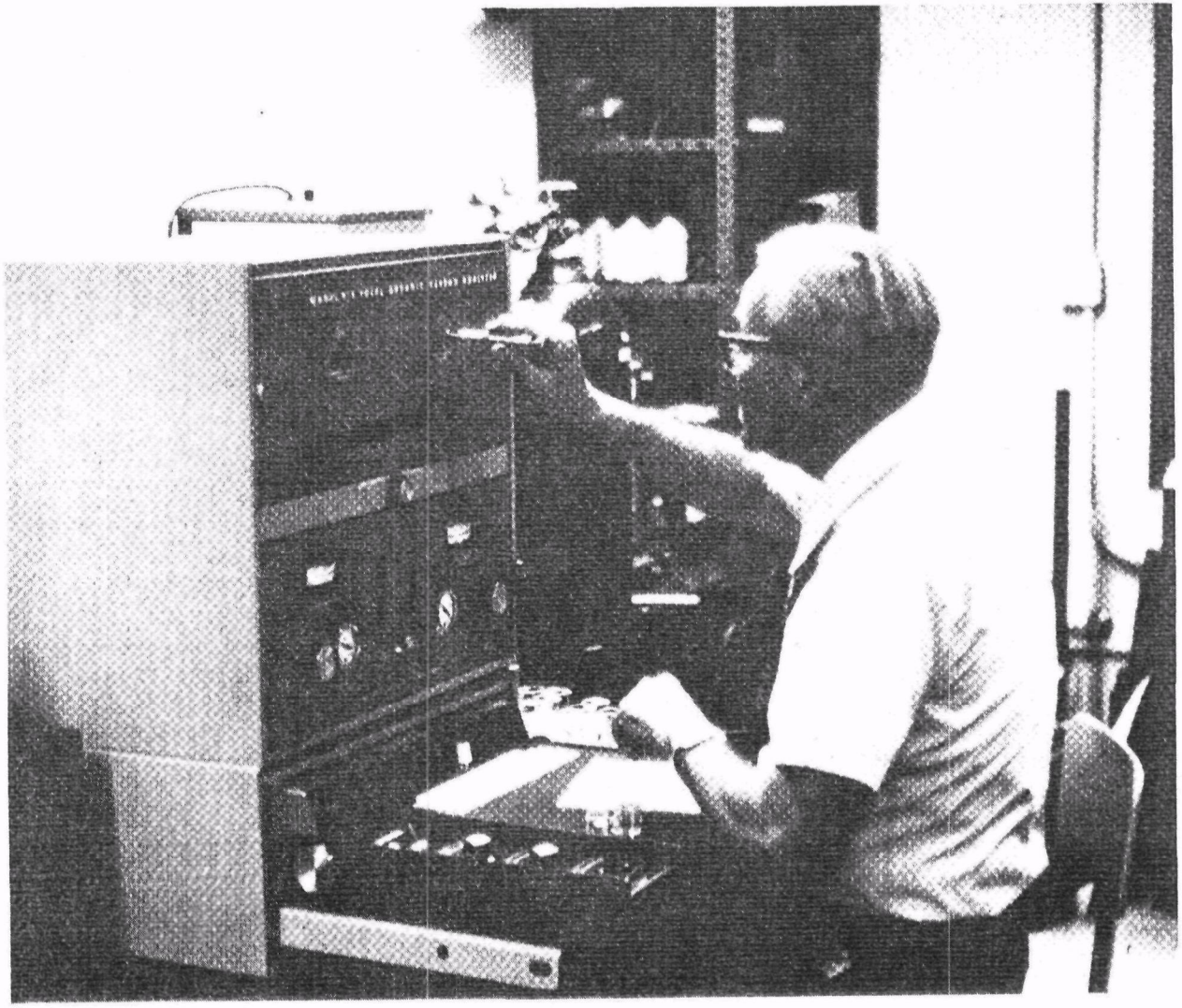

Figure 3-8. ORGANIC CARBON ANALYZER

peaks. Unsteady baseline and excessive noise is caused by a dirty or partly clogged micro filter. An accumulation of moisture in the infrared cell is indicated by loss of sensitivity and excessive noise.

### 3.8 Selective Ion Electrodes

In recent years a variety of ion-selective electrodes have been commercially available, and show great promise as fast and efficient tools for in situ monitoring and for laboratory analysis of all types of samples. A list of ions susceptible to analysis is indicative of the interest and progress being made in this field. Electrodes, or "probes" as they are popularly called, are available for measurement of monovalent cations, sulfate, nitrate, perchlorate, and a number of others. Dissolved oxygen probes should also be included in this list, although they are not technically selective ion probes. There have been a number of reviews concerning the theory and application of probes, most recently a concise article by Rechnitz (5).

Selective ion probes generally measure what they claim to measure—specific ion activity. They do not measure concentrations of un-ionized materials. For example, the probe designed to measure divalent cations promises to measure total hardness as a function of calcium and magnesium. However, since the probe does not respond to un-ionized calcium and magnesium, it does not accurately measure total hardness. As a consequence of this deficiency, much investigation is being carried out to devise means of determining total concentration of the constituent sought.

"Methods for Chemical Analysis of Water and Wastes" (4) outlined procedures for use of two probes, fluoride and DO. The chloride probe is also used on the automatic monitoring devices and will be listed as an approved procedure in future editions of EPA Methods. Evaluation of additional ion selective electrodes for cyanide, ammonium, and sulfide ions is now underway, and will be reported in the near future. Various techniques for use of the probes are reviewed by Riseman (6).

Personnel in water pollution laboratories are encouraged to investigate the use of selective ion probes as a means of reducing analytical work and improving data quality. At the present time, however, only the fluoride, DO, and chloride probes are recommended for routine use in data collection.

A basic question relating to the use of selective ion probes is the number of standards required to prepare a standard curve. It is generally agreed that the more standards used for the preparation of a colorimetric curve, the more reliable the data resulting from use of the curve. On the other hand, only one or two points are normally used in standardization of a meter for measuring pH, conductivity or DO. In the conversion of a colorimetric procedure such as fluoride to a probe-type measurement, the tendency is to prepare a millivolt vs concentration curve using the usual six to ten standards. Probe manufacturers insist that only one or two points are needed since the linearity of response has been established and only the slope of the line must be known. The alternatives are:

a. Take readings in distilled water at 0 and at some concentration approximating concentration of sample; establish slope of line.

b. Take readings in distilled water at 0 and two concentrations bracketing expected concentration of unknowns to establish curve or slope.

c. Take readings at 0 and at decade concentrations as 1, 10, 100, etc., for standard curve.

d. Take reading of sample, add known increment of measured constituent and read again; establish proportionality factor.

The method for the use of the fluoride electrode specifies use of multiple standards in the range between 0 to 2 mg/liter, because this system has supplied very precise data when compared to the colorimetric methods using the same set of standards. The system of incremental addition appears to have considerable merit since the electrode response is established in the presence of possible interferences. At the present time no single procedure for standardizing probe response has been adopted by a majority of users.

When a selective ion electrode appears to be malfunctioning, the same check system may be used as for a faulty glass pH electrode. It is unlikely, however, that the electrode will be cracked; it will probably be dry, or insufficiently filled with the necessary solution. The probe assembly and instructions for refilling customarily accompany the item when shipped by the manufacturer and said instructions should be followed by the user.

Selective ion probes are available from several manufacturers including Beekman, Corning, Coleman and Orion. The Orion organization (11 Blackstone St., Cambridge, Mass. 02139) is the largest producer in the field and offers not only selective ion probes but a sizable complement of electronic equipment for use with the probes. Figure 3-9 illustrates an Orion selective ion meter.

Dissolved oxygen probes and meters of various designs have been offered by a large number of manufacturers including Weston & Stack, Beckman, Jarrell-Ash, Union Carbide, Yellow Springs Instrument, Delta Scientific, and others.

## 3.9 References


1. 1968 Book of ASTM Standards, Part 30; Testing Single Arm Balances, pp. 1071-84: American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

2. 1968 Book of ASTM Standards, Part 23; Test for pH of Industrial Waste Water, pp. 292-3; American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

3. Black, A. P., and Hannah, S. A., "Measurement of Low Turbidities," JAWWA, 57, 901 (1965).

4. Methods for Chemical Analysis of Water and Wastes, EPA, Analytical Quality Control Laboratory, 1971.

5. Rechnitz, G. A., "Ion Selective Electrodes," Chemical Engineering News, p. 146, June 12, 1967.

6. Riseman, Jean M., "Measurement of Inorganic Water Pollutants by Specific Ion Electrode," American Laboratory, p. 32, July 1969.

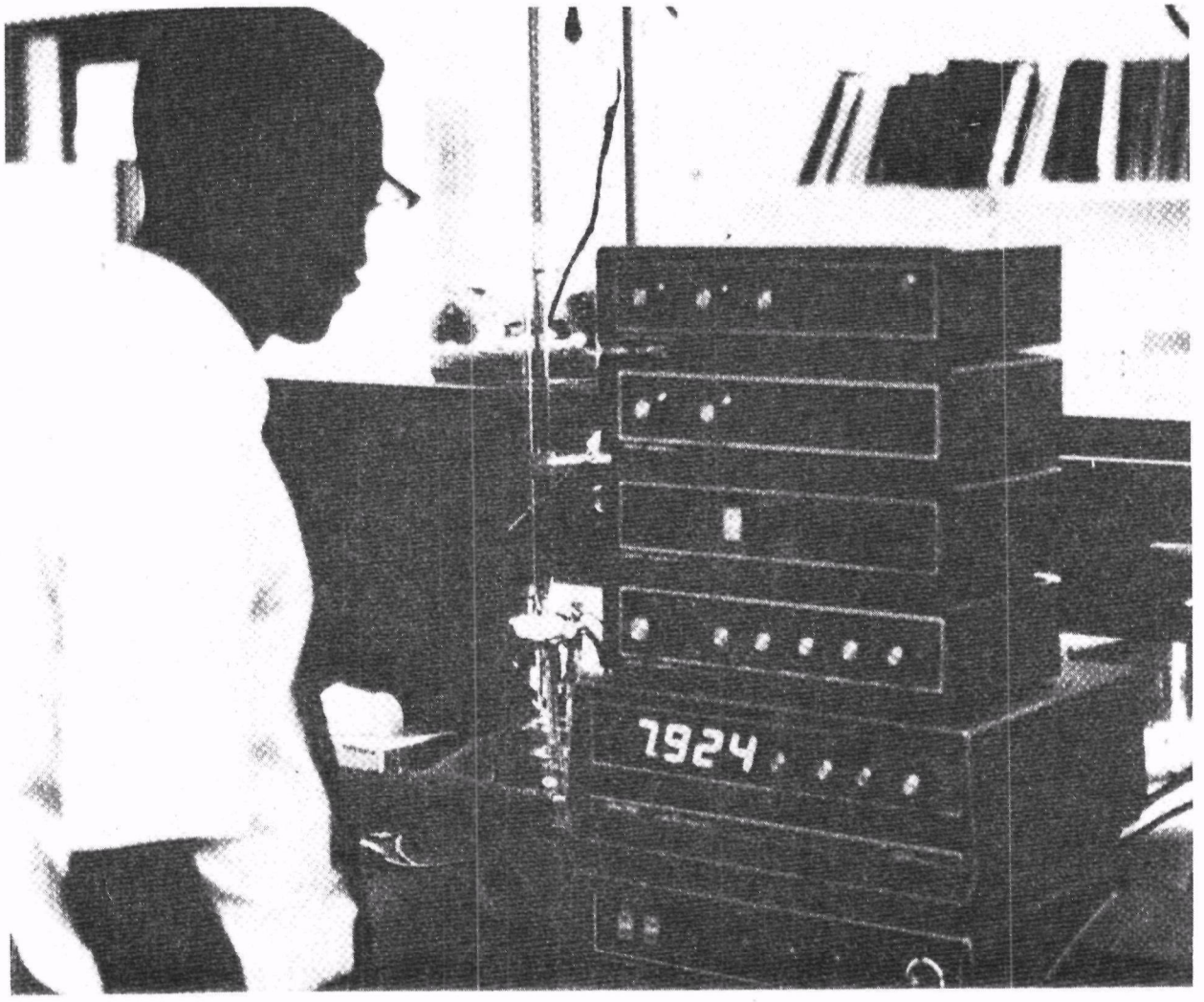


Figure 3-9. SELECTIVE ION METER

# CHAPTER 4

# GLASSWARE

## 4.1 General

The measurement of trace constituents in water demands methods capable of maximum sensitivity. This is especially true for metals and trace organics such as pesticides, as well as for the determination of ammonia and phosphorus. In addition to sensitive methods, however, there are other areas that require special consideration. One such area is that of the cleanliness of laboratory glassware. Obviously, the very sensitive analytical systems are more sensitive to errors resulting from the improper use or choice of apparatus, as well as to contamination effects due to an improper method of cleaning the apparatus. The purpose of this chapter is to discuss the kinds of glassware available, the use of volumetric ware, and various cleaning requirements.

## 4.2 Types of Glassware

Laboratory vessels serve three functions: storage of reagents, measurement of solution volumes and confinement of reactions. For special purposes, vessels made from materials such as porcelain, nickel, iron, aluminum, platinum, stainless steel, and plastic may be employed to advantage. Glass, however, is the most widely used material of construction. There are many grades and types of glassware from which to choose, ranging from student grade to others possessing specific properties such as resistance to thermal shock, alkali, low boron content, and super strength. Soft-glass containers are usually relatively soluble, and therefore are not recommended for general use, especially for storage of reagents. The mainstay of the modern analytical laboratory is a highly resistant borosilicate glass, such as that manufactured by Corning Glass Works under the name "Pyrex" or by Kimble Glass Co. as "Kimax". This glassware is satisfactory for all analyses included in "Methods for Chemical Analysis of Water and Wastes (1)."

Depending on the particular manufacturer, various trade names are used for specific brands possessing special properties such as resistance to heat, shock, alkalies, etc. Examples of some of these follow:

a. Kimax- or Pyrex-brand glass is a relatively inert all-purpose borosilicate glass.

b. Vycor-brand glass is a silica glass (96%) made to withstand continuous temperatures up to 900°C and can be down-shocked in ice water without breakage.

c. Corning brand glass is claimed to be 50 times more resistant to alkalies than conventional ware and practically boron free (max. 0.2%).

d. Ray-Sorb- or Low-Actinic-brand glass is for use with light-sensitive material.

e. Corex-brand labware is harder than conventional borosilicates, better able to resist clouding and scratching.

The use of plastic vessels, containers and other apparatus made of Teflon, polyethylene, polystyrene and polyproplylene has increased markedly over recent years. Some of these materials, such as Teflon, are quite expensive; however, Teflon stopcock plugs have

practically replaced glass plugs in burets, separatory funnels, etc. because lubrication to avoid sticking or "freezing" is not required. Polyprolylene, a methylpentene polymer, is available as laboratory bottles, graduates, beakers and even volumetric flasks. It is crystal clear, shatter-proof, autoclavable and chemically resistant.

Some points to consider in choosing glassware and/or plasticware are:

a. Generally, the special types of glass listed above are not required to perform the analyses given in "Methods for Chemical Analysis of Water and Wastes" (1).

b. Unless instructed otherwise, borosilicate or polyethylene bottles are to be used for the storage of reagents and standard solutions.

c. Certain dilute metal solutions may plate out on glass container walls over long periods of storage. Thus, dilute metal standard solutions are prepared fresh at the time of analysis.

d. For some operations, disposable glassware is entirely satisfactory. One example is the use of disposable test tubes as sample containers for use with the Technicon Automatic Sampler.

e. Plastic bottles of polyethylene and/or Teflon have been found satisfactory for the shipment of water samples. Strong mineral acids (such as sulfuric acid) and organic solvents will readily attack polyethylene and are to be avoided.

f. Borosilicate glassware is not completely inert, particularly to alkalies; therefore, standard solutions of silica, boron and the alkali metals are usually stored in polyethylene bottles.

For additional information the reader is referred to the catalogs of the various glass and plastic manufacturers. These catalogs contain a wealth of information as to specific properties, uses, sizes, etc.

### 4.3 Volumetric Analyses

By common usage, accurately calibrated glassware for precise measurements of volume has become known as volumetric glassware. This group includes volumetric flasks, volumetric pipets and accurately calibrated burets. Less accurate types of glassware including graduated cylinders, serological and measuring pipets also have specific uses in the analytical laboratory, when exact volumes are unnecessary. A typical laboratory glassware setup is shown in Figure 4-1.

The precision of volumetric work depends in part upon the accuracy with which volumes of solutions can be measured and there are certain sources of error which must be carefully considered. The volumetric apparatus must be read correctly; that is, the bottom of the meniscus should be tangent to the calibration mark. There are other sources of error, however, such as changes in temperature which result in changes in the actual capacity of glass apparatus and in the volume of the solutions.The capacity of an ordinary glass flask of 1000 ml volume increases 0.025 ml per degree rise in temperature, but if made of borosilicate glass the increase is much less. One thousand ml of water or of most 0.1 N solutions increases in volume by approximately 0.20 ml per 1°C increase at room

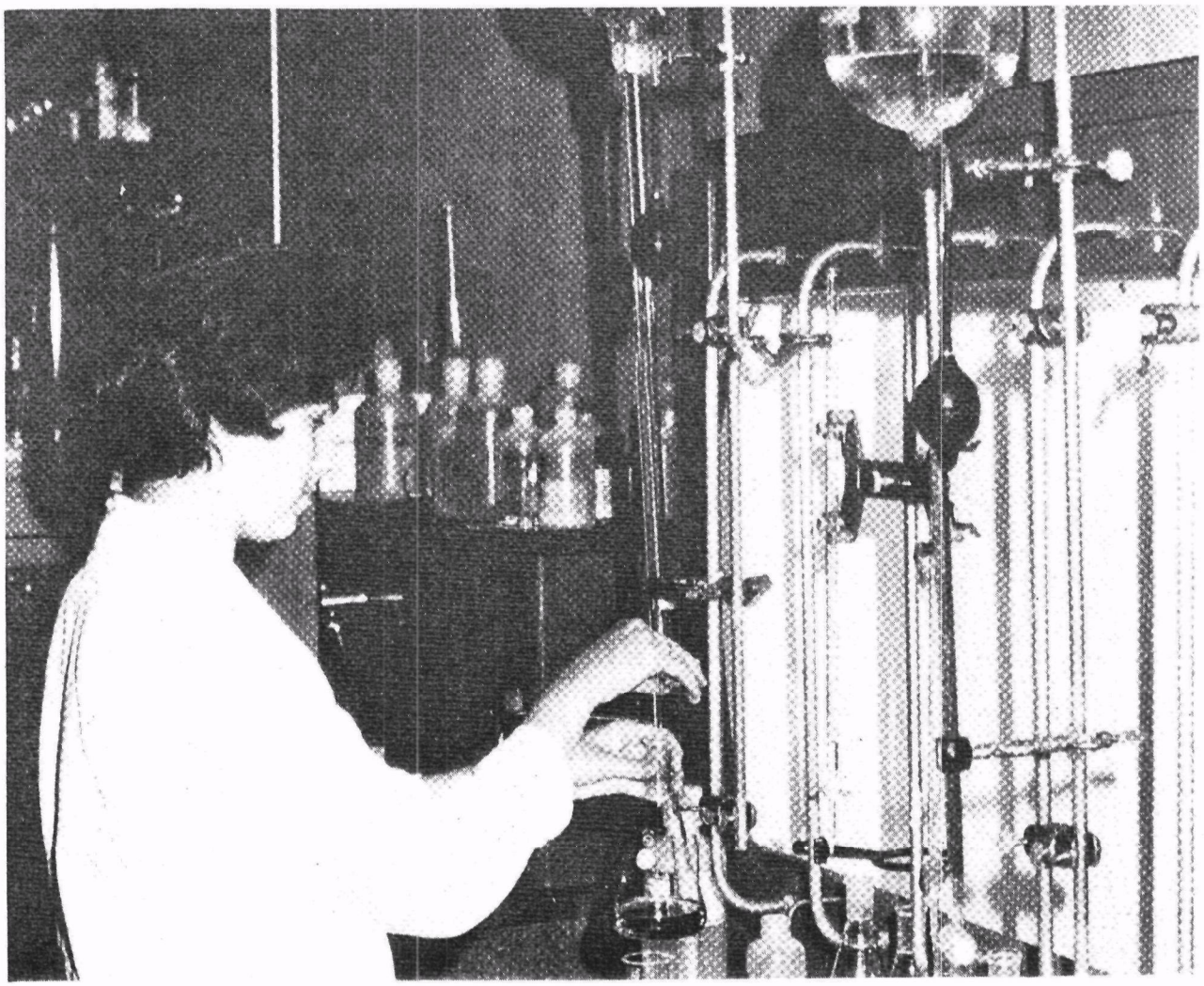

Figure 4-1. TITRATION BENCH

temperature. Thus solutions must be measured at the temperature at which the apparatus was calibrated. This temperature (usually 20°C) will be indicated on all volumetric ware. There may also be errors of calibration of the apparatus; that is, the volume marked on the apparatus may not be the true volume. Such errors can be eliminated only by recalibrating the apparatus or by replacing it.

Volumetric apparatus is calibrated "to contain" or "to deliver" a definite volume of liquid. This will be indicated on the apparatus with the letters "TC" (to contain) or "TD" (to deliver). Volumetric flasks are calibrated to contain a given volume. They are available in various shapes and sizes ranging from 1- to 2000-ml capacity.

Volumetric pipets are calibrated to deliver a fixed volume. The usual capacities are 1 thru 100 ml although micro-pipets are also available. In emptying volumetric pipets, they should be held in a vertical position and the outflow should be unrestricted. The tip of the pipet is kept in contact with the wall of the receiving vessel for a second or two after the free flow has stopped. The liquid remaining in the tip is not removed; this is most important.

Measuring and serological pipets should also be held in a vertical position for dispensing liquids; however, the tip of the pipet is only touched to the wet surface of the receiving vessel after the outflow has ceased. For those pipets where the small amount of liquid remaining in the tip is to be blown out and added, indication is made by a frosted band near the top. The band is usually located far enough down so that it will not touch the technician's lips when liquid is being drawn up or blown out.

Burets are used to deliver definite volumes. The more common types are usually of 25- or 50-ml capacity, graduated to tenths of a milliliter, and are provided with stopcocks. For precise analytical methods in microchemistry, micro-burets are also used. Micro-burets generally are of 5- or 10-ml capacity, graduated in hundredths of a milliliter division. Automatic burets with reservoirs are also available ranging in capacity from 10 to 100 ml. Reservoir capacity ranges from 100 to 4000 ml.

General rules in regard to the manipulation of a buret are as follows: Do not attempt to dry a buret which has been cleaned for use, but rinse it two or three times with a small volume of the solution with which it is to be filled. Do not allow alkaline solutions to stand in a buret, because the glass will be attacked, and the stopcock, unless made of Teflon, will tend to freeze. A 50-ml buret should not be emptied faster than 0.7 ml per second, otherwise too much liquid will adhere to the walls and as the solution drains down, the meniscus will gradually rise, giving a high false reading. It should be emphasized that improper use of and/or reading of burets can result in serious calculation errors.

In the case of all apparatus for delivering liquids, the glass must be absolutely clean so that the film of liquid never breaks at any point. Careful attention must be paid to this fact or the required amount of solution will not be delivered. The various cleaning agents and their use are described later.

### 4.4 Federal Specifications for Volumetric Glassware

Circular 602 of the National Bureau of Standards, "Testing of Glass Volumetric Apparatus", describes the Federal Specifications for volumetric glassware. The National Bureau of Standards no longer accepts stock quantities of volumetric apparatus from manufacturers or dealers for certification and return for future sale to consumers. This certification service is

still available, but apparatus will be tested only when submitted by the ultimate user, and then only after an agreement has been reached with the Bureau concerning the work to be done.

Consequently, the various glass manufacturers have discontinued the listing of NBS-certified ware. In its place catalogue listings of volumetric glass apparatus which meet the Federal Specifications are designated as Class A and all such glassware is permanently marked with a large "A". These NBS specifications are listed in Table 4-1. The ware in question includes the usual burets, volumetric flasks and volumetric pipets.

Table 4-1

TOLERANCES FOR VOLUMETRIC GLASSWARE
(Abridged from National Bureau of Standards Data, 1941)

| Capacity (ml) less than and including | Limit of error (ml) |
|---|---|
| **Graduated Flasks** | |
| 25 | 0.03 |
| 50 | 0.05 |
| 100 | 0.08 |
| 200 | 0.10 |
| 250 | 0.11 |
| 300 | 0.12 |
| 500 | 0.15 |
| 1,000 | 0.30 |
| 2,000 | 0.50 |
| **Transfer pipets** | |
| 2 | 0.006 |
| 5 | 0.01 |
| 10 | 0.02 |
| 25 | 0.025 |
| 30 | 0.03 |
| 50 | 0.05 |
| 100 | 0.08 |
| 200 | 0.10 |
| **Burets[1]** | |
| 5 | 0.01 |
| 10 | 0.02 |
| 30 | 0.03 |
| 50 | 0.05 |
| 100 | 0.10 |

[1] *Limits of error are of total or partial capacity. Customary practice is to test the capacity at 5 intervals.*

In addition to the "A" marking found on calibrated glassware and the temperature at which the calibration was made, other markings also appear. These include the type of glass, such as Pyrex, Corex, Kimax, etc., the stock number of the particular item, and the capacity of the vessel. If the vessel contains a ground-glass connection, this will also be included along with the TD or TC symbol. An example of the markings usually found on volumetric ware is shown in Figure 4-2.

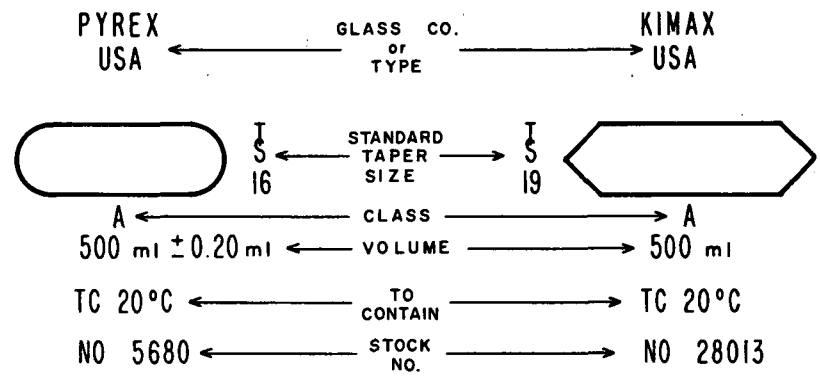


Figure 4-2. EXAMPLE OF MARKINGS ON GLASSWARE

Class A glassware need not be recalibrated before use. However, if it should become necessary to calibrate a particular piece of glassware, directions may be found in texts (2) on quantitative analysis.

### 4.5 Cleaning of Glass and Porcelain

The method of cleaning should be adapted to both the substances that are to be removed, and the determination to be performed. Water-soluble substances are simply washed out with hot or cold water, and the vessel is finally rinsed with successive small amounts of distilled water. Other substances more difficult to remove may require the use of a detergent, organic solvent, dichromate cleaning solution, nitric acid or aqua regia (25 percent v/v conc. $HNO_3$ in conc. HCl). In all cases it is good practice to rinse a vessel with tap water as soon as possible after use. Material allowed to dry on glassware is much more difficult to remove.

Volumetric glassware, especially burets, may be thoroughly cleaned by a mixture containing the following: 30 g sodium hydroxide, 4 g sodium hexametaphosphate (trade name, Calgon), 8 g trisodium phosphate, and 1 liter water. A gram or two of sodium lauryl sulfate or other surfactant will improve its action in some cases. This solution should be used with a buret brush.

Dichromate cleaning solution (chromic acid) is a powerful cleaning agent; however, due to its destructive nature upon clothing and upon laboratory furniture, extreme care must be taken when using this mixture. If any of the solution is spilled, it must be cleaned up

immediately. Chromic acid solution may be prepared in the laboratory by adding 1 liter of concentrated sulfuric acid slowly, with stirring, to 35 ml saturated sodium dichromate solution. This mixture must be allowed to stand for approximately 15 minutes in the vessel which is being cleaned and may then be returned to a storage bottle. Following the chromic-acid wash, the vessels are rinsed thoroughly with tap water, then with small successive portions of distilled water. Fuming nitric acid acts more rapidly, but is disagreeable to handle. In either case, when the acid becomes dilute, the cleaning mixture is no longer effective. A mixture of concentrated sulfuric and fuming nitric acids is even more efficient but is also hazardous to use. A persistent greasy layer or spot may be removed by acetone or by allowing a warm solution of sodium hydroxide, about 1 g per 50 ml of water, to stand in the vessel for 10-15 minutes; after rinsing with water, dilute hydrochloric acid, and water again, the vessel is usually clean. Alcoholic potassium hydroxide is also effective in removing grease. To dry glass apparatus, rinse with acetone and blow or draw air through it.

### 4.6 Special Cleaning Requirements

Absorption cells, used in spectrophotometers, should be kept scrupulously clean, free of scratches, fingerprints, smudges and evaporated film residues. The cells may be cleaned with detergent solutions for removal of organic residues, but should not be soaked for prolonged periods in caustic solutions because of the possibility of etching. Organic solvents may be used to rinse cells in which organic materials have been used. Nitric acid rinses are permissible, but dichromate solutions are not recommended because of the adsorptive properties of dichromate on glass. Rinsing and drying of cells with alcohol or acetone before storage is a preferred practice. Matched cells should be checked to see that they are equivalent by placing portions of the same solution in both cells and taking several readings of the transmittance (%T) or optical density (OD) values. If a cell is mismatched it should be discarded or reserved for rough work.

For certain determinations, especially trace metals, the glassware should also be rinsed with a 1:1 nitric acid-water mixture. This operation is followed by thoroughly rinsing with tap water and successive portions of distilled water. This may require as many as 12-15 rinses, especially if chromium is being determined. The nitric acid rinse is also especially important if lead is being determined.

Glassware to be used for phosphate determinations should not be washed with detergents containing phosphates. This glassware must be thoroughly rinsed with tap water and distilled water. For ammonia and Kjeldahl nitrogen, the glassware must be rinsed with ammonia-free water (See Chapter 2).

Glassware to be used in the determination of trace organic constituents in water, such as chlorinated pesticides, should be as free as possible of organic contaminants. A chromic acid wash of at least 15 minutes is necessary to destroy these organic residues. Rinse thoroughly with tap water, and finally with distilled water. Glassware may be dried for immediate use by rinsing with redistilled acetone. Otherwise glassware may be oven dried or drip dried. Glassware should be stored immediately after drying to prevent any accumulation of dust. Store inverted or with mouth of glassware covered with foil.

Bottles to be used for the collection of samples for organic analyses should be rinsed successively with chromic acid cleaning solution, tap water, distilled water, and finally several times with redistilled solvent (e.g., acetone, hexane, petroleum ether, chloroform).

Caps are washed with detergent, rinsed with tap water, distilled water and solvent. Liners are treated in the same way as the bottles and are stored in a sealed container.

### 4.7 Disposable Glassware

When the risk of washing a pipet for reuse becomes too great, as in the case of use with toxic materials, or when the cost of washing glassware becomes prohibitive, disposable pipets may be the answer, provided they meet the necessary specification. Various types are available including bacteriological, serological and micro-dilution pipets. Disposable glassware generally is made of soft glass.

### 4.8 Specialized Glassware

The use of vessels and glassware fitted with standard-taper, ground-glass, and ball-and-socket joints has increased because of certain advantages such as less leakage and fewer freezeups. Standard-taper, interchangeable ground joints save time and trouble in assembling apparatus. They are precision-ground with tested abrasives to insure an accurate fit and freedom from leakage. Ball and socket joints increase flexibility of operation and eliminate the need for exact alignments of apparatus. Symbols and their meaning as applied to standard joints, stoppers and stopcocks are shown below.

#### 4.8.1. Standard Taper (T)

T is the symbol used to designate interchangeable joints, stoppers and stopcocks, complying with the requirements of Commercial Standard CS-21, published by the National Bureau of Standards. All mating parts are finished to a 1:10 taper.

The size of a particular piece appears after the appropriate symbol. Due primarily to the greater variety of apparatus equipped with T fittings, a number of different types of identifications are used, as follows:

a. For joints—a two-part number, as T 24/40, with 24 being the approximate diameter in mm at the large end of the taper, and 40 the axial length of taper, also in mm.

b. For stopcocks—a single number, as T 2, with 2 mm being the approximate diameter of the hole or holes through the plug.

c. For bottles—a single number, as T 19, with 19 mm being the appropriate diameter at top of neck. However, there are differences in dimensions between the bottle and flask stoppers.

d. For flasks, etc. – a single number, as T 19, with 19 mm being the appropriate diameter of the opening at top of neck.

#### 4.8.2 Spherical Joints (S)

S is the designation for spherical (semi-ball) joints complying with CS-21. The complete designation of a spherical joint also consists of a two-part number, as 12/2, with 12 being the approximate diameter of the ball and 2 the bore of the ball and the socket, also in mm.

### 4.8.3 Product Standard (℘)

℘ is a new symbol. It will appear in a forthcoming NBS Product Standard for stopcocks with Teflon plugs, with the mating surfaces being finished to a 1:5 taper. As with ℑ stopcocks, a single number is used. Thus, ℘ 2 means a Teflon stopcock with a hole of approximately 2-mm diameter in the plug.

### 4.9 Fritted Ware

For certain laboratory operations the use of Fritted Ware for filtration (as in total dissolved solids and suspended solids determinations), gas dispersion, absorption, and/or extractions may be of an advantage.

There are six different porosities of Fritted Ware available, so that precipitates varying in size can be filtered at maximum speed with no sacrifice or retentivity. Porosity is controlled in manufacture, and discs are individually tested and graded into these classifications. The extra-coarse and coarse porosities are held toward the maximum pore diameter as listed. The medium, fine, very fine, and ultra-fine are held toward the minimum pore diameter as listed in Table 4-2.

Table 4-2

FRITTED WARE POROSITY

| Porosity Grade | Designation | Pore Size (Microns) | Principal Uses |
|---|---|---|---|
| Extra Coarse | EC | 170-220 | Coarse filtration. Gas dispersion, washing, absorption. |
| Coarse | C | 40-60 | Coarse filtration. Gas dispersion, washing, absorption. |
| Medium | M | 10-15 | Filtration and extraction. |
| Fine | F | 4-5.5 | Filtration and extraction. |
| Very Fine | VF | 2-2.5 | General bacterial filtration. |
| Ultra-Fine | UF | 0.9-1.4 | General bacterial filtration. |

Pore sizes are determined by the method specified in ASTM E 128, "Maximum Pore Diameter and Permeability of Rigid Porous Filters for Laboratory Use." (3)

### 4.9.1 Recommended Procedures for Maximum Filter Life

a. New Filters. Wash new filters by suction with hot hydrochloric acid, followed by a water rinse.

b. Pressure Limits. The maximum safe differential pressure on a disc is 15 pounds per square inch.

c. Thermal Shock. Fritted ware has less resistance to thermal shock than non-porous glassware. Hence, excessive, rapid temperature changes and direct exposure to a flame should be avoided. Heating in a furnace to 500°C may be done safely, provided the heating and cooling are gradual. Dry ware may be brought to constant weight by heating at 105-110°C.

Never subject a damp filter of ultra-fine porosity to a sudden temperature change. Steam produced in the interior may cause cracking.

### 4.9.2 Cleaning of Used Filters

In many cases, precipitates can be removed by rinsing with water, passed through from the underside, with the pressure not exceeding 15 pounds per square inch. The suggestions that follow in Table 4-3 will be helpful in dealing with material that will not be removed by the reverse water-wash. The use of strong alkalies, strong hydrofluoric acid and phosphoric acid should be avoided. Also, scratching of the surfaces will weaken the discs.

Table 4-3

CLEANING OF FILTERS

| Material | Removal Agent |
|---|---|
| Albumen | Hot ammonia or hydrochloric acid |
| Aluminous and siliceous residues | 2% Hydrofluoric acid followed by concentrated sulfuric acid. Rinse immediately with water until no trace of acid can be detected. |
| Copper or iron oxides | Hot hydrochloric acid plus potassium chlorate. |
| Fatty materials | Carbon tetrachloride |
| Mercuric sulfide | Hot aqua regia |
| Organic matter | Hot concentrated cleaning solution, or hot concentrated sulfuric acid with a few drops of sodium nitrite. |
| Silver chloride | Ammonium or sodium hyposulfite. |

## 4.10 References


1. "Methods for Chemical Analysis of Water and Wastes," EPA, Analytical Quality Control Laboratory, 1971.

2. Willare, H. H., and Furman, N. H., Elementary Quantitative Analysis--Theory and Practice, D. Van Nostrand Co., Inc., New York (1947).

3. 1968 Book of ASTM Standards, Part 30, American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

# Chapter 5

# REAGENTS, SOLVENTS, AND GASES

## 5.1 Introduction

The objective of this chapter is to provide general information and suggestions that will serve to keep the analyst conscious of his responsibilities in analytical quality control, as they relate to reagents, solvents and gases. While the material presented here will assist the analyst in producing high quality data, it is by no means complete. It is incumbent on the analyst to obtain details of special precautions required to insure proper selection, preparation, and storage of reagents, solvents and gases from the descriptions of individual methods.

## 5.2 Reagent Quality

Chemical reagents, solvents, and gases are available in a wide variety of grades of purity, ranging from technical grade to various "ultra pure" grades. The purity of these materials required in analytical chemistry varies with the type of analysis. The parameter being measured and the sensitivity and specificity of the detection system are important factors in determining the purity of the reagents required. For many analyses, e.g., most inorganic analyses, analytical reagent grade is satisfactory. Other analyses, e.g., trace organic and radiological, frequently require special "ultra pure" reagents, solvents, and gases. In methods where the purity of reagents is not specified it is intended that analytical reagent grade be used. Reagents of lesser purity than that specified by the method should not be used. The labels on the container should be checked and the contents examined to verify that the purity of the reagents meets the needs of the particular method involved. The quality of reagents, solvents, and gases required for the various classes of analyses: inorganic, metals, radiological, and organic, are discussed below.

Reagents must always be prepared and standardized with the utmost of care and technique, against reliable primary standards. They must be restandardized or prepared fresh as often as required by their stability. Stock and working standard solutions must be checked regularly for signs of deterioration, e.g., discoloration, formation of precipitates, and concentration. Standard solutions should be properly labeled as to compound, concentration, solvent, date, and preparer.

Primary standards must be obtained from a reliable source, pretreated, e.g., dried, under specified conditions, accurately prepared in calibrated volumetric glassware, and stored in containers that will not alter the reagent. A large number of primary standards are available from the National Bureau of Standards (NBS). A complete listing of available standards is given in NBS Special Publication 260 (1). Primary standards may also be obtained from many chemical supply companies. Suppliers for special quality reagents, solvents, and gases are noted in later discussions of the various classes of analyses. Reagents and solvents of all grades are available from many chemical supply houses.

There is some confusion among chemists as to the definition of the terms ANALYTICAL REAGENT GRADE, REAGENT GRADE, and ACS ANALYTICAL REAGENT GRADE. A review of the literature and chemical supply catalogs indicates that the three terms are synonymous. Hereafter, in this document, the term ANALYTICAL REAGENT GRADE

(AR) will be used. It is intended that AR chemicals and solvents shall conform to the current specifications of the Committee on Analytical Reagents of the American Chemical Society (2).

The ASTM Manual on Industrial Water and Industrial Waste Water (3), Part 23 of ASTM Standards (4), and "Standard Methods for the Examination of Water and Wastewater" (5) devote separate chapters to problems related to preparation, standardization, and storage of reagents. The information provided therein is particularly appropriate to inorganic determinations. The type of volumetric glassware to be used, the effect of certain reagents on glassware, the effect of temperature on volumetric measurements, purity of reagents, absorption of gases and water vapor from the air, standardization of solutions, instability, and need for frequent standardization of certain reagents are among the topics discussed. It is recommended that the analyst become thoroughly familiar with these publications.

### 5.2.1 General Inorganic Analyses

In general, AR-grade reagents and solvents are satisfactory for inorganic analyses. Primary standard reagents must, of course, be used for standardizing all volumetric solutions. Commercially prepared reagents and standard solutions are very convenient and may be used when it is demonstrated that they meet the method requirements. All prepared reagents must be checked for accuracy.

The individual methods specify the reagents that require frequent standardization, or other special treatment, and the analyst must follow through with these essential operations. To avoid waste, the analyst should prepare a limited volume of such reagents, depending on the quantity required over a given period of time. Examples and brief discussions of the kind of problems that occur are given under Paragraph 5.3, "Elimination of Determinate Errors".

As far as possible, distilled water used for preparation of reagent solutions must be free of measurable amounts of the constituent to be determined. Special requirements for distilled water are given in Chapter 3 of this manual and in individual method descriptions.

Compressed gases, such as oxygen and nitrogen, used for total organic carbon determination may be of commercial grade.

### 5.2.2 Metals Analyses

All standards used for atomic absorption and emission spectroscopy should be of spectroquality. It is recommended that other reagents and solvents also be of spectroquality, although AR grade is sometimes satisfactory. Standards may be prepared by the analyst in the laboratory or prepared, spectrographically standardized materials may be purchased commercially. Standards required for determination of metals in water are not generally available from the National Bureau of Standards.

Analytical reagent grade nitric and hydrochloric acids must be specially prepared by distillation in borosilicate glass and diluted with deionized distilled water. All other reagents and standards are also prepared in deionized water.

In general, fuel and oxidant gases used for atomic absorption can be of commercial grade. Air supplied by an ordinary laboratory compressor is quite satisfactory, if adequate pressure is maintained and necessary precautions are taken to filter oil, water, and possible trace

metals from the line. For certain determinations, e.g., aluminum, reagent-grade nitrous oxide is required.

### 5.2.3 Radiological Analyses

The great sensitivity of radioactive counting instruments requires that scintillation grade reagents and solvents, or equivalent, be used for all radioactivity determinations. Some of the reagents, for example, strontium carbonate and yttrium oxide carriers used for the determination of strontium 90 and yttrium 90, must be stable, that is free of radioactivity. Barium sulfate, used for coprecipitation of radium must be free from all traces of radium. These reagents and solvents are commercially available from chemical supply houses.

Calibrated standard sources of specific radioactive materials with known count and date of counting are available from various suppliers. No single company supplies all standards.

Gases used for radioactive counting must be of high purity and extra dry. Gases such as helium and air are aged for about 30 days to allow radioactive background to decay. All gases are checked for background before use. Some cylinders contain inherent radioactivity which is imparted to the gas. When this background is above normal, the gas should not be used for radioactivity determinations.

### 5.2.4 Organic Chemical Analyses

The minimum purity of reagents and solvents that can be used for organic analyses is AR grade. Reference grade standards should be used whenever available. Special note should be taken of the assay of standard materials. Owing to the great sensitivity (nanogram and subnanogram quantities) of gas chromatography (GC), which is often used to quantitate organic results, much greater purity is frequently required (6). The specificity of some GC detectors requires that reagents and solvents be free of certain classes of compounds. For example, analyses by electron capture require that reagents and solvents be free of electronegative materials that would interfere with the determination of specific compounds in the sample. Similarly, use of the flame photometric detector requires that reagents and solvents be free from sulfur and/or phosphorus interference. Pesticide quality solvents are available from several sources. These are often satisfactory for many organic GC determinations. However, the contents of each container must be checked to assure its suitability for the analyses. Similarly, all analytical reagents and other chemicals must also be checked routinely.

The quality of gases required for GC determinations varies somewhat with the type of detector. In general, the compressed gases are a prepurified dry grade. Grade A helium from the U.S. Bureau of Mines has always been satisfactory. The Dohrmann nitrogen-detection system requires the use of ultra-pure hydrogen for satisfactory results. The use of molecular-sieve, carrier-gas filters and drying tubes is required on combustion gases. They are recommended for use on all other gases. It is recommended that the analyst familiarize himself with an article by Burke (7) on practical aspects of gas chromatography.

All reagents, solvents, and adsorbents used for thin-layer chromatography must be checked to be certain that there are no impurities present that will react with the chromogenic reagent or otherwise interfere with subsequent qualitative or quantitative determinations. Glass-backed layers prepared in the laboratory or precoated layers supplied by a manufacturer may be used. However, precoated layers are more difficult to scrape.

Therefore, it is recommended that layers prepared in the laboratory be used when zones are to be scraped in order to recover isolated compounds. Plastic-backed layers are generally unsatisfactory for this type of analysis.

Adsorbents most commonly used for column chromatographic clean-up of sample extracts are Florisil, silica gel, and alumina. These must be pre-activated according to the method specifications and checked for interfering constituents.

### 5.3 Elimination of Determinate Errors

In order to produce high quality analytical data, determinate errors must be eliminated or at least minimized. For purposes of this discussion, we assume that a competent analyst and reliable equipment, in optimum operating condition, are available. Thus, determinate errors that might result from an inexperienced or careless analyst and poor equipment are eliminated. The remaining sources of error are the reagents, solvents, and gases that are used throughout the analyses. The quality of these materials, even though they are AR grade or better, may vary from one source to another, from one lot to another, and even within the same lot. Therefore, the analyst must predetermine that all of these materials are free of interfering substances under the conditions of the analyses. To do this he must have a regular check program. Materials that do not meet requirements are replaced or purified so that they can be used.

#### 5.3.1 Reagent Blank

The first step the analyst must take is to determine the background or blank of each of the reagents and solvents used in a given method of analysis. The conditions for determining the blank must be identical to those used throughout the analysis, including the detection system. If the reagents and solvents contain substances that interfere with a particular determination, satisfactory reagents and solvents must be found. Where possible and practical, they should be treated so that they can be used.

#### 5.3.2 Method Blank

After determining the individual reagent or solvent blanks, the analyst must determine the method blank to see if the cumulative blank interferes with the analyses. The method blank is determined by following the procedure step by step, including all of the reagents and solvents, in the quantity required by the method. If the cumulative blank interferes with the determination, steps must be taken to eliminate or reduce the interference to a level that will permit this combination of solvents and reagents to be used. If the blank cannot be eliminated, the magnitude of the interference must be considered when calculating the concentration of specific constitutents in the samples being analyzed.

A method blank should be determined whenever an analysis is made. The number of blanks to be run is determined by the method of analysis and the number of samples being analyzed at a given time. In some methods, such as the AutoAnalyzer procedures, the method blank is automatically and continuously compensated for since a continuous flow of the reagents passes through the detector. In other procedures, such as the gas chromatographic determination of pesticides, a method blank is run with each series of samples analyzed. Usually this is one blank for every nine samples.

### 5.3.3 Elimination of Interferences and Other Sources of Error

Procedures for eliminating or at least minimizing impurities that produce specific interferences or high general background, vary with the reagent and method involved. These procedures may include: recrystallization, precipitation, distillation, washing with an appropriate solvent, or a combination of these. Examples of procedures used for various types of analyses are given below. For complete information, the analyst should consult the individual methods.

#### 5.3.3.1 General Inorganic Analyses

Analytical reagent grade chemicals and solvents usually present no interference problems in inorganic analyses. However, some reagents do not always meet methods requirements. An example is potassium persulfate used in phosphorus and nitrogen determinations. This reagent is frequently contaminated with ammonia. Therefore, it is routinely purified by passing air through a heated water solution of the reagent. The purified potassium persulfate is recovered by recrystallization.

A problem more commonly encountered in inorganic analyses is the rapid deterioration of the standard reagents and other ingredients. To minimize or eliminate this problem some reagents, for example, ferrous ammonium sulfate, must be standardized daily. Others, such as sodium thiosulfate used for dissolved oxygen determination, may require a substitute reagent, e.g., phenyl arsene oxide. Solid phenol which readily oxidizes and acquires a reddish color can be purified by distillation. Starch indicator used for idiometric titrations may be prepared for each use or preserved by refrigeration, or by addition of zinc chloride or other suitable compounds.

#### 5.3.3.2 Metals Analyses

In general, spectrograde chemicals, solvents, and gases present no interference problems in atomic absorption or emission spectrographic determinations. However, standards which do not meet the requirements of the method are sometimes obtained. Ordinarily, no effort is made to purify them. They are simply replaced by new reagents of sufficient purity. Some reagents may form precipitates on standing. Such reagents will reduce the accuracy of quantitative analyses and should not be used.

#### 5.3.3.3 Radiological Analyses

In general, reagents that do not meet the purity requirements for radiological determinations are replaced with reagents that are satisfactory. However, in some instances (for example, barium sulfate used for coprecipitation of radium), it may be necessary to carry out repeated recrystallization to remove all forms of radium, and reduce the background count to a useable level. In some instances, solvents that do not meet requirements may be distilled to produce adequate purity. In some cases, gases having background counts may be useable after aging as described earlier. If not, they should be replaced with gases that are satisfactory.

#### 5.3.3.4 Organic Analyses

Many AR-grade chemicals and solvents, and at times pesticide quality solvents, do not meet the specifications required for the determination of specific organic compounds. Impurities

that are considered trace, or insignificant, for many analytical uses, are often present in greater quantities than the organic constituents being measured. Coupled with the several-hundred-fold concentration of the sample extract that is usually required, such impurities can cause very significant interferences in trace organic analyses. Reagents and solvents found to be unsatisfactory, under the conditions of the analyses, must be replaced or cleaned up so that they are useable. Some useful clean-up procedures are:

a. Washing the inorganic reagents with each solvent that the reagent contacts during the analysis,

b. Washing the adsorbents, such as silica gel G and Florisil, with the solvents that are used for a specified column or thin-layer chromatographic procedure,

c. Pre-extracting distilled water with solvents used for the particular analysis involved,

d. Pre-extracting aqueous reagent solutions with the solvents involved,

e. Redistilling solvents in all-glass systems using an efficient fractionating column,

f. Recrystallizing reagents and dyes used in colorimetric or thin-layer determinations,

If the reagents and solvents thus produced are not of sufficient purity, they should be replaced.

Dirty gases (quality less than specified) are particularly troublesome in gas chromatographic analyses. They may reduce the sensitivity of the detector, and produce a high or noisy baseline. If this occurs, the cylinder should be replaced immediately. Similarly, if cylinders of compressed gases are completely emptied in use, the end volumes of the gas may produce a similar and often more severe effect. Oils and water may get into the system and foul the detector. When this occurs the system must be dismantled and cleaned. Overhaul of the detector may be required. To reduce chances of this, it is recommended that all gas cylinders be replaced when the pressure falls to 100-200 psi. Filter driers are of little help in coping with this type of contamination.

### 5.3.4 Storing and Maintaining Quality of Reagents and Solvents

Having carried out the tasks of selecting, preparing, and verifying the suitability of reagents, solvents, and gases, the analyst must properly store them to prevent contamination and deterioration prior to their use. Borosilicate glass bottles with ground glass stoppers are recommended for most standard solutions and solvents. Plastic containers, e.g., polyethylene, are recommended for alkaline solutions. Plastic containers must not be used for reagents or solvents intended for organic analyses. However, plastic containers may be used for reagents not involved with organic analyses if they maintain a constant volume, and it is demonstrated that they do not produce interferences and do not absorb constitutents of interest. It is important that all containers be properly cleaned and stored prior to use. (Refer to Chapter 4 for details).

Standard reagents and solvents must always be stored according to the manufacturer's directions. Reagents or solvents that are sensitive to the light should be stored in dark bottles and/or stored in a cool, dark place. It is particularly important to store materials used for radiological determinations in dark bottles, since photoluminescence will produce

high background if light sensitive detectors are used for counting. Some reagents require refrigeration.

Adsorbents for thin-layer and column chromatography are stored in the containers that they are supplied in, or according to the requirements of individual methods. Activated carbon, used for collection of samples for organic analyses, must be stored and processed in areas protected from atmospheric and other sources of contamination (8).

The analyst should pay particular attention to the stability of the standard reagents. Standards should not be kept longer than recommended by the manufacturer, or in the method. Some standards are susceptible to changes in normality due to absorption of gases or water vapor from the air. Provisions for minimizing this effect are given in Part 23 of ASTM Standards (4).

The concentration of the standards will change as a result of evaporation of solvent. This is especially true of standards prepared in volatile organic solvents. Therefore, the reagent bottles should be kept stoppered, except when actually in use. The chemical composition of certain standards may change on standing. Certain pesticides, for instance, will degrade if prepared in acetone that contains small quantities of water. Thus, it is essential that working standards be frequently checked to determine changes in concentration or composition. Stock solutions should be checked before preparing new working standards from them.

### 5.4 References


1. National Bureau of Standards, Special Publication 260, "Standard Reference Materials", July 1969.

2. "Reagent Chemicals, American Chemical Society Specifications", American Chemical Society, Washington, D.C.

3. ASTM Special Technical Publication No. 148-H, "Manual on Industrial Water and Industrial Waste Water", 2nd Edition, p. 869 (1965).

4. 1968 Book of ASTM Standards, Part 23; p. 897: American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

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7. Burke, J., JAOAC, 48, 1037 (1965).

8. Breidenbach, A. W., et al, "The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Waters", Publication WP-22, Federal Water Pollution Control Administration, Washington, D.C. (November 1966).

## Chapter 6

# CONTROL OF ANALYTICAL PERFORMANCE

### 6.1 Introduction

This chapter is limited to a discussion of the control of analytical performance in the laboratory. It is assumed that a valid sample has been properly taken, preserved, and delivered to the laboratory for analyses; that the laboratory analyses were done according to currently-recognized methods; and that the recording and reporting of subsequent laboratory results were done in a systematic, uniform, and permanent fashion (See Chapter 7). It must be recognized (and practiced!), however, that quality control begins with the sample collection and does not end until the resulting data are reported. The laboratory control of analytical performance is but one vital link in obtaining valid data. A continuous rapport and conscientious use of quality control between field sampling, laboratory analyses, and management decisions are necessary to insure this validity.

Earlier chapters have discussed such key elements as laboratory services, instrumentation, glassware, reagents, solvents, and gases; the reader should refer to these sections to determine the necessary specifications and requirements required for quality control. On the assumption that these variables are under control, that a single method is being used, and that the complete system is initially under control, what should be done in the evaluation of daily performance to document that valid data are being produced? First, valid precision and accuracy data should be available on the method and analyst. Thereafter, systematic daily checks are required to show that reproducible results are being obtained, and that the methodology is actually measuring what is in the sample. These items are discussed in detail in the following sections.

### 6.2 Precision and Accuracy

Precision refers to the reproducibility among replicate observations. In an Analytical Quality Control Program, it is determined, not on reference standards, but by the use of actual water samples which cover a range of concentrations and a variety of interfering materials usually encountered by the analyst. Obviously, such data should not be collected until the analyst is thoroughly familiar with the method, and has obtained a reproducible standard curve. For colorimetric analyses, the initial standard curve should include a blank and a series of at least eight standards encompassing the full concentration range to be used for routine sample analyses. Subsequently, at least two standards (a high and a low) should be analyzed to verify the original standard curve. For other measurements, such as pH, conductivity, turbidity, etc., instruments should be standardized according to manufacturer's instructions (See Chapter 3) and sound, scientific practice.

There are a number of different methods available for the determination of precision. One method that has been successfully employed by experienced AutoAnalyzer users, and can be adapted to many other analytical instrumentation and chemical procedures, is described as follows:

a. Four separate concentration levels should be studied, including a low concentration near the sensitivity level of the method, two intermediate concentrations, and a concentration near the upper limit of application of the method.

b. Seven replicate determinations should be made at each of the concentrations tested.

c. To allow for changes in instrument conditions, the precision study should cover at least two hours of normal laboratory operation.

d. In order to permit the maximum interferences in sequential operation, it is suggested that the samples be run in the following order: high, low, intermediate, intermediate. This series is then repeated seven times to obtain the desired replication.

e. The precision statement should include a range of standard deviations over the tested range of concentration. Thus, four standard deviations will be obtained over a range of four concentrations, but the statement should contain only the extremes of standard deviations and concentrations studied.

An example of data generated from such an approach is shown in Table 6-1.

Table 6-1

PRECISION DATA ON RIVER WATER SAMPLES FOR PHOSPHORUS AUTOANALYZER METHOD

| | Conc., (mg P/1) | | | |
|---|---|---|---|---|
| Sample | Kanawha | Klamath | Arkansas | Big Souix |
| 1 | 0.05 | 0.10 | 0.48 | 0.62 |
| 2 | 0.06 | 0.10 | 0.48 | 0.62 |
| 3 | 0.06 | 0.10 | 0.49 | 0.62 |
| 4 | 0.06 | 0.11 | 0.48 | 0.63 |
| 5 | 0.06 | 0.11 | 0.48 | 0.62 |
| 6 | 0.06 | 0.11 | 0.48 | 0.62 |
| 7 | 0.06 | - | - | 0.62 |
| Avg. | 0.059 | 0.105 | 0.482 | 0.621 |
| s | 0.004 | 0.005 | 0.004 | 0.004 |

The resulting precision statement would read as follows:

> "In a single laboratory, using surface water samples at concentrations of 0.06 and 0.62 mg P/1, the standard deviation was ±0.004 (Analytical Quality Control Laboratory)."

Thus, the statement contains the number of laboratories involved, the type of samples, the concentrations used, the resulting standard deviation (s) and the reference source.

Accuracy refers to a degree of difference between observed and known, or actual, values. Again, accuracy should be determined on actual water samples routinely analyzed, and preferably, on the same series as those used in the precision determinations. The method employed by experienced AutoAnalyzer users consists of the following key steps:

a. Known amounts of the particular constituent should be added to actual samples at concentrations where the precision of the method is satisfactory. It is suggested that amounts be added to the low-concentration sample, sufficient to double that concentration, and that an amount be added to one of the intermediate concentrations, sufficient to bring the final concentration in the sample to approximately 75% of the upper limit of application of the method.

b. Seven replicate determinations at each concentration should be made.

c. Accuracy should be reported as the percent recovery at the final concentration of the spiked sample. Percent recovery at each concentration should be the mean of the seven replicate results.

Data were obtained with this approach by using two of the water samples previously used in the precision study reported in Table 6-1 (Kanawha and Arkansas Rivers). They are reported in Table 6-2.

Table 6-2

ACCURACY DATA ON RIVER WATER SAMPLES FOR PHOSPHORUS AUTOANALYZER METHOD

| | Conc., (mg P/1) | |
|---|---|---|
| Sample | Kanawha (Added 0.06 mg/1P) | Arkansas (Added 0.3 mg/1 P) |
| 1 | 0.105 | 0.74 |
| 2 | 0.105 | 0.75 |
| 3 | 0.105 | 0.75 |
| 4 | 0.110 | 0.73 |
| 5 | 0.110 | 0.74 |
| 6 | 0.110 | 0.75 |
| 7 | 0.105 | 0.75 |
| Avg. | 0.107 | 0.74 |

% Recovery $\left[\frac{0.107}{0.059 + 0.06}\right] \times 100 = 90$ $\left[\frac{0.74}{0.48 + 0.30}\right] \times 100 = 95$

Again, in order to contain the key elements, the accuracy statement would read as follows:

> "In a single laboratory, using surface water samples at concentrations of 0.11 and 0.74 mg P/1, recoveries were 90% and 95%, respectively (Analytical Quality Control Laboratory)".

Once collected and documented, these precision and accuracy data may be used in a number of ways. Two important examples are: (1) They present clearcut evidence that the analyst in question is indeed capable of analyzing the water samples for that particular parameter. That is, he has the standard method under control, and is capable of generating valid data; and (2) the data can be used in the evaluation of daily performance in reference to replicate samples, spiked standards and samples, and in the preparation of quality control charts.

As observed, the above methods can be adapted to other chemical procedures and analytical instruments. They have been used on manual titration methods for such parameters as alkalinity, chloride, and hardness; on general inorganic instruments such as pH, conductivity, selective ion, and turbidity meters; and on the Beckman Carbonaceous Analyzer. Other instruments, such as atomic absorption and flame emission spectrophotometers, could also be evaluated by these methods; however, radiological instrumentation and gas chromatography systems (See Chapter 8) require special techniques.

### 6.3 Evaluation of Daily Performance

Once valid precision and accuracy data are available on the method and the analyst, systematic daily checks are necessary to insure that valid data are being generated. First of all, verification of the originally-constructed standard curve is mandatory. As previously noted, at least two standards (a high and a low) should be analyzed routinely along with a blank to determine that comparable operating conditions exist. If the data do not substantiate such control, the analyst must systematically trouble-shoot his system until the problem is corrected.

In order to document that reproducible results are being obtained (i.e., precision of the method), it is necessary to run replicate samples. Although frequency of such replicate analyses is, by nature, dependent on such factors as the original precision of the method, the reliability of the instrumentation involved, and the experience of the analyst, good laboratory technique is to run duplicate analyses at least ten percent of the time. The resulting data should agree favorably with the known precision of the method. If they do not, the system is not under control, and results are subject to question.

Concurrently, quality control should include assurance that the daily system is actually measuring what is in the sample (i.e., accuracy of the method). Although it is far preferable to have obtained values check with known or actual values, it should be recognized that inaccuracy does not destroy the value of data if the degree and precision of the error is known and taken into account. In order to account for background contamination and/or sample interferences, and as a matter of routine practice, spiked samples should be used in addition to standards. As in the case of duplicate sample analyses, good laboratory technique dictates that spiked samples be run at least ten percent of the time.

Thus, daily control of analytical performance in the laboratory requires approximately 15-20 percent of the analyst's time. Considering the elapsed time and combined efforts of

skilled personnel that are represented in a final laboratory result, this is a comparatively small price to pay for, not a "number", but a valid concentration value.

A most convenient way of recording the obtained precision and accuracy data is through the preparation of quality control charts. Plotting of said data systematically answers the question as to whether the laboratory analyses are under control, and is useful in observing developing trends of positive or negative bias. Because of its importance in documenting the quality control being practiced daily in the laboratory, the construction and uses of quality control charts are treated as a separate topic in the next section.

A broader and somewhat different form of evaluation of daily performance may be made through routine participation in interlaboratory round-robin studies. Samples analyzed in such a cooperative program should be treated as part of the routine sample load. In so doing, the analyst is able to compare his individual performance against other laboratory personnel, and to have a reliable measure of the particular method's capabilities. In many respects such samples can be regarded as reputable "blind samples"; a necessary ingredient in the quality control of laboratory results.

### 6.4 Quality Control Charts

Quality control charts were originally developed for the control of production processes where large numbers of items were being manufactured and inspected on an essentially continuous basis. As shown in Figure 6-1, a control chart consists of a graphical chart with the vertical scale plotted in units of the test result and the horizontal scale in units of time or sequence of results. The upper and lower control limits shown on the chart are used as criteria for action, or for judging the significance of variations between duplicate samples. The central line represents the average or the standard value of the statistical measure being plotted.

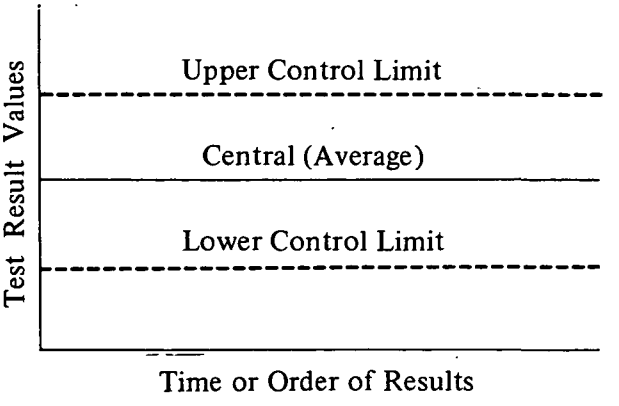



Figure 6-1 ESSENTIALS OF CONTROL CHART

As observed in the previous section on the evaluation of daily performance, daily precision and accuracy data can be plotted by means of these quality control charts to determine if valid, questionable, or invalid data are being generated from day to day. There are several techniques available for actually constructing quality control charts and plotting subsequent data. Two currently in use are the Shewhart technique (1,2) and the CuSum technique (3). In both techniques, precision control charts are constructed from duplicate sample

analyses, whereas, accuracy control charts are constructed from spiked samples or standards data generated in monitoring recovery efficiencies. At least 15 to 20 sets of duplicate and 15 to 20 sets of spiked sample data from an in-control process are necessary for the initial construction. A system is initially said to be in control when the standard deviation and recovery efficiency data for a given parameter are comparable to those obtained by other experienced laboratories. It is also necessary that the initial and subsequent sets of data be obtained under normal laboratory operation conditions, that the same analyst or group of analysts run the analysis, and that the same analytical method is used.

### 6.4.1 Cumulative-Summation (CuSum) Quality Control Charts

There are various systems currently available for plotting data in the form of cumulative sum charts (4). One system that has been in continuous use within EPA Region VI is that of Harkins and Crowe (3). It has proved most useful in monitoring the validity of data generated by a contracting laboratory and is currently being used routinely to daily record intra-laboratory performance in technical operations. The following material has been excerpted from their manual (3), in order to accurately describe the construction and use of these charts:

#### 6.4.1.1 Construction of CuSum Quality Control Charts

The control charts are derived from three basic calculations:

a. Standard deviations ($S_d$) of the differences between duplicates or, in the case of spiked or standard samples, between the known quantity and the quantity obtained.

b. The upper control limit (UL)

c. The lower control limit (LL)

Prior to these calculations, two decisions must be made:

a. The $\alpha$ and $\beta$ levels

b. The allowable variability levels

Mathematical Equations

$$S_d^2 = \frac{\sum_{i=1}^{n} di^2 - \frac{(\sum_{1}^{n} di)^2}{N}}{N-1} = \text{Variance of the differences}$$

$$S_d = \sqrt{S_d^2} = \text{Standard deviation of the differences} \qquad \text{(a)}$$

$$S_0^2 = (.8S_d)^2 = .64\, S_d^2 \text{ (estimates } \sigma_0^2)$$

$$S_1^2 = (1.2S_d)^2 = 1.44\, S_d^2 \text{ (estimates } \sigma_1^2)$$

$$UL(M) = \frac{2\log_e\left[\frac{1-\beta}{\alpha}\right]}{\frac{1}{S_0^2} - \frac{1}{S_1^2}} + M \frac{\log_e\left[\frac{S_1^2}{S_o^2}\right]}{\frac{1}{S_0^2} - \frac{1}{S_1^2}}$$

$$LL(M) = \frac{2\log_e\left[\frac{\beta}{1-\alpha}\right]}{\frac{1}{S_0^2} - \frac{1}{S_1^2}} + M \frac{\log_e\left[\frac{S_1^2}{S_0^2}\right]}{\frac{1}{S_0^2} - \frac{1}{S_1^2}}$$

Where: UL(M) = upper limit at M sets of samples

LL(M) = lower limit at M sets of samples

di = the difference between the $i^{th}$ set of duplicates or spiked samples

N = the total number of sets of duplicates or spiked samples used to construct the control charts

$S_0^2$ = minimum amount of variation allowed in the system

$S_1^2$ = maximum amount of variation allowed in the system

$\alpha$ = percent (decimal fraction) of time you are willing to judge the procedure out of control when it is in control

$\beta$ = percent (decimal fraction) of time you are willing to judge the procedure in control when it is out of control

M = number of sets of duplicates or spiked samples used in calculating the value to be plotted on the chart

By definition, $\alpha$ is the probability of judging the process to be out of control when in fact, it is in control. It is recommended that $\alpha$ be chosen to lie between the boundaries of .05 and .15, that is, the laboratory personnel are willing to stop the laboratory process somewhere between 5 and 15% of the time, judging it to be out of control, when in fact, it is in control. If the cost of examining a process to determine the reason or reasons for being out of control is considerable, then it may be desirable to choose a low $\alpha$. Likewise, if the cost is negligible, it may be desirable to choose a larger $\alpha$ value, and thus stop the process more frequently. (See Figure 6-2)

On the other hand, $\beta$ is defined as the probability of judging the process to be in control when it is not. Again, it is recommended that $\beta$ be chosen to lie between the values of .05 and .15; thus, the laboratory personnel are willing to accept out of control data somewhere between 5 and 15% of the time. The economic considerations used for choosing $\alpha$ are also applicable to the choice of B. (See Figure 6-2.)

It is also essential to set maximum and minimum allowable variability levels. It is necessary to specify a value for the minimum and maximum amount of variation that will be allowable in the system. These minimum and maximum amounts are referred to as $\sigma_0^2$ and $\sigma_1^2$ respectively. The values used should be based on a knowledge of the variation in the procedure under consideration. However, if such knowledge is not available, the values may be arbitrarily set at $\sigma_0^2 = (\sigma - .20\sigma)^2$ and $\sigma_1^2 = (\sigma + .20\sigma)^2$.

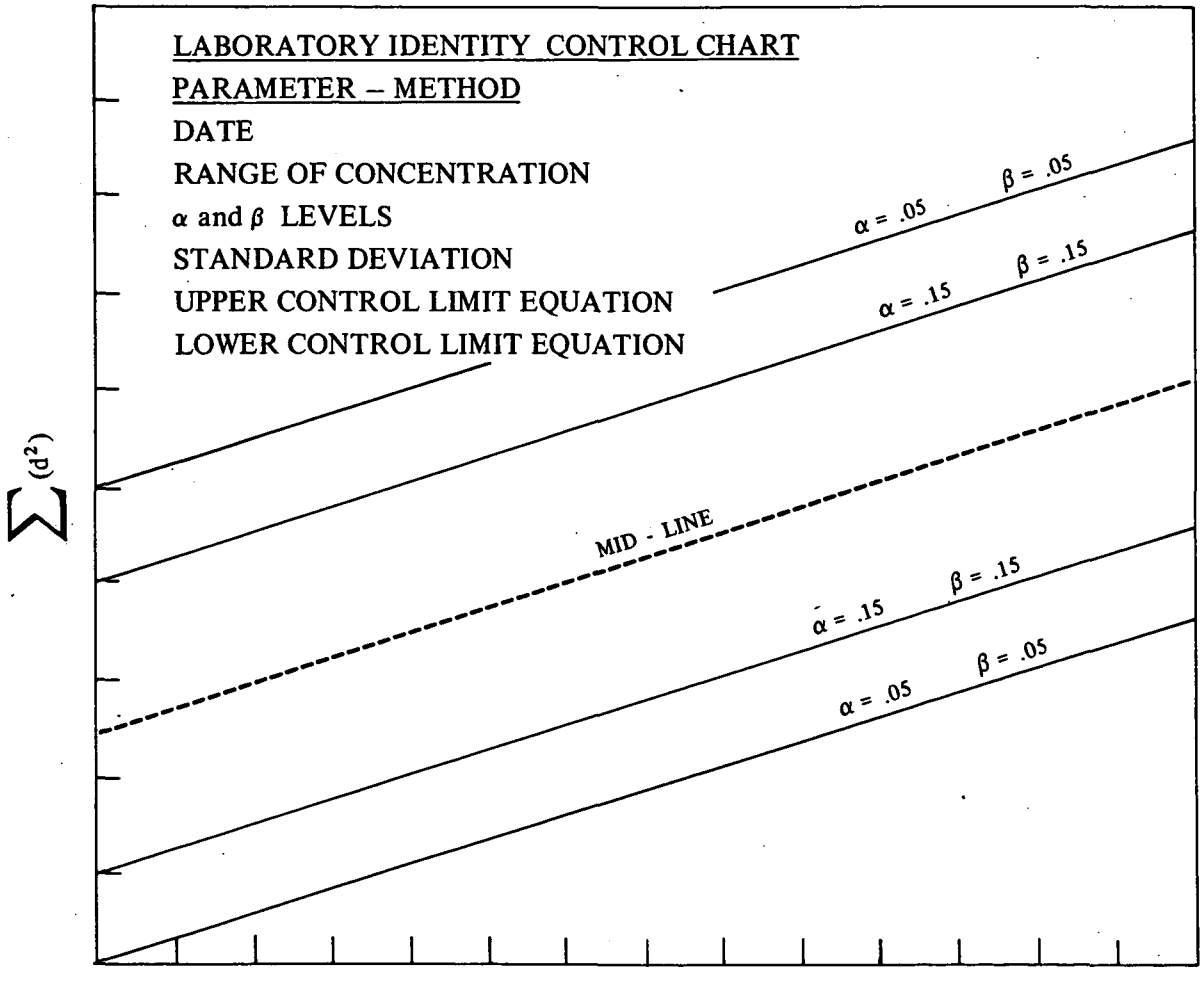



Figure 6-2. EFFECT OF $\alpha$ AND $\beta$ LEVELS ON STANDARD CONTROL CHART

#### 6.4.1.2 Use of CuSum Control Charts

Once the control charts are constructed, and prior to their use, consideration must be given to the number of duplicate analyses to be conducted during a series of samples; likewise, the same decision must be made on spiked or standard samples.

In considering the number of duplicate and spiked sample analyses to be conducted in a series of samples, it is necessary to weight the consequences when the data go out of control. The consequences of this situation are reanalyzing a series of samples or discarding the questionable data obtained. The samples to be reanalyzed are those lying between the last in-control point and the present out-of-control point. A realistic frequency for running duplicate and spiked samples would be every fifth sample; however, economic consideration and experience may require more or less frequent duplicate and spiked sample analyses.

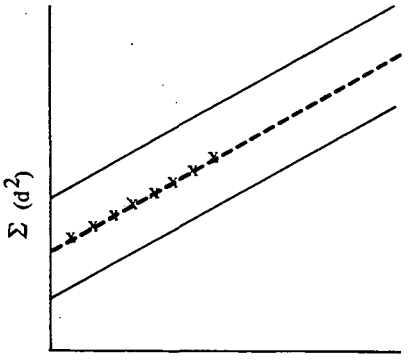
Once the frequency of duplicate and spiked samples has been determined, it is then necessary to prepare spiked or standard samples in concentrations relative to the concentration of the control charts, which should be similar to those of the environmental samples. These spiked or standard samples must be intermittently dispersed among the series of samples to be analyzed and without the analyst's knowledge of concentration. Similarly, duplicate samples must be intermittently dispersed throughout the series of samples to be analyzed, and ideally, without the analyst's knowledge; however, this is sometimes very difficult to accomplish.

The results of the duplicate and spiked sample analyses should be calculated immediately upon analyzing the samples to allow for early detection of problems that may exist in the laboratory. An example of these calculations follows:

| Duplicate Sample No. M | Results No. 1 | No. 2 | Difference (di) | $di^2$ | $\Sigma (di^2)$ |
|---|---|---|---|---|---|
| 1 | 5.4 | 5.2 | .2 | .04 | .04 |
| 2 | 4.8 | 4.7 | .1 | .01 | .05 |
| 3 | 6.1 | 5.8 | .3 | .09 | .14 |

Upon plotting the summation or $\Sigma(di^2)$, one of three possibilities can occur (See Figure 6-3):

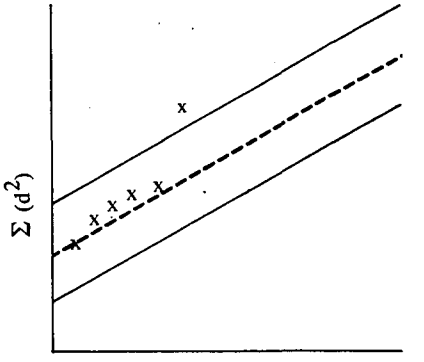
a. Out of control on the upper limit
When data goes out of control on the upper limit the following steps should be taken:
    1. Stop work immediately
    2. Determine problems
        (a) Precision control chart
            (1) The analyst
            (2) Nature of the sample
            (3) Glassware contamination




SAMPLE SET NO.

ANALYSIS IN CONTROL

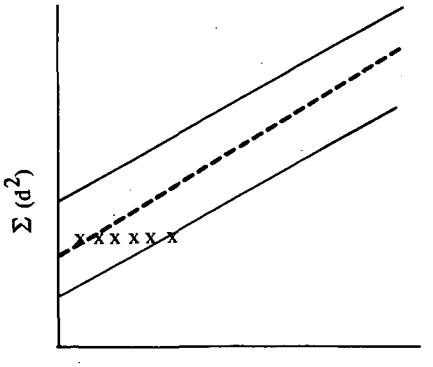
NO PROBLEMS:
CONTINUE ANALYSIS




SAMPLE SET NO.

ANALYSIS OUT OF CONTROL
UPPER LIMIT

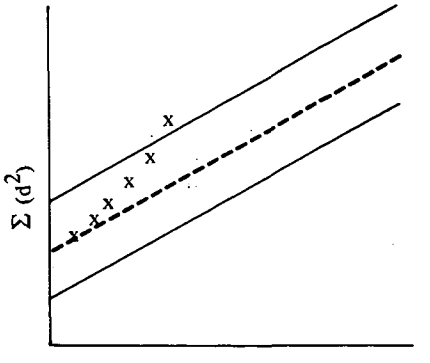
PROCEDURES:
1. STOP ANALYSIS
2. LOCATE PROBLEM
3. CORRECT PROBLEM
4. RERUN SAMPLES
5. START CHART AT SAMPLE SET NO. 1.




SAMPLE SET NO.

ANALYSIS OUT OF CONTROL
LOWER LIMIT

INCREASED EFFICIENCY OR
FALSE REPORTING
PROCEDURES:
1. CONTINUE ANALYSIS
2. CONSTRUCT NEW CHART WITH RECENT DATA
3. OBSERVE ANALYST




SAMPLE SET NO.

ANALYSIS OUT OF CONTROL
UPPER LIMIT

CONTINUOUS ERROR TREND
PROCEDURES:
SAME AS ABOVE BUT STOP ANALYSIS WHEN TREND IS DETECTED.

Figure 6-3. LABORATORY QUALITY CONTROL CHARTS

(b) Accuracy control chart
   (1) The analyst
   (2) Glassware contamination
   (3) Contaminated reagents
   (4) Instrument problems
   (5) Sample interference with the spiked material

3. Rerun samples represented by that sample set number, including additional duplicate and spiked samples.
4. Begin plotting at sample No. 1 on chart.

b. In control within the upper and lower limit lines

When data continuously fall in between the upper and lower control limits, the analyses should be continued until an out-of-control trend is detected.

c. Out of control on the lower limit

When data fall out of control on the lower limit, the following steps should be taken:

1. Continue analyses unless trend changes
2. Construct new control charts on recent data
3. Check analyst's reporting of data

### 6.4.2 Shewhart Quality Control Charts

Dr. Walter A. Shewhart of Bell Telephone Laboratories developed the basic theory of control charts in the 1920's. His book on statistical quality control (1) grew out of this original work. Since then, industrial acceptance of these control chart concepts and other statistical techniques have refined and quantitated the quest for quality in manufacturing. Although originally developed for control of production processes when large numbers of articles were being manufactured and inspected on an essentially continuous basis, these same concepts have been readily adapted to laboratory operations where the analyst produces comparatively fewer results on an intermittent basis.

As in the CuSum approach, precision control charts are prepared from data resulting from duplicate sample analyses and accuracy control charts from duplicate spiked standards or samples. Once the control charts are constructed, however, data are plotted as individual values rather than cumulative sums.

Certain constants (factors) are also involved in the preparation of Shewhart Charts. Depending upon how the data are grouped, what the size of each grouping is, and what control limit formulation is being calculated, Table 6-3 will serve as a basic reference point:

Table 6-3

FACTORS FOR COMPUTING CONTROL CHART LINES (5, 6)

| Observations in Subgroup ($n$) | Factor $A_2$ | Factor $D_4$ |
|---|---|---|
| 2 | 1.88 | 3.27 |
| 3 | 1.02 | 2.58 |
| 4 | 0.73 | 2.28 |
| 5 | 0.58 | 2.12 |
| 6 | 0.48 | 2.00 |
| 7 | 0.42 | 1.92 |
| 8 | 0.37 | 1.86 |

Inherent in the Shewhart approach is recognition of the basic assumption that variations exist in every method. That is, no procedure is so perfect, so unaffected by its environment, that it will always give exactly the same assay value or product. Where such situations seem to exist, either the device used to measure the process is not sensitive enough or the person making the measurements is not performing properly. For our purposes, the recorded difference between paired samples should never be less than one-half the minimum detectable limit of the parameter under consideration. In the following outlines for preparing precision and accuracy control charts, nitrate data were used to develop the examples. Therefore the minimum values shown are 0.05 (one-half the observed minimum detectable limit of 0.1 mg/l as N).

### 6.4.3 Precision Control Charts

These charts are developed by collecting data for many samples, a minimum of 15 to 20, run in duplicate under assumed controlled conditions. Once these data have been generated, preferably over an extended period of laboratory time, the following steps should be followed to construct the control chart:

a. List the range (R) for each set of samples. That is, the absolute value of the difference between each set of duplicate samples.

Note: The following ranges were observed in the nitrate data:

| | | |
|---|---|---|
| 0.1 | 0.1 | 0.05 |
| 0.05 | 0.05 | 0.05 |
| 0.05 | 0.05 | 0.05 |
| 0.05 | 0.05 | 0.05 |
| 0.05 | 0.30 | 0.05 |
| 0.05 | 0.10 | 0.05 |
| 0.05 | 0.05 | 0.05 |
| 0.05 | 0.05 | 0.05 |
| 0.05 | 0.05 | 0.05 |
| 0.05 | 0.05 | 0.05 |
| 0.05 | 0.05 | 0.05 |
| 0.05 | 0.05 | |

b. Calculate the average range ($\bar{R}$) by summing the list of R values and dividing by the number of sets of duplicates:

$$\bar{R} = \frac{\Sigma R}{\eta}$$

$$\bar{R} = \frac{2.15}{35} = 0.06$$

c. Calculate the Upper Control Limit (UCL) on the range according to the formula:

$$UCL_R = D_4 \bar{R},$$

where $D_4$ is a constant dependent on the number of units in the subgroup. In this case, since two observations are in the subgroup, $d_4 = 3.27$ (see Table 6-3).

$$UCL_R = 3.27\ \bar{R} = 0.20$$

d. Calculate the Upper Warning Limit (UWL) on the range according to the formula:

$$UWL_R = 2/3\ (D_4 \bar{R} - \bar{R}) + \bar{R},$$

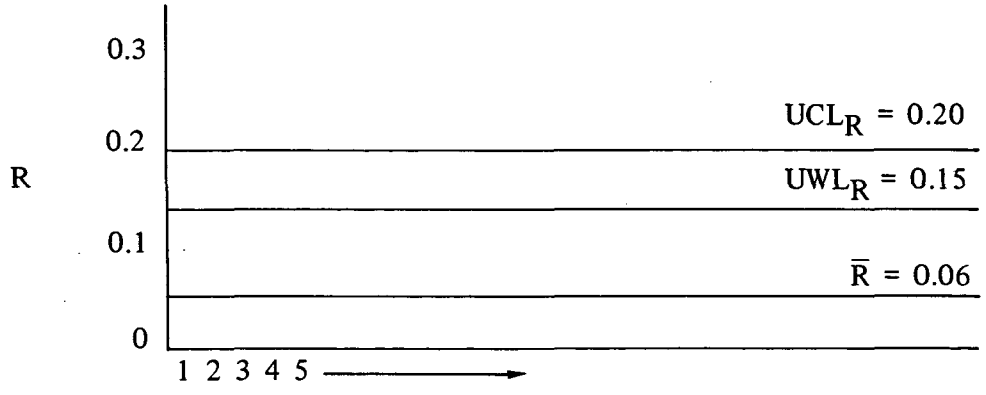
which for duplicate samples reduces to

$$UWL_R = 2.51\ \bar{R}$$

$$UWL_R = 0.15$$

This UWL corresponds to the 95% confidence level.

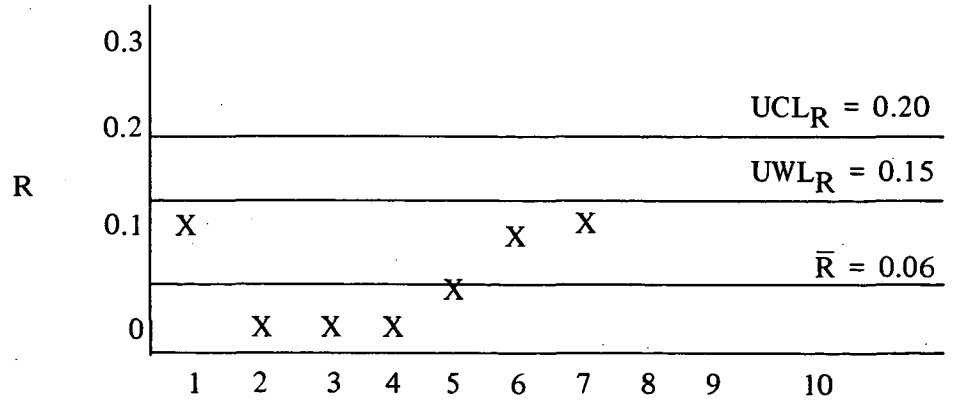
e. Now graph $\bar{R}$, $UWL_R$, and $UCL_R$ in the following manner:




Order of Results
(e.g., duplicate sample sets)

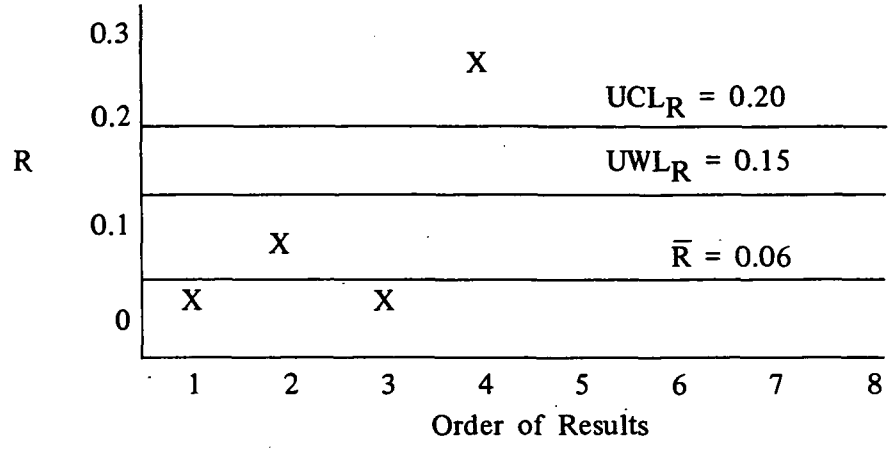
f. The above precision control chart for nitrates is now complete, and can be used to plot R values on subsequent duplicate samples to determine if the system is in control, out of control (plotted R value beyond the UCL), and/or to detect any trends developing within the system.

1. In this example, a trend has developed between duplicate sample sets 4 thru 7. Although the system is not out of control, all variables in the procedure should be checked in an attempt to stop this obvious trend before the UCL is reached.




Order of Results

2. In this example, the system has clearly gone out of control between duplicate samples 3 and 4. At this point, the system can be stopped and all variables in the system checked, or another set of duplicate samples can be run to verify the observed difference. Once the system has been corrected, all samples between set 3 and 4 should be rerun to insure the validity of the data.




### 6.4.4 Accuracy Control Charts

As in the above system, these charts are developed by collecting data for many samples, a minimum of 15 to 20, but on spiked samples (preferably) or standards under assumed controlled conditions. Again, these data should be generated over an extended period of laboratory time, and be representative of normal operating conditions (7). The following steps should be followed to construct accuracy control charts:

a. List the range (R) and the average ($\bar{X}$)* of each subgroup of data.
Note: In the following example of nitrate data, subgroups of monthly data involving four observations were used:

$$*\bar{X} = \frac{\Sigma d \quad \text{Found - Actual}}{n}$$

| Month | Actual | Found | R | $\bar{X}$ |
|---|---|---|---|---|
| Sept. | 1.1 | 1.1 | | |
| | 1.1 | 1.1 | 0 | 0 |
| | 1.1 | 1.1 | | |
| | 1.1 | 1.1 | | |
| Nov. | 1.1 | 1.1 | | |
| | 1.1 | 1.1 | 0.1 | -0.025 |
| | 1.2 | 1.2 | | |
| | 1.2 | 1.1 | | |
| Dec. | 1.0 | 1.1 | | |
| | 1.0 | 1.0 | 0.1 | +0.025 |
| | 1.0 | 1.0 | | |
| | 1.0 | 1.0 | | |
| Jan. | 1.0 | 0.9 | | |
| | 1.0 | 1.0 | 0.2 | 0 |
| | 1.0 | 1.1 | | |
| | 1.0 | 1.0 | | |

b. Calculate the average range ($\bar{R}$) by summing the list of R values and dividing by the number of subgroups:

$$\bar{R} = \frac{\Sigma R}{\eta}$$

$$\bar{R} = \frac{0.4}{4} = 0.10$$

c. Calculate the Upper Control Limit (UCL) on the range according to the formula:

$$UCL_R = D_4 \bar{R},$$

where $D_4$ is a constant dependent on the number of units in the subgroup. In this case, since four observations are in the subgroup, $D_4 = 2.28$ (see Table 6-3).

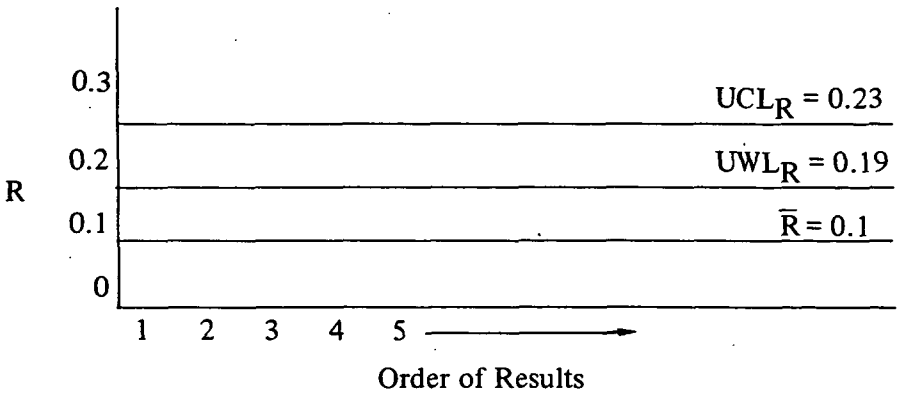
$$UCL_R = 2.28\ \bar{R} = 0.23$$

d. Calculate the Upper Warning Limit (UWL) on the range according to the formula:

$$UWL_R = 2/3\ (D_4 \bar{R} - \bar{R}) + \bar{R}$$

$$UWL_R = 2/3\ [2.28\ (0.1) - 0.1] + 0.1 = 0.19$$

e. Now graph $\bar{R}$, $UWL_R$, and $UCL_R$ in the following manner:




f. Turning now to the $\bar{X}$ values, calculate the $UCL_{\bar{X}}$ by the formula:

$$UCL_{\bar{X}} = A_2 \bar{R}$$

where $A_2$ is a constant dependent on the number of units in the subgroup. In this case, since four observations are in the subgroup, $A_2 = 0.73$ (see Table 6-3).

$$\therefore UCL_{\bar{X}} = 0.73\bar{R} = 0.07$$

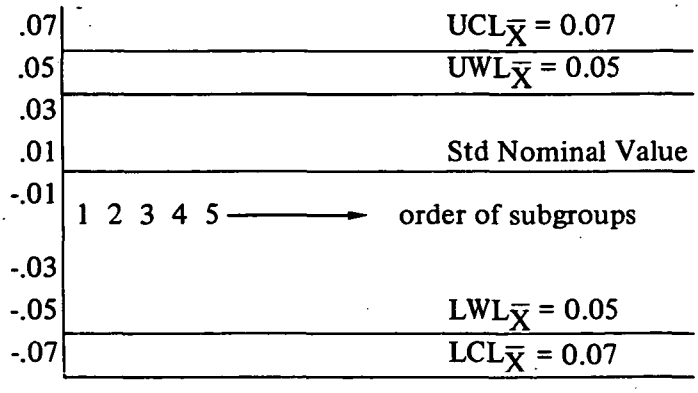
g. Calculate the $UWL_{\bar{X}}$ by the formula:

$$UWL_{\bar{X}} = 2/3\ A_2(\bar{R})$$

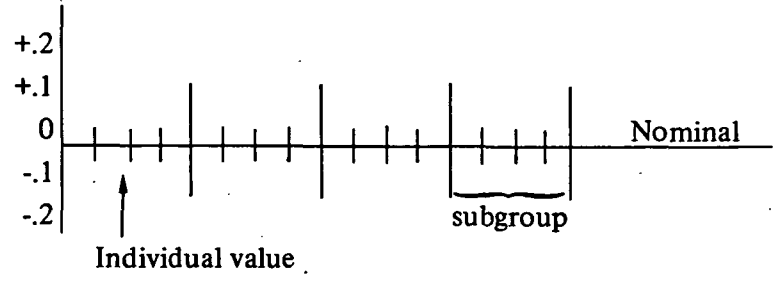
$$UWL_{\bar{X}} = 2/3\ [\ 0.73\ (0.1)] = 0.05$$

Note: Lower Warning Limit ($LWL_{\bar{X}}$) and Lower Control Limit ($LCL_{\bar{X}}$) are simply the negative values of $UWL_{\bar{X}}$ and $UCL_{\bar{X}}$, respectively.

h. Now graph the standard Nominal Value (set equal to zero), $UWL_{\bar{X}}$, and $UCL_{\bar{X}}$, $LWL_{\bar{X}}$, and $LCL_{\bar{X}}$ in the following manner:




i. In order to detail any trends forming within each subgroup, individual differences may be plotted by preparing the following graph:




j. Thus, as in the precision control charts, once all of the above accuracy control charts have been constructed, all future data can be plotted on each set of duplicate spiked samples or standards, to determine if the system is in control, out of control, and/or to detect any trends developing within the system.

## 6.5 References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# CHAPTER 7

# DATA HANDLING AND REPORTING

## 7.1 Introduction

To obtain meaningful data on water quality, the laboratory must first collect a representative sample and deliver it unchanged for analysis. The analyst must then complete the proper analysis in the prescribed fashion. Having accomplished these steps, one other important step must be completed before the data are of use. This step includes the permanent recording of the analytical data in meaningful, exact terms, and reporting it in proper form to some storage facility for future interpretation and use.

The brief sections that follow discuss the data value itself, recording and reporting the value in the proper way, means of quality control of data, and storage and retrieval.

## 7.2 The Analytical Value

### 7.2.1 Significant Figures

The term significant figure is used rather loosely to describe some judgment of the number of reportable digits in a result. Often the judgment is not soundly based and meaningful digits are lost or meaningless digits are accepted.

Proper use of significant figures gives an indication of the reliability of the analytical method used. The following definitions and rules are suggested for retention of significant figures:

A number is an expression of quantity. A figure or digit is any of the characters 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, which, alone or in combination, serves to express a number. A significant figure is a digit that denotes the amount of the quantity in the place in which it stands.

Reported values should contain only significant figures. A value is made up of significant figures when it contains all digits known to be true and one last digit in doubt. For example, if a value is reported as 18.8 mg/1, the "18" must be firm values while the "0.8" is somewhat uncertain and may be "7" or "9".

The number zero may or may not be a significant figure:

a. Final zeros after a decimal point are always significant figures. For example, 9.8 grams to the nearest mg is reported as 9.800 grams.

b. Zeros before a decimal point with other preceding digits are significant. With no other preceding digit, a zero before the decimal point is not significant.

c. If there are no digits preceding a decimal point, the zeros after the decimal point but preceding other digits are not significant. These zeros only indicate the position of the decimal point.

d. Final zeros in a whole number may or may not be significant. In a conductivity measurement of 1000 $\mu$mhos/cm, there is no implication that the conductivity is 1000 ± 1 $\mu$mho. Rather, the zeros only indicate the magnitude of the number.

A good measure of the significance of one or more zeros before or after another digit is to determine whether the zeros can be dropped by expressing the number in exponential form. If they can, the zeros are not significant. For example, no zeros can be dropped when expressing a weight of 100.08 grams in exponential form; therefore the zeros are significant. However, a weight of 0.0008 grams can be expressed in exponential form as $8 \times 10^{-4}$ grams, and the zeros are not significant. Significant figures reflect the limits of the particular method of analysis. It must be decided beforehand whether this number of significant digits is sufficient for interpretation purposes. If not, there is little that can be done within the limits of normal laboratory operations to improve these values. If more significant figures are needed, a further improvement in method or selection of another method will be required to produce an increase in significant figures.

Once the number of significant figures is established for a type of analysis, data resulting from such analyses are reduced according to set rules for rounding off.

### 7.2.2 Rounding Off Numbers

Rounding off of numbers is a necessary operation in all analytical areas. It is automatically applied by the limits of measurement of every instrument and all glassware. However, it is often applied in chemical calculations incorrectly by blind rule or prematurely, and in these instances, can seriously affect the final results. Rounding off should normally be applied only as follows:

#### 7.2.2.1 Rounding-Off Rules

a. If the figure following those to be retained is less than 5, the figure is dropped, and the retained figures are kept unchanged. As an example: 11.443 is rounded off to 11.44.

b. If the figure following those to be retained is greater than 5, the figure is dropped, and the last retained figure is raised by 1. As an example: 11.446 is rounded off to 11.45.

c. When the figure following those to be retained is 5, and there are no figures other than zeros beyond the 5, the figure is dropped, and the last place figure retained is increased by 1 if it is an odd number, or it is kept unchanged if an even number. As an example: 11.435 is rounded off to 11.44, while 11.425 is rounded off to 11.42.

#### 7.2.2.2 Rounding Off Single Arithmetic Operations

a. Addition: When adding a series of numbers, the sum should be rounded off to the same numbers of decimal places as the addend with the smallest number of places. However, the operation is completed with all decimal places intact and rounding off is done afterward. As an example:

11.1
11.12
11.13
---
33.35 The sum is rounded off to 33.4.

b. Subtraction: When subtracting one number from another, rounding off should be completed before the subtraction operation, to avoid invalidation of the whole operation.

c. Multiplication: When two numbers of unequal digits are to be multiplied, all digits are carried through the operation, then the product is rounded off to the number of significant digits of the less accurate number.

d. Division: When two numbers of unequal digits are to be divided, the division is carried out on the two numbers using all digits. Then the quotient is rounded off to the number of digits of the less accurate of the divisor or dividend.

e. Powers and Roots: When a number contains n significant digits, its root can be relied on for n digits, but its power can rarely be relied on for n digits.

#### 7.2.2.3 Rounding Off the Results of a Series of Arithmetic Operations

The rules for rounding off are reasonable for simple calculations, however, when dealing with two nearly equal numbers, there is a danger of loss of all significance when applied to a series of computations which rely on a relatively small difference in two values. Examples are calculation of variance and standard deviation. The recommended procedure is to carry several extra figures through the calculation and then to round off the final answer to the proper number of significant figures.

### 7.2.3 Glossary of Terms

To clarify the meanings of reports and evaluations of data, the following terms are defined. They are derived in part from American Chemical Society and American Society for Quality Control usage (1, 2).

#### 7.2.3.1 Accuracy Data

Measurements which relate to the difference between the average test results and the true result when the latter is known or assumed. The following measures apply:

Bias is defined as error in a method which systematically distorts results. The term is used interchangeably with accuracy in that bias is a measure of inaccuracy.

Relative error is the mean error of a series of test results as a percentage of the true result.

#### 7.2.3.2 Average

In ordinary usage, the arithmetic mean. The arithmetic mean of a set on $n$ values is the sum of the values divided by $n$.

#### 7.2.3.3 Characteristic

A property that can serve to differentiate between items. The differentiation may be either quantitative (by variables), or qualitative (by attributes).

#### 7.2.3.4 Error

The difference between an observed value and its true value.

#### 7.2.3.5 Mean

The sum of a series of test results divided by the number in the series. Arithmetic mean is understood ($\bar{X}$).

#### 7.2.3.6 Population

Same as Universe. (See subparagraph 7.2.3.13).

#### 7.2.3.7 Precision

Degree of mutual agreement among individual measurements. Relative to a method of test, precision is the degree of mutual agreement among individual measurements made under prescribed, like conditions.

#### 7.2.3.8 Precision Data

Measurements which relate to the variation among the test results themselves, i.e., the scatter or dispersion of a series of test results, without assumption of any prior information. The following measures apply:

a. Standard Deviation ($\sigma$). The square root of the variance.

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} X_1^2}{n} - \bar{X}^2}$$

b. Standard Deviation, estimate of universe (s).

$$s = \sqrt{\frac{\sum_{i=1}^{n} X_1^2 - \frac{\left(\sum_{i=1}^{n} X_i\right)^2}{n}}{n-1}}$$

c. Coefficient of Variance (V). The ratio of the standard deviation (s) of a set of numbers, n, to their average, $\bar{X}$, expressed as a percentage:

$$V = \frac{S}{\bar{X}}$$

d. Range. The difference between the largest and smallest values in a set.

e. 95% Confidence Limits. The interval within which one estimates a given population parameter to lie, 95% of the time.

#### 7.2.3.9 Sample

A group of units, or portion of material, taken from a larger collection of units, or quantity of material, which serves to provide information that can be used as a basis for judging the quality of the larger quantity as a basis for action on the larger quantity or on the production process. Also used in the sense of a "sample of observations."

#### 7.2.3.10 Series

A number of test results which possess common properties that identify them uniquely.

#### 7.2.3.11 Skewness (k)

A measure of the lopsidedness or asymmetry of a frequency distribution defined by the expression:

$$\frac{(X_i - \overline{X})^3}{n\sigma^3}$$

This measure is a pure signed number. If the data are perfectly symmetrical, the skewness is zero. If k is negative, the long tail of the distribution is to the left. If k is positive, the long tail extends to the right.

#### 7.2.3.12 Unit

An object on which a measurement or observation may be made.

#### 7.2.3.13 Universe

The totality of the set of items, units, measurements, etc., real or conceptual, that is under consideration.

#### 7.2.3.14 Variable

A term used to designate a method of testing, whereby units are measured to determine, and to record for each unit, the numerical magnitude of the characteristic under consideration. This involves reading a scale of some kind.

### 7.3 Report Forms

The analytical information reported should include the parameter, the details of the analysis such as burette readings, absorbance, wavelength, normalities of reagents, correction factors, blanks, and finally, the reported value.

To reduce errors in manipulation of numbers, a good general rule is to keep data transposition to an absolute minimum. If this were pursued, the ideal report form would include all preliminary information of the analysis, yet it would be possible to use the same form through to the final reporting of data into a computer or other storage device. However, the ideal report form is not usually in use. Rather, a variety of methods are used to record data. They are:

### 7.3.1 Loose Sheets

Reporting of data onto loose or ring-binder forms is an older, but much used means of recording data. It does allow easy addition of new sheets, removal of older data, or collection of specific data segments. However, the easy facility for addition or removal also permits easy loss or misplacement of sheets, mix-ups as to date sequence, and questionable status in formal display, or for presentation as evidence.

### 7.3.2 Bound Books

An improvement in data recording is use of bound books which force the sequence of data insertion. Modification beyond a simple lined book improves its effectiveness with little additional effort. Numbering of pages encourages use in sequence and aids also in referencing data, through a table of contents, according to time, type of analysis, kind of sample, analyst, etc.

Validation can be easily accomplished by requiring the analyst to date and sign each analysis on the day completed. This validation can be strengthened further by providing space for the laboratory supervisor to sign off as to the date and acceptability of the analysis.

A further development of the bound notebook is the commercially available version designed for research-type work. These note books are preprinted with book and page numbers and spaces for title of project, project number, analyst signature, witness signature and dates. Each report sheet has its detachable duplicate sheet which allows for up-to-date review by management without disruption of the book in the laboratory. The cost is about four times that of ordinary notebooks.

Use of bound notebooks is essentially limited to research and development work where an analysis is part of a relatively long project, and where the recording in the notebook is the prime disposition of the data until a status or final report is written.

### 7.3.3 Pre-Printed Report Forms

Most field laboratories or other installations doing repetitive analyses for many parameters day in and day out, develop their own system of recording and tabulating laboratory data. This may include bound notebooks; but a vehicle for forwarding data is also required. In many instances, laboratory units tailor a form to fit a specific group of analyses, or to report a single type of analysis for series of samples, with as much information as possible preprinted to simplify use of the form. With loose-sheet multicopy forms (use of carbon or NCR paper) information can be forwarded daily, weekly, or on whatever schedule is necessary, while allowing retention of all data in the laboratory. Still, the most common record is an internal bench sheet, or bound book, for recording of all data in rough form. The bench sheet or book never leaves the laboratory but serves as the source of information for all subsequent report forms (See Figure 7-1).

In most instances the supervisor and analyst wish to look at the data from a sample point in relation to other sample points on the river or lake. This review of data by the supervisor, prior to release, is a very important part of the laboratory's quality control program; however, it is not easily accomplished with bench sheets. For this purpose, a summary sheet can be prepared which compares a related group of analyses from a number of stations. An example is shown in Figure 7-2. Since the form contains all of the information necessary for

Figure 7-1. EXAMPLE OF BENCH SHEET

NL-C-88
(7-68)
Spectrographic Analyses Bench Data

Sample #____ Date_____ Source_____ TDS_____

_____ ml. conc. to_____ ml. Factor _____

| Test Count | Sec |
|---|---|
| 1. | |
| 2. | |
| 3. | |

| | Count | | | Rerun Count | | | Av. Count | PPM In Conc. Sample | Less Than | (μg/l)PPB Orig. Sample |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. Zn | | | | | | | | | | |
| 2. Cd | | | | | | | | | | |
| 3. As | | | | | | | | | | |
| 4. B | | | | | | | | | | |
| 5. P | | | | | | | | | | |
| 6. Fe | | | | | | | | | | |
| 7. Mo | | | | | | | | | | |
| 8. Mn | | | | | | | | | | |
| 9. Al | | | | | | | | | | |
| 10. Be | | | | | | | | | | |
| 11. Cu | | | | | | | | | | |
| 12. Ag | | | | | | | | | | |
| 13. Ni | | | | | | | | | | |
| 14. Co | | | | | | | | | | |
| 15. Pb | | | | | | | | | | |
| 16. Cr | | | | | | | | | | |
| 17. V | | | | | | | | | | |
| 18. Ba | | | | | | | | | | |
| 19. Sr | | | | | | | | | | |

Figure 7-2. EXAMPLE OF SUMMARY REVIEW SHEET

Table 2. MINERALS ANALYSES OF ZONE B, OHIO RIVER SAMPLES, CONC., mg/1.

| STATION | Storet Number | Date, 1969 | Alkalinity | Hardness | Chloride | Sulfate | Fluoride | SOLIDS | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Total | Diss. | Susp. |
| Ohio at Ironton | 200152 | | | | | | | | | |
| Ohio at Greenup Dam | 200001 | | | | | | | | | |
| Ohio at Portsmouth | 200139 | | | | | | | | | |
| Scioto at Lucasville | 381710 | | | | | | | | | |
| Ohio at Maysville | 200153 | | | | | | | | | |
| Ohio at Meldahl Dam | 383070 | | | | | | | | | |
| Little Miami at Cincinnati | 380090 | | | | | | | | | |
| Ohio at Cincinnati | 380037 | | | | | | | | | |
| Licking at 12th Street | 200523 | | | | | | | | | |
| Ohio at Miami Fort | 383072 | | | | | | | | | |
| Ohio at Markland Dam | 200521 | | | | | | | | | |
| Kentucky at Dam I | 200522 | | | | | | | | | |
| Ohio at Madison | 174304 | | | | | | | | | |
| Great Miami at Eldean | | | | | | | | | | |
| Great Miami at Sellars Road | 383047 | | | | | | | | | |
| Great Miami at Liberty-Fairfield Road | 383015 | | | | | | | | | |
| Great Miami at American Materials Bridge | 383007 | | | | | | | | | |
| Whitewater at Suspension | | | | | | | | | | |
| Great Miami at Lawrenceburg (Lost Bridge) | 383071 | | | | | | | | | |

reporting data it is used also to complete the data forms forwarded to the storage and retrieval system.

The forms used to report data to data storage systems require a clear identification of the sample point, the parameter code, the type of analysis used, and the reporting terminology. Failure to provide the correct information can result in rejection of the data, or insertion in an incorrect parameter. As a group of analyses is completed on one or more samples, the values are reported in floating decimal form, along with the code numbers, for identifying the parameter and the sampling point (station). Figure 7-3 shows an example of a preprinted report form for forwarding data to keypunch.

### 7.3.4 Digital Read-out

Instrumental analyses, including automated, wet-chemistry instruments, such as Technicon AutoAnalyzer, atomic absorption spectrophotometer, pH meter, selective electrode meter, etc., now can provide direct digital readout of concentration, which can be recorded directly onto report sheets without further calculation. Electronics manufacturers now produce computer-calculators that will construct best-fit curves, integrate curves, and/or perform a pre-set series of calculations required to obtain the final reported value for recording by the analyst.

### 7.3.5 Key Punch Cards and Paper Tape

Since much of the analytical data generated in laboratories is recorded on bench sheets, transferred to data report forms, key-punched, then manipulated on small terminal computers, or manipulated and stored in a larger data storage system, there is a built-in danger of transfer error. This increases with each transposition of data. It is suggested that the analyst can reduce this error by recording data onto punch cards directly from bench sheets. The cards can be retained, or forwarded immediately to the data storage system as desired. IBM now offers a small hand-operated key-punch for this purpose.

It is anticipated that in future water quality systems, the intermediate report sheets will be eliminated and the data will be punched automatically by the analytical instrument system onto key-punch cards and/or paper tapes for direct use as computer input.

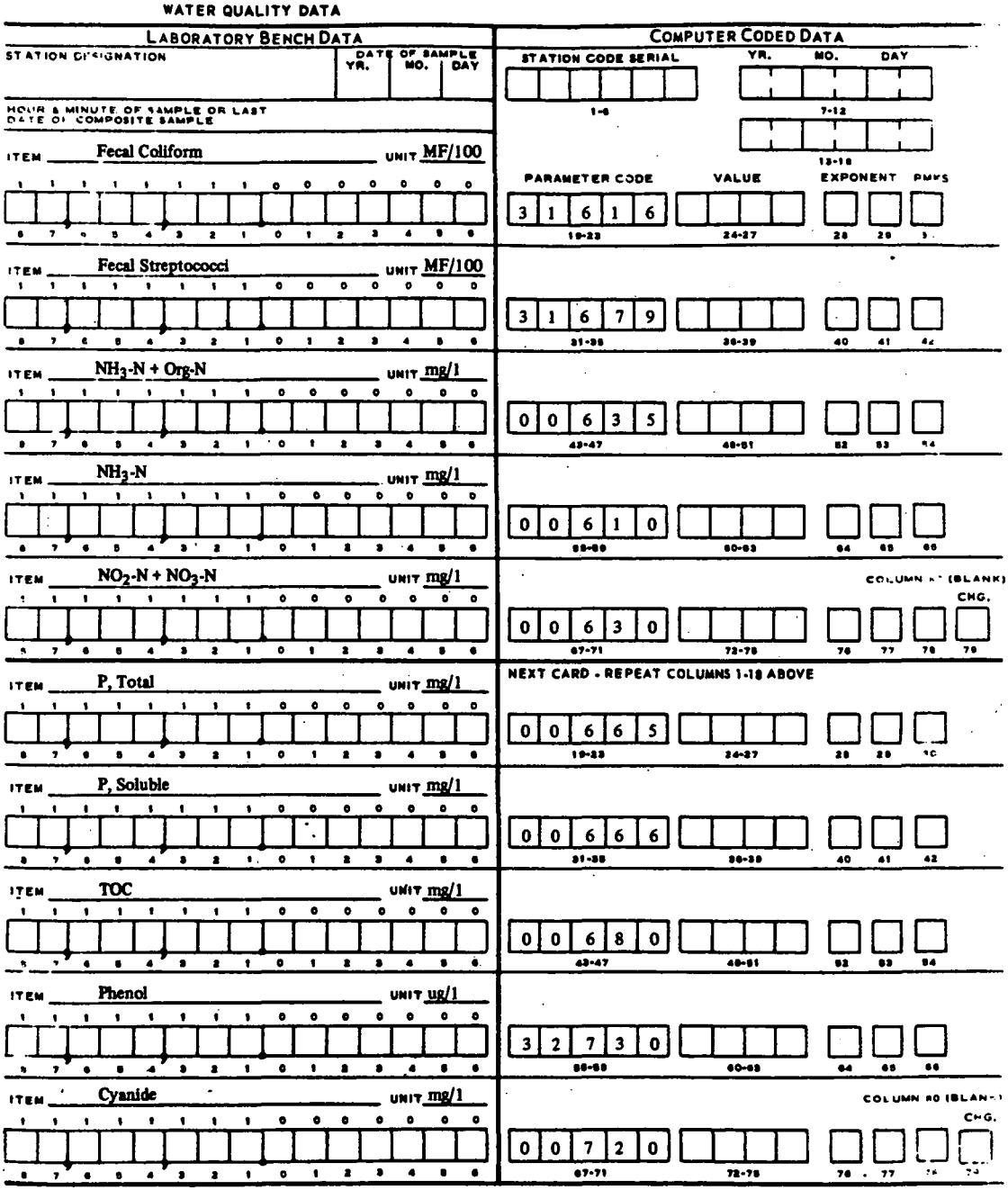
## 7.4 STORET–Computerized Storage and Retrieval of Water Quality Data

The use of computers with their almost unlimited ability to record, store, retrieve, and manipulate huge amounts of data is a natural outgrowth of demands for meaningful interpretation of the great masses of data generated in almost any technical activity.

In August 1961, an informal conference was held in the Basic Data Branch, Division of Water Supply and Pollution Control, U.S. Public Health Service. A number of ideas were brought together in the basic design of a system for storage and retrieval of data for water pollution control, called STORET. In 1966, the STORET system was transferred, with the Division, into the Federal Water Pollution Control Administration, U.S. Department of the Interior. A refinement of this system is now operated by the Technical Data and Information Branch, Division of Applied Technology, EPA.

If properly stored, the data can be retrieved according to the point of sampling, the date, the specific parameters stored, etc., or all data at a sample point or series of points can be

Figure 7-3. EXAMPLE OF STORET REPORT FORM



extracted as a unit.

There is a State/Federal cooperative activity which provides State water pollution control agencies with direct, rapid access into a central computer system for the storage, retrieval, and analysis of water quality control information.

Full details on use of the STORET system are given in the STORET handbook recently revised (3).

### 7.4 SHAVES–A Consolidated Data Reporting and Evaluation System

Information systems have been developed to bridge the gap between the analyst and his raw data, and a complex data storage and control system. These systems include preprinted report forms, computerized verification, and evaluation of data and data storage. An example is the SHAVES system.

The term, SHAVES, is an acronym for "Sample Handling and Verification System," which originated at the Great Lakes-Illinois River Basin Comprehensive Project Laboratory at Grosse Isle, Michigan. Although the system's original purpose was verification of the calculations following laboratory analyses, it now includes data storage, checks for completeness and consistency of data, procedures for submitting analytical requests, a set of forms for recording sampling and analytical information, and a clerical procedure to account for analyses completed and pending. The primary purposes of SHAVES are the standardization, automation and control of reporting analyses. All samples received at the Pacific Northwest Water Laboratory for routine analysis are processed through the system.

Although SHAVES uses a computer to perform its operations, it is not primarily a computer program. It is intended for use as an intra-laboratory quality control tool, and as such compliments the STORET system. It is described in detail elsewhere (4).

### 7.6 References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# CHAPTER 8

# SPECIAL REQUIREMENTS FOR TRACE ORGANIC ANALYSIS

## 8.1 Introduction

The high sensitivity of the instrumentation used in trace organic chemical analysis, and the low concentration of compounds being investigated, dictate that special attention be given this field of analytical endeavor. Contamination of the sample from any possible source must be diligently guarded against, and interferences in the sample must be carefully controlled. Finally, strict attention to method and highly refined technique are required to produce valid quantitative results.

## 8.2 Discrete Bottled Samples

Sample collection should be done with wide-mouth glass bottles, equipped with screw caps fitted with Teflon liners. The use of a screw cap without a Teflon liner may cause contamination of the sample by the liner or adhesive used in sealing the liner to the cap. Plastic bottles (polyethylene) are not used because traces of plasticizer may be leached from the plastic by the water, and can be a source of analytical interference. Moreover, organics from the sample may be adsorbed on the plastic. It has been suggested that high grade Teflon bottles may be satisfactory for this use; however, the cost is prohibitive at present. Many investigators avoid the use of glass sample bottles, because breakage in shipment frequently causes loss of sample. This is overcome by the use of relatively inexpensive, expanded polystyrene foam shipping containers molded to fit the bottle. These shipping containers can be purchased from Preferred Plastics Corp., North Grosvenordale, Connecticut.

To insure freedom from organic contaminants, bottles are rinsed successively with chromate cleaning solution, running tap water, distilled water, and finally several times with redistilled solvent (e.g., acetone, hexane, petroleum ether, chloroform). Caps are washed with detergent, rinsed with tap water, distilled water, and solvent. Liners are treated in the same way as the bottles and are stored in a sealed container.

Each method designates a recommended sample size for surface water analysis. Duplicate samples are recommended. If analysis by more than one method is to be requested on the same sample, sufficient sample must be simultaneously obtained to supply the needs of each analysis.

It is also recommended that when requesting a non-specific analysis, any information that could help direct the analytical approach, or aid in interpretation of results, be supplied. Such information could include industrial or agricultural activities in the area from which the sample was obtained, spills, or other accidents that may have occurred in the area. Also mention of similar upstream activity could provide valuable assistance.

Samples should be stored in a cool, dark place, and analyzed as soon as possible. If the sample cannot be analyzed immediately, reporting the holding time can help in interpreting results where die-off rates are known.

### 8.3 Carbon Adsorption Samples

The affinity of carbon for organic substances requires that supplies of carbon be protected from extraneous sources of contamination. For example, carbon can adsorb organic substances such as paint vehicles and insecticides from the air. Therefore, the carbon is stored and processed in an area adequately protected from such sources of contamination. As an additional precaution, the ventilating, heating, and air-conditioning systems for the laboratories in which carbon adsorption samples are processed are completely isolated from all other laboratories. All carbon is obtained from the manufacturer in sealed metal drums. Obviously, spraying with pest-control chemicals is not permitted in these areas. Carbon blank determinations supplement these precautions.

### 8.4 Glassware

Proper calibration of volumetric glassware is essential to valid analytical results, because quantitation is performed by comparison to measured amounts of standard compounds, and by accurate measurement of sample volume.

Individual concentrator tubes, used to measure final concentrate volumes, must first be calibrated at the working volume. This is particularly important for volumes less than 1 ml. Calibration should be made by noting the number of microliters of solvent required to bring the liquid level (lower miniscus) up to a particular graduation mark. A precision 100 $\mu$l syringe should be used to measure calibration volume.

It is also very important in trace organic analysis that glassware be as free as possible of any organic contaminants. A chromic acid cleaning solution is required for removing all traces of organic material from glassware.

### 8.5 Reagents and Chemicals

The minimum purity of reagents and chemicals should be analytical reagent grade. Analytical standards should be reference grade, when available. The analyst should take special note of the assay of less pure materials (most often pesticides). All reagents and chemicals should be stored according to manufacturer's instructions to prevent degradation. Proper storage is especially important if the chemical is to be used in preparing an analytical standard. Refrigerated chemicals should be allowed to come to room temperature before exposing them to the atmosphere.

When preparing stock solutions, it is recommended that at least 0.100 gram of material be used for greater accuracy in weighing. Solutions should be carefully stored so as to preserve their concentration, and to protect them from ultraviolet radiation. Usually storage in ground-glass stoppered bottles, either amber-colored, or out of the line of direct lighting, is sufficient.

Standard solutions should be prepared using precision syringes, preferably equipped with a Cheney adapter, to measure the volume of stock solution to be diluted. The syringe barrel should be pre-wetted with solvent and air bubbles expelled. Dilution should be done in a Class A volumetric flask to insure accurate measurement. If these solutions are to be used frequently, they are best stored in a screw-cap, septum, sealed vial. These vials allow instant access to the solution and offer good protection against concentration changes of the standard solution. Evaporation of the solvent caused by repeated removal of the cap is a

serious problem with other containers. If septum vials are not available it is advisable to prepare standard solutions in a volumetric container of 100 ml or more and transfer a small portion to a separate container for daily use, then discard that portion at the end of the day.

All stock or standard solutions should be carefully watched for signs of changes in concentration or deterioration. As an aid to monitoring these solutions, it is wise to label them as to compound, concentration, solvent used, date, and preparer. Also, in the case of GC solutions, it is necessary to retain some evidence of its chromatographic behavior as a fresh solution for comparison at a later date.

Distilled water used as dosed, control samples must be free of organic interferences. A very effective way of removing organic interferences from distilled water is to pre-extract the water with the solvent that is to be used in the analysis, then boil the water to remove the residual solvent.

Organic solvents used in pesticide analysis should be pesticide quality, and demonstrated to be free of interferences in a manner compatible with whatever analytical operation is to be performed. Solvents can be checked by analyzing a volume equivalent to that used in the analysis and concentrated to the minimum final volume. Possible interferences are noted in terms of factors such as relative retention times, peak geometry, peak intensity, and width of solvent response. Interferences noted under these conditions can be considered maximum. If necessary, a solvent must be redistilled in glass using a 60-cm column packed with 1/8" glass helices, or an equivalent system.

Hexane - ethyl ether and benzene are commonly used in the extraction of water and wastewater in conjunction with analysis by electron-capture gas-liquid chromatography. Because electron-capture detection methods are extremely sensitive to interferences normally found in these solvents, the cleanest possible reagent grade or pesticide-quality solvents must be used. Redistillation in the lab in an all-glass system is usually necessary. Experience in the Analytical Quality Control Laboratory has shown Burdick-Jackson hexane and Baker Chemical Co. ethyl ether to be satisfactory. All solvents vary from lot to lot. Therefore, when a good lot is found, subsequent order should specify that lot number. Solvents in the same lot may also vary and therefore each container should be checked.

### 8.6 Common Analytical Operations

Adequate steps must be taken to eliminate or minimize interferences from solvents and other materials. A blank should be run simultaneously under the same analytical conditions as any block of samples analyzed. A block of samples is defined as any group of one or more samples analyzed using a common batch of analytical supplies. Should any one of the supplies be changed (i.e. solvent, silica gel, Florisil, etc.) a new blank is required.

Quantitation of micro amounts of organic materials requires extremely careful technique to avoid loss of sample. Quantitative transfers are essential to obtain accurate and precise results. Practice in these manipulations is recommended for the inexperienced analyst.

Concentration of sample extracts to very small volumes for micro analysis requires great care to avoid loss of constituents. A Kuderna-Danish evaporator is a very useful apparatus to accomplish this operation. Instructions in the use of this evaporator must be strictly followed to avoid loss of desired sample (1). Final concentration in an ampule or calibrated tube is accomplished in a warm water bath with a gentle stream of clean, dry air, if air

oxidation is not a problem; otherwise, nitrogen should be used. During the final concentration, the inside walls should be rinsed repeatedly with the working solvent to insure the total sample is contained in the bottom of the tube. Complete evaporation of solvent must be avoided to prevent loss of sample constituents. The step should be accomplished within 10 or 12 minutes for best results.

### 8.7 Gas-Liquid Chromatography

To obtain reproducible results, it is necessary to have very accurate control of the column oven temperature. The temperature should be reproducible within ± 1°C, and have minimum gradients of 2°C throughout the oven. For temperature-programmed operation, a low mass oven is required to allow rapid heating and cooling of the column. Most manufacturers produce gas chromatographs which meet these requirements. Many organic compounds decompose when they come in contact with hot stainless steel. For this reason the injection block should be capable of accepting a quartz or glass tube to prevent the compounds from contacting hot metal. Memory peaks observed using direct, aqueous-injection, gas chromatography can be eliminated or greatly reduced by employing direct on-column injection. A Bio-med injection kit manufactured by Tracor Instrument Co. is very suitable for this purpose.

The septum should be changed at the end of each day's use. Changing at the end of each day allows overnight purging of the system of any bleed-off of contaminants from the septum. To avoid most of this bleed-off the septa can be preconditioned by heating at 250°C in a vacuum oven for two hours.

The gas chromatograph should be equipped with accurate needle-valve, gas-flow controls. If these flow controls are not previously calibrated this can easily be done using a soap-bubble flow meter and a stopwatch.

The nature of constituents to be measured dictates the type of detector to be employed. The electron capture detector is extremely sensitive to electronegative functional groups and substituents, such as: halogens, conjugated carbonyls, nitrites, nitrates, and organometals. It is virtually insensitive to hydrocarbons, alcohols, and ketones. The selective sensitivity to halides makes this detector particularly valuable for the analysis of many pesticides.

Electron capture detectors employing two sources of ionization are available: tritium ($H^3$) and radioactive nickel ($Ni^{63}$). Each has its advantages. Most $H^3$ detectors possess greater sensitivity than $Ni^{63}$ detectors. However, the $H^3$ detector is limited to 225°C because of radioactive leakage. This temperature limitation makes the $H^3$ detector susceptible to a buildup of high-boiling contaminants which reduce its sensitivity. The $Ni^{63}$ detector can be operated up to 400°C to prevent this buildup of contamination.

The microcoulometric detector is specific for halogen-, sulfur-, or nitrogen-containing compounds depending on the conditions used. Although the sensitivity of this detection system is not as great as others available, the extreme specificity makes it a very valuable device in indentification, minimizing the need for cleanup of the extract.

Another selective detector, with about the same sensitivity for chlorinated compounds as microcoulometric titration, is the Coulson electrolytic conductivity detector. The specificity of this detector makes it very useful in the identification of pesticides, and sample cleanup and pre-treatment are less critical. However, if chlorinated compounds are being detected, a

scrubber tube must be used with this instrument to remove any $SO_2$ produced by sulfur-containing compounds in the sample.

The flame ionization detector (FID) responds to virtually all compounds. Some notable exceptions are air, water, carbon disulfide, and the fixed gases. This makes the FID very useful for direct- aqueous-injection gas chromatography. However, since the detector is sensitive to such a wide range of compounds, it is also subject to interference from extraneous material. This means that the extract must be cleaned up considerably before analysis. The flame photometric detector (FPD) is used to analyze residues of phosphorus- and sulfur-containing pesticides and their metabolities. Little or no cleanup of the sample extract is required, and extraneous material causes no appreciable interference.

The alkali flame detector (Thermionic) can also be used in the analysis of organophosphate pesticides. Even though this detector gives an enhanced response to phosphorus, a large amount of extraneous material demands that the extract be cleaned up before the phosphorus-containing constituents are quantitated. An enhanced response to sulfur is not obtained with the alkali flame.

A one-millivolt, one-second, full-scale-response, strip-chart recorder should be used to maintain a permanent record of the results.

The type of detector employed dictates the type of carrier gas that must be used. Nitrogen can be used for the FID, electron-capture, flame-photometric, and microcoulometric titration detectors. The Analytical Quality Control Laboratory has used nitrogen from various suppliers, and has found the J. T. Baker Chemical Company's extra dry grade to be satisfactory. However, to avoid the risk of system or detector contamination from materials possibly present in the gas cylinder, the cylinder should be replaced when the tank pressure reaches 200 psi. It is also recommended that some type of gas purifier which is composed of a molecular sieve, desiccant, and filter be used on all combustion gases and carrier gases used with electron capture detectors.

A precision, gas-tight, microliter syringe, which can be accurately filled, will deliver reproducible injections, and can be easily and thoroughly cleaned, is recommended for gas chromatographic use. A Teflon plunger seal to prevent backwash, and no dead volume are desirable features. These types of syringes are available from various suppliers, but the Glencoe syringes have additional design features to prevent bending plungers.

It is not possible to enumerate here the many types and applications of liquid phases and solid supports. The literature (2, 3) will provide helpful guidance in the selection of these materials.

The type of column tubing required to perform a specific GC analysis is prescribed by the method for that analysis. Aluminum has been found suitable for chlorinated organic pesticides and stainless steel for phenols. Generally 1/8" or 1/4" OD tubing is used. The 1/4" column is capable of accepting larger injections but the 1/8" column is more efficient at chromatographing small injections. It should be noted that the use of glass columns will prevent the degradation of material often associated with metal columns.

Silanized glass wool should be used to minimize degradation or absorption of organic compounds by the glass-wool plugs. Glass wool can be silanized by treating with 10%

dimethyldichlorosilane in toluene for 10 minutes, or the prepared material can be purchased from Applied Science Laboratories.

Column packings can be prepared by the analyst, or purchased already prepared and conditioned. If prepared in the laboratory a highly refined technique should be developed by the analyst. A well prepared packing is essential to an acceptable chromatographic analysis. Particular care should be taken to accurately measure loadings, uniformly distribute the liquid phase, and preserve the structure of the fragile solid support. One method used successfully is the beaker slurry method in which the proper amounts of stationary phase and solid support are mixed together in a solvent in a beaker. The solvent can be evaporated by immersing the beaker in a warm water bath or filtering it through a Buchner funnel. The mixture is then dried at 110° C to remove residual solvent. A fluidized dryer (4) may also be used to remove the remaining solvent. Details on techniques for preparation of Teflon packings may be obtained from Applied Science Laboratories (Bulletin FF124).

An important consideration in packing a column is obtaining a uniform density, not so compact as to restrict gas flow, and not so loose as to create voids during use. Also important is the need to exercise care not to crush particles during packing.

It cannot be overemphasized that column conditioning is essential to obtain acceptable GC analysis. Proper conditioning further distributes the liquid phase over available active sites, and removes excess liquid phase that may bleed off the column and impair GC performance. Unstable performance in the form of baseline drift, varying sensitivity, and wide solvent peaks are usually the result of column bleed due to improper conditioning. This also contaminates the detector and makes frequent cleaning necessary.

A good general procedure for conditioning columns is as follows: install the column, leaving it disconnected from the detector; heat the column in the GC oven to just above maximum recommended operating temperature for the liquid phase, without gas flow, for 2 hours; follow with a half-hour equilibration period at a temperature at least 40 degrees below the maximum recommended temperature, still without flow; raise the temperature 20 degrees above operating conditions (do not exceed the maximum recommended operating temperature for the liquid phase), adjust gas flow to 50 ml/min, and maintain these conditions 24 to 48 hours.

There are many factors that must be considered in any attempt to obtain optimum operating conditions. As indicated, optimum conditions begin with the proper selection of column materials including tubing, glass wool, liquid phase, and solid support. Each method dictates the selection of these items including the percent liquid phase. Liquid load and operating temperature are critical to optimum resolution and elution rate. Uniformly coating the solid support, properly packing the column, and properly conditioning the column all contribute greatly to attaining optimum conditions. Improper attention to these factors contributes to either column bleed, which fouls the detector, or tailing peaks, which prevent resolution of closely eluting compounds.

The system must be free of gas leaks since these affect sensitivity and reproducibility. The proper selection of a carrier gas is important. Injection blocks should be of proper flow design, and should be kept clean. Temperature control of injection port, column oven, and detector must be accurate and constant, and must be at equilibrium to obtain reproducible results.

Considerable attention must be given to those factors affecting detector response, such as gas flows and regulated current. Also, standard solutions and condition of electrodes for MCT detectors, condition of radioactive source for EC detectors, and ratio of combustion gases in flame ionization detectors must be carefully attended to.

The gas chromatograph must either be operated within the linear range of the detector, or a suitable calibration curve must be employed. The linear range can be determined by chromatographing different amounts of a compound and observing the response. The peak area of the responses should be proportional to the amount of compound injected throughout the full range of the recorder. If these conditions are not fulfilled at the attenuation investigated, a more sensitive attenuation should be selected until the linear range is found.

Once having obtained optimum operating conditions, the analyst must assure sustained optimum performance through a routine maintenance program, as prescribed in the instrument manual. Such common practices as inspecting for gas leaks, changing the septum after daily use, replacing gas tanks before they run too low, and keeping a close check on injection block, oven, and detector temperatures must be performed frequently.

Column performance can be monitored by observing daily response to a selected group standard, and comparing it to response to that same standard under previously determined optimum conditions. Changes in elution pattern, relative proportions of peaks, and peak geometry are signs of a deteriorating column if the rest of the system has been properly maintained. A column should be replaced as soon as deterioration is observed.

A good syringe-handling technique is important when doing GC analysis. Before measuring the volume to be injected into the gas chromatograph, first wet the barrel of the syringe and expel all air bubbles. The volume injected is determined by drawing a selected portion of the extract entirely into the glass, calibrated, syringe barrel, noting the volume, injecting the sample, partially withdrawing the plunger, and measuring the liquid remaining in the syringe barrel. Injections are made rapidly after the needle is in the gas chromatographic injection port. The needle is immediately removed from the system to prevent volatilization of any sample in the needle. This method improves the analytical accuracy since any absolute volume is injected and reproducible technique is not required. It also eliminates the built-in bias of injecting slightly more than expected each time, when direct barrel readings are used exclusively.

The standard solutions can be more accurately used if prepared at a concentration that allows injection volumes similar to those of the sample.

### 8.8 Qualitative Analysis

The retention time ($R_t$) of a component on a given column, under given conditions, is characteristic of that particular component, and is used for qualitative identification. The standard way of reporting retention data is to give the relative retention ($RR_t$) defined as $R_t$ (component) ÷ $R_t$ (reference compound). The retention time may be measured as the time elapsed from an unrestrained solute peak elution, to the apogee of the peak of interest. This works well when a flame ionization or electron-capture detector are used. However, when the microcoulometric titration or the flame photometric detector are used (the flame is extinguished by the injection), no unrestrained solute peak is observed. In such cases, the injection point is manually or electrically marked, and used as the point of reference for

retention times. Caution must be exercised when manual marking is practiced, so that an accurate reference point is provided.

Tentative identification is made by matching the relative retention time of the unknown component with that obtained from a known compound analyzed under identical conditions, provided that peak geometry is also similar. It must be pointed out that similarity of retention time and peak geometry to a known compound does not unequivocally identify an unknown. Additional gas chromatographic columns of different polarity and other detectors may be used for confirmation. If retention time and peak geometry match on two or more columns, the identity is corroborated. However, further corroboration using analytical tools such as infrared spectroscopy, mass spectrometry, or thin-layer chromatography should be used whenever possible.

Proof of identity of compounds which produce a multi-peak response should be evidenced, not only by relative retention and peak geometry, but also by the correct number and relative proportion of each peak in the total chromatogram. This is called a "fingerprint" comparison of the known standard chromatogram with that of the unknown constituent.

### 8.9 Quantitative Analysis

The quantitative interpretation of a gas chromatogram is based either on the peak height or the peak area. The area measurement is generally preferred because peak height is extremely sensitive to small changes in the operating conditions, particularly in the column temperature. However, in chromatograms where the peaks are extremely sharp and narrow, the error involved in the area measurement makes height measurements more reliable.

Peak area measurement should be carried out using height x width at half height, disc integrator, or electronic digital integrator. These techniques are rapid and simple, and give good results with symmetric peaks of reasonable width. The use of a planimeter, although less precise than other methods, is presently found to be the best method for measuring the area of unsymmetric peaks which do not originate at the baseline. Precision is improved by tracing each peak several times and taking an average value. The electronic integrator is also recommended for those unsymmetric peaks which originate at the original baseline.

Concentrations of constituents are determined from standard calibration curves obtained under identical conditions. An absolute calibration curve is obtained from peak areas or peak heights plotted against known weights of a compound chromatographed under identical conditions. These standard injections must be made during the sample run to detect any change in instrument conditions or response which would invalidate the calibration.

The use of an internal standard is the most accurate method of quantitating constituents in a sample. A calibration curve can be obtained by simultaneously chromatographing the previously identified sample constituent and a standard, in known weight ratios, and plotting the weight ratios versus area ratios. An accurately known amount of the standard is then added to the unknown sample and the mixture chromatographed. The area ratios are calculated, and the weight ratio of the sample constituent to the standard is read from the curve. Since the amount of standard added is known, the amount of the sample constituent can be calculated. Using this method, injection volumes need not be accurately measured

and detector response need not remain constant since any change in response will not alter the ratio.

If the following requirements can be met, the internal standard is the preferred method of calibration. The internal standard must be well resolved from other peaks, must elute close to peaks of interest, should approximate the concentration of unknown, and should have structural similarity to unknown.

### 8.10 Thin-Layer Chromatography

Special equipment for thin-layer chromatography as supplied by the Brinkman Instrument Company has been found by the Analytical Quality Control Laboratory to be particularly convenient to use. Adsorbents supplied by Warner-Chilcott Laboratories, particularly Silica Gel G and Alumina G, are found to be free of impurities. In any case, it is best to check the adsorbent for impurities, and discard it if necessary. Plates precoated by the manufacturer can often be used when quantitation is visual; however, trials of plastic-backed plates in the Analytical Quality Control Laboratory have shown that they often develop unevenly and very slowly. The use of precoated glass-backed plates is therefore recommended.

Prepared plates should be carefully inspected for flaws that might interfere with proper chromatographic development. Plates should be marked in such a way that any grain lines would be perpendicular to the direction of development when used. Prepared plates are best activated by heating at 110°C in a convection oven for 30 minutes. Phosphorous pentoxide is recommended as the desiccant for use when storing plates.

Two important points to remember before using the developing chambers are to give the chamber atmosphere time to become saturated with solvent vapor, and to keep the chamber in constant temperature surroundings shielded from drafts. Failure to observe these points could result in an erratic chromatographic development, generally off line.

When spotting a plate, care must be taken not to overload the spot. Overloading results in poor chromatographic efficiency. Also, when applying a gentle stream of air or other gas to evaporate the solvent, care must be taken not to blow away any of the layer or sample droplets. The analyst should also be aware of whether or not the use of air to evaporate the solvent could cause any oxidation of the sample material, thereby causing erroneous results. If oxidation is a problem an inert gas should be substituted.

When spraying a plate with aqueous or other non-volatile sprays, care should be taken not to soak the plate. Soaking with such sprays may cause spots to run as the plate stands vertically. When using any spray it is better to use a stronger solution, then apply increased amounts to develop the spots. Overspraying can produce a background color which impairs the visibility of the developed spot.

Quantitation by visual comparison of sample response to a series of standards is semi-quantitative at best. However, by making the spot size the same for samples and standards,by using the same solvent for both, and by using careful development techniques, a fair degree of reliability can be obtained, when comparing the factors of spot size and color intensity.

When zone scraping and collecting the layer material with the aid of a vacuum, the collection apparatus is usually plugged with glass wool to trap the adsorbent. Since poorly

packed glass wool can cause high loss of material by failure to trap the dust, the scraping of the periphery of the zone should be done first. A clear tygon vacuum line, or a glass section near the end of the tube makes it easy to monitor for untrapped adsorbent.

### 8.11 Column Chromatography

The features of a chromatographic column which define its utility are shape, liquid capacity, and elution rate. The liquid capacity is principally a matter of convenience and should be weighed against the increased labor required for cleaning equipment. A flow-control device is critical to an efficient separation because increased flow decreases resolution between emerging components. A Teflon, flow-control stopcock is required because lubricant will produce an analytical interference.

Most adsorbents used in column chromatography are preactivated by the manufacturer and shipped in air-tight containers. Storage in the laboratory should also be in an air-tight container because moisture will greatly affect the activation state of an adsorbent. If a second activation step is required, the material should be heated at 130°C for at least five hours. The activity of the adsorbent can be monitored by eluting a mixture of chromatographically pure dyes (5). The elution rate and degree of separation of the individual dyes is a function of the activation state of the adsorbent. This enables the analyst to accurately attain the same activation for different batches of adsorbent.

When the adsorbent is added to the column, gentle tapping or a vibrator should be used to settle the material. This minimizes the space between particles and prevents channeling of the eluting solvent through the adsorbent which reduces separation efficiency.

Liquids should be added slowly, down the inside wall of the column, to avoid disturbing the packing surface. Mixing of the solution above the adsorbent with the fresh eluting solvent can be minimized by introducing the new solvent just as the last of the solution reaches the packing surface. The column top must not go dry or air may be introduced which will lower the separation efficiency of the system.

Before addition of the sample, columns should be pre-eluted with 50-75 ml of the solvent prescribed by the procedure. This is done to remove trapped air and to clean the column material of trace contaminants. During this pre-elution it is often necessary to tap the column to free all trapped air, especially if a volatile solvent is used in the pre-elution.

### 8.12 References


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# CHAPTER 9

# SKILLS AND TRAINING

## 9.1 General

Analytical operations in the laboratory can be graded according to the degree of complexity. Some analyses require no sample treatment, with the measurement performed in minutes on a simple instrument. Other determinations require extensive sample preparation prior to complex instrumental examination. Consequently, work assignments in the laboratory should be clearly defined. Each analyst should be completely trained and fully understand all the assignments of his job before being given new responsibilities. In this regard, all analysts, sub-professional or professional, should be thoroughly instructed in basic laboratory operations, according to the degree of professional maturity. Some of the basic operations that should be reviewed periodically with laboratory personnel follow:

a. Sample Logging

   Emphasize the routine procedure for recording of samples entering the laboratory, and assign primary responsibility. Establish what information is required, and how sample is routed to analyst. Discuss stability of samples, and how they should be stored prior to analysis.

b. Sample Handling

   The analyst should understand thoroughly when the sample is to be settled, agitated, poured, pipetted, etc., before removal from the container.

c. Measuring

   The analysts, especially new employees and sub-professionals, should be instructed in the use of volumetric glassware. The correct use of pipettes and graduates should be emphasized as discussed in Chapter 4.

d. Weighing

   Because almost every measuring operation in the analytical laboratory is ultimately related to a weighing operation, the proper use of the analytical balance should be strongly emphasized. Maintenance of the balance, including periodic standardization, should be reiterated to all personnel.

e. Glassware

   All glassware should be washed and rinsed following the requirements of the analysis to be performed. Not only must the personnel assigned to this task be instructed, but all lab personnel should know the routine for washing glassware, and also special requirements for particular uses. In addition, the precision tools of the laboratory such as pipets, burets, graduates, Nessler tubes, etc., should be inspected before use for cleanliness, broken delivery tips, and clarity of marking. Defective glassware should be discarded or segregated.

In summary, quality control begins with basic laboratory techniques. Individual operator error and laboratory error can be minimized if approved techniques are consistently practiced. To insure the continued use of good technique, laboratory supervisors should periodically review the basic techniques with each analyst and point out, when necessary, areas of needed improvement.

Continuing improvement of technical competence for all laboratory personnel is, of course, the final responsibility of the laboratory supervisor. In a well organized laboratory, however, a big brother attitude of higher ranking to lower grade personnel should be encouraged; each person should be eager to share experience, tricks-of-the-trade, special skills, and special knowledge with subordinates. Obviously, improved efficiency and improved data quality will result.

## 9.2 Skills

The cost of data production in the analytical laboratory is based largely upon two factors—the pay scale of the analyst, and the number of data units produced per unit of time. However, estimates of the number of measurements that can be made per unit of time are difficult, because of the variety of factors involved. If the analyst is pushed to produce data at a rate beyond his capabilities, unreliable results may be produced. On the other hand, the analyst should be under some compulsion to produce a minimum number of measurements per unit of time, lest the cost of data production become prohibitive. In the following table, estimates are given for the number of determinations that an analyst should be expected to perform on a routine basis. The degree of skill required for reliable performance is also indicated. The arbitrary rating numbers for the degree of skill required are footnoted in the tables, but are explained more fully below:

a. Rating 1—indicates an operation that can be performed by a semi-skilled sub-professional with limited background; comparable to GS-3 through GS-5.

b. Rating 2—operation requires an experienced aide (sub-professional) with background in general laboratory technique and some knowledge of chemistry, or a professional with modest training and experience; comparable to GS-4 through GS-7.

c. Rating 3—indicates a complex procedure requiring a good background in analytical techniques; comparable to GS-7 through GS-11.

d. Rating 4—a highly involved procedure requiring experience on complex instruments; determination requires specialization by analyst who interprets results; comparable to GS-9 through GS-13.

The time limits presented in the table are based on use of EPA methods.

A tacit assumption has been made that multiple analytical units are available for measurements requiring special equipment, as for cyanides, phenols, ammonia, nitrogen and COD. For some of the simple instrumental or simple volumetric measurements, it is assumed that other operations such as filtration, dilution or duplicate readings are required; in such cases the number of measurements performed per day may appear to be fewer than one would normally anticipate.

Table 9-1

SKILL-TIME RATING OF STANDARD ANALYTICAL OPERATIONS

| Measurement | Skill Required (Rating No.) | No./Day |
|---|---|---|
| | (Simple Instrumental) | |
| pH | 1 | 100-125 |
| Conductivity | 1 | 100-125 |
| Turbidity (HACH 2100) | 1 | 75-100 |
| Color | 1 | 60-75 |
| DO (Probe) | 1, 2 | 100-125 |
| Fluoride (Probe) | 1, 2 | 100-125 |
| | (Simple Volumetric) | |
| Alkalinity (Potentiometric) | 1 | 50-75 |
| Acidity (Potentiometric) | 1 | 50-75 |
| Chloride | 1 | 100-125 |
| Hardness | 1 | 100-125 |
| DO (Winkler) | 1, 2 | 75-100 |
| | (Simple Gravimetric) | |
| Solids, Suspended | 1, 2 | 20-25 |
| Solids, Dissolved | 1, 2 | 20-25 |
| Solids, Total | 1, 2 | 25-30 |
| Solids, Volatile | 1, 2 | 25-30 |
| | (Simple Colorimetric) | |
| Nitrite N (Manual) | 2 | 75-100 |
| Nitrate N (Manual) | 2 | 40-50 |
| Sulfate (Turbidimetric) | 2 | 100-125 |
| Silica | 2 | 100-125 |
| Arsenic | 2, 3 | 20-30 |

SKILL REQUIRED

1 - aide with minimum training, comparable to GS-3 through GS-5

2 - aide with special training or professional with minimum training, comparable to GS-5 through GS-7.

3 - experienced analyst, professional, comparable to GS-9 through GS-12.

Table 9-1 (continued)

SKILL-TIME RATING OF STANDARD ANALYTICAL OPERATIONS

| Measurement | Skill Required (Rating No.) | No./Day |
|---|---|---|
| | (Complex, Volumetric or Colorimetric) | |
| BOD | 2, 3 | 30-40* |
| COD | 2, 3 | 25-30 |
| TKN | 2, 3 | 25-30 |
| Phosphorus, Total | 2, 3 | 50-60 |
| Phenol (Dist'n only) | 2, 3 | 20-30 |
| Oil & Grease (Soxhlet) | 2, 3 | 15-20 |
| Fluoride (Dist'n) | 2, 3 | 25-30 |
| Cyanide | 2, 3 | 10-15 |
| | (Special Instrumental) | |
| Metals by AA (No preliminary treatment) | 2, 3 | 150 |
| Metals by AA (With preliminary treatment) | 2, 3 | 60-80 |
| Pesticides by GC (Without cleanup) | 3, 4 | 3-5 |
| Pesticides by GC (With cleanup) | 3, 4 | 2-4 |

SKILL REQUIRED

2 - aide with special training or professional with minimum training, comparable to GS-5 through GS-7.

3 - experienced analyst, professional, comparable to GS-9 through GS-12.

4 - experienced analyst, professional, comparable to GS-11 through GS-13.

* - depends on type of sample.

### 9.3 Training

For more experienced, higher grade personnel, formal training in special fields, possibly leading to specialization, should be almost mandatory. Such training can be fostered through local institutions and through the training courses provided by the Environmental Protection Agency. Regional policies on after-hours, government-supported training should be properly publicized.

Formalized training for lower grade personnel, comparable to GS-3 to GS-5, is relatively scarce. However, skills can be most efficiently improved at the bench level on a personal, informal basis by more experienced analysts working in the same area. Exposure to pertinent literature should also be a definite program policy.


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# Automated Determination of Mercury in Sediments

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An automated method for the determination of total mercury in sediment samples is reported. Aqueous suspensions of sediment samples are automatically analyzed using the cold-vapor detection method following a persulfate oxidation and stannous chloride reduction. The method completely recovers mercuric sulfide and produces data that are comparable to those obtained by the standard method. The method is safer, faster, and easier to perform than the standard method. Samples are analyzed at the rate of 30 per hour, with a routine detection limit of 0.1 mg Hg/kg of sample, and an average relative standard deviation of 6% at the level of 20–30 mg/kg.

In recent years, mercury has been recognized as a toxic contaminant to the environment. Reliable and efficient methods are required for determining mercury in all types of environmental samples. Most of the reported methods are for the determination of mercury in water samples. Nearly all of these methods involve the cold-vapor detection method described by Hatch and Ott (*1*). The measurement of total mercury involves a rigorous digestion procedure.

In lakes and streams, mercury can collect in the bottom sediments, where it may remain for long periods of time. It is difficult to release the mercury from these matrices for analysis. Several investigators have liberated mercury from soil and sediment samples by application of heat to the samples and collection of the released mercury on gold surfaces. The mercury was then released from the gold by application of heat or by absorption in a solution containing oxidizing agents (*2*, *3*).

Bretthaur, Moghissi, Snyder, and Mathews described a method in which samples were ignited in a high-pressure oxygen-filled bomb (*4*). After ignition, the mercury was absorbed in a nitric acid solution. Pillay, Thomas, Sondel, and Hyche used a wet-ashing procedure with sulfuric acid and perchloric acid to digest samples (*5*). The released mercury was precipitated as the sulfide. The precipitate was then redigested using aqua regia.

Feldman digested solid samples with potassium dichromate, nitric acid, perchloric acid, and sulfuric acid (*6*). Bishop, Taylor, and Neary used aqua regia and potassium permanganate for digestion (*7*). Jacobs and Keeney oxidized sediment samples using aqua regia, potassium permanganate, and potassium persulfate (*8*). The approved U.S. Environmental Protection Agency (EPA) digestion procedure requires aqua regia and potassium permanganate as oxidants (*9*).

These digestion procedures are slow and often hazardous because of the combination of strong oxidizing agents and high temperatures. In some of the methods, mercuric sulfide is not adequately recovered. The oxidizing reagents, especially the potassium permanganate, are commonly contaminated with mercury, which prevents accurate results at low concentrations.

El-Awady, Miller, and Carter have described an automated method for the determination of total mercury in waters and wastewaters (*10*). In that method, potassium persulfate and sulfuric acid were used to digest samples for analysis by the cold-vapor technique. The use of potassium permanganate as an additional oxidizing agent was proved unnecessary, which reduced the level of contamination in the system, and allowed a routine detection limit of 0.1 μg Hg/L to be attained. Use of the described method resulted in significant savings in time, reagent costs, and laboratory space when analyzing water and wastewater samples.

The same advantages can be realized when analyzing sediment samples by use of the method described here.

## EXPERIMENTAL

**Apparatus.** A Tekmar model SDT homogenizer was used to blend samples prior to analysis. All other apparatus used was described by El-Awady (*10*). Additional air lines were added to the analytical system, and an air-bar was used for all air lines. The GO fitting on the sample line was changed to G3. The analytical manifold is shown in Figure 1.

**Reagents.** A preservative solution was prepared by addition of 250 mL of concd $HNO_3$ and 25 g of $K_2Cr_2O_7$ to 500 mL of distilled water and dilution to 1000 mL. All other reagents were those described by El-Awady (*10*).

**Procedure.** Sediment samples were passed through a No. 10 polypropylene sieve to remove large debris. If necessary, the samples were blended using a Waring blender. Approximately 1 g of wet sediment was accurately weighed into a 360-mL polyethylene bottle. Five mL of preservative solution was added to drive off or oxidize any free sulfides as well as to preserve the sample (*10*, *11*). If the $K_2Cr_2O_7$ was entirely reduced, as indicated by a green color, additional preservative solution was added. Then 245 mL of distilled water was added, and the aqueous samples were blended using a Teckmar blender. The samples were allowed to stand overnight. Additional preservative solution was added if the dichromate was entirely reduced after standing. The aqueous samples were then analyzed, using the modified automated analytical system in the manner described by El-Awady (*10*).

To convert the mercury concentrations in the aqueous samples to the concentrations in the original sediments, separate determinations of percent solids were made, and the following formula was applied:

$$\text{mg Hg/kg (dry sample)} = \frac{\mu\text{g Hg/L (aqueous)} \times 25}{(\text{g sediment}) \times (\%\ \text{solids})}$$

## RESULTS AND DISCUSSION

**Recovery of Mercuric Sulfide.** Jacobs and Keeney observed that mercuric sulfide required digestion prior to analysis, using aqua regia and $KMnO_4$ plus $K_2S_2O_8$ for complete recovery of mercury (*8*). El-Awady observed variable results when analyzing $HgCl_2$ solutions which contained more than 20 mg $S^{2-}$/L, but he reported no interference for $HgCl_2$ solutions containing less than 20 mg $S^{2-}$/L (*10*).

To determine the recovery of HgS when analyzed by the automated method, organic and inorganic mercury standards were analyzed after they were spiked with $Na_2S$. Inorganic mercury reacts with sulfide:

$$S^{2-} + Hg^{2+} \rightarrow HgS$$

To ensure that the reaction was complete under the conditions studied, a standard containing 1000 μg $Hg^{2+}$/L was reacted with $Na_2S$ and filtered to remove HgS. The filtrate was diluted 1/10, and dichromate preservative solution was

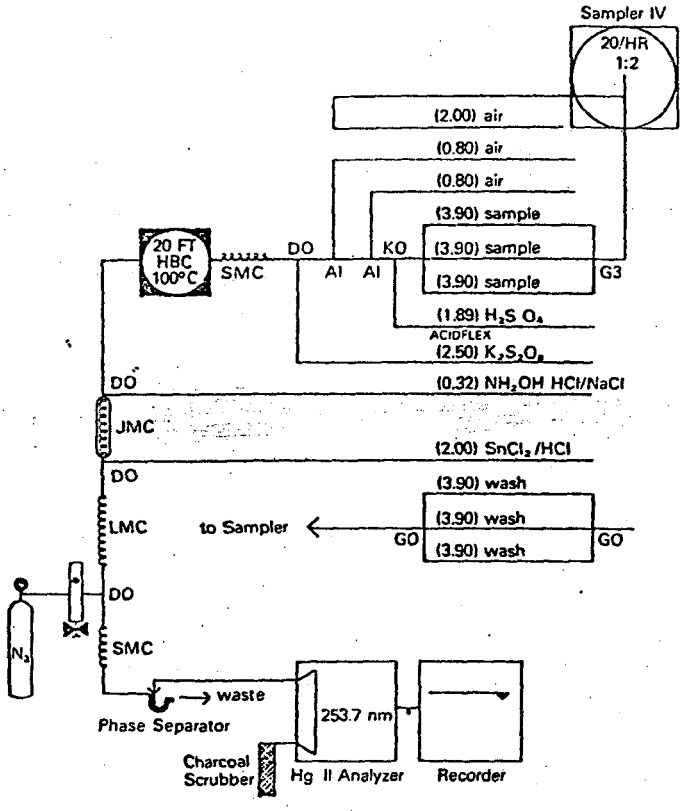


Figure 1. Modified automated total mercury manifold. Numbers in parentheses correspond to the flow rate of the pump tubes in mL/min. Numbers adjacent to glass coils and fittings are Technicon Corp. part numbers

added. Less than 0.1 μg Hg/L was recovered from analysis of the filtrate, which indicated that the formation of the insoluble HgS was complete.

When the same experiment was performed using organic mercury, no precipitate was observed, and 80% of the mercury was recovered in the filtrate. The loss of 20% was probably due to mercury which adhered to the glassware during filtration, since oxidizing conditions were not maintained.

Table I contains the results of analyses for organic and inorganic mercury standards which were spiked with sulfide. There was no significant interference due to $S^{2-}$ in the solutions containing 10 mg $S^{2-}$/L. However, a negative interference was observed for both organic and inorganic standards containing 100 mg $S^{2-}$/L which is equivalent to 25 000 mg $S^{2-}$/kg in the sediment. The spiked blank also resulted in a small negative interference.

It is interesting to note that exactly the same interference occurred for both organic and inorganic mercury standards, since methyl mercuric chloride does not react directly with sodium sulfide to form mercuric sulfide. Therefore, the interference could not be the result of incomplete digestion of HgS or $CH_3Hg^+$.

Sulfur, ozone and $H_2S$ were investigated as possible causes for the interference. Sulfur added to a standard did not cause an interference. Ozone and $H_2S$ were introduced directly into the mercury detector. No interference was observed.

When the automated method was used, the interferences which were observed for the standards and blank spiked with 100 mg $S^{2-}$/L, occurred when an excess of dichromate did not exist in the solutions. As excess of dichromate exists when the sulfide concentration is less than 36 mg/L. Above that concentration, there is no dichromate. When no additional dichromate preservative solution was added, a negative interference was observed between 10 and 50 mg $S^{2-}$/L for both organic and inorganic mercury standards. However, no interference was observed for sulfide concentrations as high as

Table I. Recovery of Mercury Standards Spiked with Sulfide

| Standard solution | $S^{2-}$ spike, mg/L | Observed Hg conc, μg/L |
|---|---|---|
| Blank | ... | 0.0 |
| 1 μg Hg/L ($HgCl_2$) | ... | 1.0 |
| 1 μg Hg/L ($CH_3HgCl$) | ... | 1.0 |
| 1 μg Hg/L ($HgCl_2$) | 10 | 1.0 |
| 1 μg Hg/L ($CH_3HgCl$) | 10 | 1.0 |
| Blank | 10 | 0.0 |
| 1 μg Hg/L ($HgCl_2$) | 100 | 0.4[a] |
| 1 μg Hg/L ($CH_3HgCl$) | 100 | 0.4[a] |
| Blank | 100 | −0.1 |
| 1 μg Hg/L ($HgCl_2$) + 4 times normal preservative | 100 | 1.1 |
| 1 μg Hg/L ($CH_3HgCl$) + 4 times normal preservative | 100 | 1.1 |
| Blank + 4 times normal preservative | 100 | 0.0 |

[a] Odd shaped peak.

100 mg/L if additional dichromate preservative solution was added and oxidizing conditions were maintained.

Therefore, it is our conclusion that the negative interference which was observed for the standards when oxidizing conditions were not maintained was caused by the adsorption of mercury on the inside surfaces of the culture tubes and the analytical system prior to the introduction of $K_2S_2O_8$. The small negative interference in the blank can be explained by the fact that a small amount of mercury was present in [redacted] analytical reagents. When the blank, which contained $S^{2-}$ [redacted] pumped through the system, oxidizing conditions were [redacted] maintained and a slight negative signal was observed.

The mercury which was adsorbed on the surfaces of the analytical system was released when oxidizing conditions were again present. This was observed as a peak which occurred when sulfide was removed from the system, or by a positive baseline drift.

**Organic Interferences.** Aromatic organic compounds such as benzene, which are not oxidized in the digestion, absorb at the same wavelength as mercury. This represents a positive interference in all cold-vapor methods for the determination of mercury. For samples containing aromatics, i.e., those contaminated by some industrial wastes, a blank analysis must be performed, and the blank results must be subtracted from the sample results. The blank analysis is accomplished by replacing the $K_2S_2O_8$ reagent and the $SnCl_2$ reagent with distilled water, and re-analyzing the sample.

**Comparison of Methods.** A diverse group of 25 sediments were analyzed by the automated method, and also after digestion by the standard EPA method. The standard EPA method was a micro modification of an aqua regia/$KMnO_4$ digestion (9). The results are shown in Table II. For those samples containing detectable concentrations of mercury, the automated method results averaged 94% of the standard EPA method results. The mean results for the first sample listed under "high organics" are quite different, with the automated method producing low results. However, when these results are compared to the standard deviations, 41 ± 11 mg/kg and 23 ± 5 mg/kg, the bias is not significant when compared [redacted] the data scatter. The variation in detection limits reported for the standard method is the result of the different weights of the samples taken for analysis.

Table III contains the results of inter-laboratory comparison testing for five sediment samples containing low mercury concentrations. The results which were obtained using the automated method with the analytical system as described by [redacted]

Table II. Comparison of Methods for Mercury Determination in Sediments

| Sample type | Standard method (7) mg Hg/kg (S) | Standard method (7) No. of detns | Automated method mg Hg/kg (A) | Automated method No. of detns | % Recovery (A/S x 100) |
|---|---|---|---|---|---|
| Sand | | | | | |
| Industry | 1.2 | 4 | 1.4 | 8 | 117 |
| Industry | 2.6 | 2 | 2 | 2 | 77 |
| Harbor | 0.5 | 2 | 0.4 | 2 | 80 |
| Harbor | <0.2 | 2 | <0.1 | 2 | ... |
| Harbor | <0.2 | 2 | <0.1 | 2 | ... |
| Harbor | <0.1 | 2 | <0.1 | 2 | ... |
| Harbor | <0.1 | 2 | <0.1 | 2 | ... |
| Harbor | <0.2 | 2 | <0.1 | 2 | ... |
| Harbor | <0.1 | 2 | <0.1 | 2 | ... |
| Clay | | | | | |
| Creek | <0.1 | 2 | 0.1 | 2 | ... |
| Harbor | 1 | 4 | 0.8 | 6 | 80 |
| Harbor | <0.2 | 2 | <0.1 | 2 | ... |
| Harbor | <0.5 | 4 | 0.2 | 6 | ... |
| Island | <0.2 | 2 | <0.1 | 2 | ... |
| Island | <0.6 | 2 | <0.1 | 2 | ... |
| Island | <0.5 | 2 | <0.1 | 2 | ... |
| Harbor | <0.2 | 2 | <0.1 | 2 | ... |
| High organics | | | | | |
| Industrial | 41 | 4 | 23 | 8 | 56 |
| Creek | <0.2 | 2 | 0.1 | 2 | ... |
| Sludge | 28 | 2 | 24 | 2 | 86 |
| Sludge | 16 | 2 | 18 | 2 | 112 |
| Sludge | 30 | 2 | 27 | 2 | 90 |
| Industrial | 0.5 | 2 | 0.7 | 2 | 140 |
| Harbor | 0.3 | 2 | 0.3 | 2 | 100 |
| Island | <0.9 | 2 | <0.1 | 2 | ... |
| Mean | | | | | 94 |

Table III. Comparison of Methods for Mercury Determination in Sediments

| Sample | Results of interlaboratory study (28 labs)[a] Mean, mg Hg/kg | Results of interlaboratory study (28 labs)[a] Acceptable range, mg Hg/kg | Automated method Without modification, mg Hg/kg | Automated method With modification, mg Hg/kg |
|---|---|---|---|---|
| 1 | 0.0873 | 0.063-0.111 | 0.072 | 0.1 |
| 2 | 0.179 | 0.126-0.232 | 0.14 | 0.2 |
| 3 | 0.229 | 0.166-0.292 | 0.12 | ... |
| 4 | 0.667 | 0.515-0.820 | 0.42 | 0.60 |
| 5 | 0.625 | 0.495-0.754 | 0.45 | 0.61 |

[a] Unpublished results obtained from Aspila and Carron (*12*).

did not flow uniformly through the system. Some of the sediment settled in the GO fitting before the pump. However, when additional air lines were added, an air-bar was used for all air lines, and the GO fitting was changed to a G3 fitting, the sediments were easily pumped through the system, and comparable results were obtained.

There was a limit to the amount of sediment that could be pumped through the manifold. If precipitation of sediment was observed in the sample lines, a smaller amount of sample was taken for analysis. If this precaution was not taken, results were biased low.

**Precision, Accuracy, and Spike Recovery.** The precision of the automated method at low levels was evaluated by analyzing four sand samples (which contained less than 1 mg Hg/kg) five times. The mean values ranged from 0.13 to 0.21 mg Hg/kg. The standard deviations ranged from 0.011 to 0.020 mg Hg/kg, with relative standard deviations ranging from 8.4% to 12%. (See Table IV).

Table IV. Precision at Low Levels

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Hg, mg/kg | 0.22 | 0.11 | 0.13 | 0.15 |
| | 0.19 | 0.12 | 0.15 | 0.18 |
| | 0.22 | 0.14 | 0.13 | 0.14 |
| | 0.22 | 0.15 | 0.12 | 0.16 |
| | 0.18 | 0.13 | 0.13 | 0.14 |
| Mean | 0.21 | 0.13 | 0.13 | 0.15 |
| Std dev | 0.020 | 0.016 | 0.011 | 0.017 |
| Rel std dev, % | 9.5 | 12 | 8.4 | 11 |

Table V. Precision and Spike Recovery for Sludge Samples

| | Sample 5 | Sample 6 | Sample 7 | Sample 8 |
|---|---|---|---|---|
| Hg, mg/kg | 18 | 16 | 35 | 30 |
| | 19 | 17 | 34 | 32 |
| | 18 | 18 | 30 | 33 |
| | 20 | 18 | 30 | 30 |
| | 16 | 17 | 32 | 31 |
| Mean | 18.2 | 17.2 | 32.2 | 31.2 |
| Std dev | 1.48 | 0.84 | 2.28 | 1.30 |
| Rel std dev | 8.1% | 4.9% | 7.1% | 4.2% |

| Sample | mg Hg/kg Mean | Spike | Sample + spike | Spike recovery | Spike recovery, % |
|---|---|---|---|---|---|
| 5 | 18.2 | 6.02 | 26 | 8 | 130 |
| 5 | 18.2 | 5.43 | 24 | 6 | 110 |
| 6 | 17.2 | 5.18 | 22 | 5 | 93 |
| 6 | 17.2 | 4.93 | 21 | 4 | 77 |
| 7 | 32.2 | 22.5 | 61 | 29 | 130 |
| 7 | 32.2 | 23.6 | 61 | 29 | 120 |
| 8 | 31.2 | 9.52 | 36 | 5 | 50 |
| 8 | 31.2 | 16.0 | 53 | 22 | 140 |

The precision of the automated method at high levels was evaluated by analyzing four sludge samples five times. The mean values ranged from 17.2 to 32.3 mg Hg/kg (Table V). These data indicate that results should be reported to no more than two significant figures. The same sludge samples were spiked in duplicate with methyl mercuric chloride, and then analyzed by the automated method. The average spike recoveries were 85%, 95%, 120%, and 125% (Table V).

**Detection Limit.** The detection limit for the automated method is dependent upon the weight of sample taken for analysis. It is 0.1 μg Hg/L in the aqueous samples. The results for the automated method are routinely reported to a lower limit of 0.1 mg/kg which corresponds to a dry sample weight of 0.25 g.

If a lower detection limit is necessary, the samples may be pulverized and a larger weight may be used. However, if any material settles in the manifold, the results will be biased low.

## ACKNOWLEDGMENT

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# Enzymic Substrate Determination in Closed Flow-Through Systems by Sample Injection and Amperometric Monitoring of Dissolved Oxygen Levels

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**Repetitive determinations using injection of the sample containing the sought-for species (substrate, S) into a continuously circulated reagent mixture (enzyme + buffer), is described. The glucose oxidase-catalyzed oxidation of β-D-glucose has been chosen to illustrate methods of substrate determination based on: $S + H_2O + O_2 \xrightarrow{E} P + H_2O_2$, with P = oxidation product(s), E = enzyme. Oxygen depletion is monitored by a three-electrode amperometric system allowing determination rates as high as 700 determinations/h, and with due precautions even about 1700 determinations/h, and relative standard deviations (population) of less than 2%. The method was compared with the "Beckman Oxygen Rate Analyzer" technique, which requires about 1 min per determination. The correlation factor between the two methods was found to be $r = 0.97$ (for 44 samples of human blood serum). The proposed approach allows continuous use of the enzyme and more than 10 000 serum samples have been injected into the same reservoir solution without any observable interference.**

Reagent recirculation in closed flow-through systems has been shown to be a useful ancillary device in repetitive determinations by sample injection (*1–3*). Enzymes, because of their catalytic nature, suggest themselves as main reagents to be recirculated in the implementation of such procedures. Several enzymic methods are based on the general scheme illustrated by the equation below:

$$S\ (\text{substrate}) + H_2O + O_2 \xrightarrow{E} \text{Product(s)} + H_2O_2 \quad (1)$$

in which E is the appropriate enzyme. Determination of glucose, uric acid, galactose, and D- and L-amino acids can be cited as examples of substrate determinations currently done with methods based on Equation 1. Both equilibrium and kinetic methods are available in which the $H_2O_2$ produced is measured by coupling reaction 1 with a second reaction in which the $H_2O_2$ oxidizes an organic compound whose color change(s) or fluorescence is monitored. Methods based on the measurement of oxygen decrease (as a result of reaction 1), mainly amperometrically, with membrane–Clark type–electrodes or a platinum disk covered with an immobilized enzyme layer acting as a thin reaction zone (*4, 5*), can also be found in the literature, and are both useful and popular. Details of these and similar procedures can be fou[illegible] monographs dedicated to enzymic determinations (*6*, [illegible] recent reviews on the subject (*8*). Specificity and regeneration (through the catalytic cycle) are, perhaps, the most significant properties of an enzyme as an analytical reagent. The first of these properties is widely recognized and used; the second, however, has not yet been thoroughly exploited. To our knowledge only a few papers report the re-use of the enzyme and attention has been mainly focused on immobilized enzymes (*1, 9*). An interesting exception to the use of immobilized enzymes is a Letter to the Editor recently published by Case and Phillips (*10*), when the work reported here was well under way, reporting recycling of the enzyme solution in the Beckman Glucose Analyzer.

The major objective of the work reported here was to effect recycling of the enzyme solution with sample injection techniques in continuously flowing streams with amperometric monitoring of changes in oxygen levels as a result of a reaction of the type illustrated in Equation 1. The advantages of enzyme recirculation are obvious and have been briefly discussed above; sample injection affords the use of small sample volumes and in conjunction with flow-through systems allows processing of a large number of samples per unit time. To take advantage of the latter, fast detection is needed, and in this paper we describe the application of the three-electrode nonmembrane system reported previously (*11*). Because of the relatively low price of glucose oxidase, the determination of glucose in:

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{D-Gluconic acid} + H_2O_2$$

in which GOD = glucose oxidase, was chosen as a model to illustrate the application that is the subject of this paper. The method illustrated here is another example of the analytical use of transient signals generated by series reactions and/or processes (*2, 3, 12*). As such it involves signal measurements made under dynamic conditions in a system approaching

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APPENDIX I

# Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue

U.S. Environmental Protection Agency
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

APPENDIX I

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APPENDIX I

# Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue

U.S. Environmental Protection Agency
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

# FORWORD

The accomplishment of our objective in protecting the environment requires a reliable assessment of the present condition and a determination of the effectiveness of corrective measures. Decisions which must be made on the need for pollution abatement and the most efficient means of achieving environmental quality depend upon the availability of sound data. Test procedures for measurement of the presence and concentration of substances hazardous to human health as well as an evaluation of the quality of the environment are essential to satisfactory decision-making.

These guidelines for sample preparation and analysis of sediment and fish have been prepared by the staff of the Environmental Monitoring and Support Laboratory - Cincinnati, at the request of the Monitoring and Data Support Division, Office of Water and Hazardous Wastes, with the cooperation of many US EPA Regional Laboratories, the Food and Drug Administration, the Southeast Water Research Laboratory, the Environmental Research Laboratory - Duluth, and the National Institute for Occupational Safety and Health.

The procedures represent the current state of the art, but improvements are anticipated as more experience is obtained. Users of these methods are encouraged to identify problems to assist in updating the test procedures by contacting the Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45263.

The Manual is published and distributed by the Environmental Research Information Center (ERIC), as one of a series designed to insure that the latest technologies developed by EPA and private

industry are disseminated to states, municipalities, and industries who are responsible for environmental pollution control.

The other manuals in this series are:

Methods for Chemical Analysis of Water and Wastes

Handbook for Analytical Quality Control in Water and Wastewater Laboratories

These are available from ERIC, Environmental Research Center, Cincinnati, Ohio 45268.

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SAMPLE HANDLING

1. Collection

1.1 Samples shall be collected according to recognized procedures. Preferably, all analyses should be performed on the same sample. A minimum of 250-grams are required for the total protocol.

1.2 The recommended container for the sediment sample is a standard one-quart, wide-mouth, screw-cap, glass bottle with a Teflon lid liner. It is particularly important that glassware used in organic residue analyses be scrupulously cleaned before initial use. At the time of collection the bottle should be filled nearly to the top with the sediment sample. If the sample is collected below a water column, the threads and sealing surfaces should be washed off with sample water. "Top off" the collected sediment sample with sample water and seal with the Teflon-lined screw cap. Maximum effort must be made to seal the sample with a minimum of gaseous head space. The sample must remain sealed until the aliquots for volatile organics are taken for analyses.

1.3 In the case of small fish, a sufficient number should be combined by sampling site location and species to obtain the minimum weight. The collected samples are wrapped in aluminum foil, labeled with freezer tape, and placed in the freezer chest with dry ice.

2. Preservation

2.1 The sediment sample should be labeled with freezer tape and transfered to the laboratory in an ice chest maintained at or near 4°C. The samples should be processed as soon as possible.

2.2 Fish samples are to be frozen at the time of collection and must remain frozen until the subsamples are taken for purgeable organics.

3. Processing

3.1 Sediment

3.1.1 Decant the water from the top of the sediment. Transfer the sediment into a Pyrex tray and mix thoroughly with a Teflon spatula. Discard sticks, stones and other foreign objects if present. Weigh five 10.0-gram portions of the sample into separate 125-ml vials. Using a crimper, tightly secure a septum to each bottle with an aluminum seal. Store these sample aliquots in a freezer until ready for volatile organics analysis.

3.1.2 Determine the percent solids in the sediment by drying a 10-25-g portion in a tared evaporating dish, overnight, at 103°C.

Calculate the % solids using the equation:

$$\% \text{ solids} = \frac{A}{B} \times 100$$

where: A = weight of dry residue in grams B = weight of wet sample in grams

3.1.3 Transfer half of the remaining sediment sample back to the original sample bottle and store at 4°C. This

portion will be used for those analyses requiring a wet sample. Spread the other half of the sample uniformly in the tray and allow to dry at room temperature for four or five days in a contaminant-free environment. When dry--less than 10% water--grind the sample with a large mortar and pestle to a uniform particle size. Discard any foreign objects found during grinding and transfer the powdered sediment into a wide-mouthed glass jar and seal with a Teflon lined lid. This air dried sample will be used for those analyses requiring an air dried sample.

3.2 Fish

3.2.1 To prepare the fish sample for analytical pretreatment, unwrap and weigh each fish. Combine small fish by site and species until a minimun conbined weight of 250-g is obtained Chop the sample into 1 in chunks using a sharp knife and mallet.

3.2.2 Grind the sample using a large commercial meat grinder that has been precooled by grinding dry ice. Thoroughly mix the ground material. Regrind and mix material two additional times. Clean out any material remaining in the grinder; add this to the sample and mix well.

3.2.3 Weigh five 10.0-g portions of the sample into separate 125-ml vials. Using a crimper, tightly secure a septum to each bottle with a seal. Store

these sample aliquots in a freezer until ready for volatile organics analysis.

3.2.4 Transfer the renaining fish sample to a glass container and store in a freezer for later subsampling and analysis.

4. Special Equipment and Materials

4.1 Ice chest.

4.2 wide-mouth quart bottles with Teflon lid liners.

4.3 Teflon coated or porcelain spatula.

4.4 Pyrex glass tray, 8 x 12 x 2 inch.

4.5 Mortar and pestle (large).

4.6 Knife, heavy blade (or meat cleaver).

4.7 Mallet, plastic faces, 2 to 3-lb.

4.8 Electric meat grinder, 1/2 HP.

4.9 Dry ice.

4.10 Aluminum foil.

4.11 Freezer tape, for labels.

4.12 Freezer.

4.13 Vials, 125-ml Hypo-Vials (Pierce Chemical Co., #12995), or equivalent.

4.14 Septa, Tuf-Bond (pierce #12720), or equivalent.

4.15 Seals, aluminum (Pierce #13214), or equivalent.

4.16 Crimper, hand (Pierce #13212), or equivalent.

# Analysis of Sediments for Chlorinated Pesticides, Polychlorinated Biphenyls and non-polar Neutrals

1. Scope

1.1 The compounds listed in Table I are extracted from air-dried sediment by the soxhlet extraction technique. The extract is subsequently analyzed for pesticides and PCBs using approved methods$ul,2$p as cited in the Federal Register$u3$p. The remaining compounds are determined using the methods described in "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants" (4). While the above referenced methods have been proven for pesticides and PCBs, they have not been sufficiently tested through extensive experimentation for the non-polar neutral compounds in Table I.

2. Special Apparatus and Materials

2.1 Soxhlet extractor, 40-mm ID, with 500-ml round bottom flask.

2.2 Kuderna-Danish, 500-ml, with 10-ml graduated receiver and 3-ball Snyder column.

2.3 Chromatographic column - Pyrex, 20-mm ID x approximately 400-mm long, with coarse fritted plate on botton.

3. Procedure

3.1 Extraction

3.1.1 Weigh 30.0-grams of the previously air-dried sample into a tared 200-ml beaker. Add 3-ml distilled water

(10% of sample weight), mix well and allow to stand for 2 hours while mixing occasionally.

3.1.2 Place about 1/2" of pre-extracted glass wool in the bottom of the soxhlet extractor chamber and quantitatively transfer the contents of the beaker into the chamber. Place a second glass wool plug on top of the sample. Wash the 200-ml beaker and all mixing tools several times with a 1:1 hexane acetone mixture. Cycle the wash mixture through the extractor using a total of 300-ml of the mixed solvent.

3.1.3 Attach the extractor to a 500-ml round bottom flask containing a boiling stone and extract the solids for 16 hours.

3.1.4 After extraction is complete, dry and filter the extract by passing it through a 4" column of hexane-washed sodium sulfate. Wash the 500-ml flask and the sodium sulfate with liberal amounts of hexane. Collect the eluate in a 500-ml K-D evaporative flask with a 10-ml ampul. Concentrate the sample extract to 6-10-ml.

3.2 Clean up and seperation.

3.2.1 Adjust the sample extract volume to 10-ml and cleanup the extract by Florisil column chromatography according to the 304 (g) methodology for PCBs, part 10.3. For sulfur removal continue with part 10.5.3.4 of that method. NOTE: If sulfur crystals are

present in the extract seperate the crystals from the sample by decantation.

3.2.2 Analyze the Florisil eluates for the pesticides appearing in Table I, according to the approved method for pesticides. The PCBs appearing in Table I, should be determined as described in Reference 2.

3.2.3 Analyze remaining compounds of Table I, column C, by "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants" (4).

3.3 Standard quality assurance protocols should be employed, including blanks, duplicates and dosed samples as described in the "Analytical Quality Control Handbook". Dosing can be accomplished by injecting 1-20-ul of a standard into the homogenized sediment contained in the soxhlet extractor chamber.

4. Reporting of Data

4.1 Report results in ug/kg on a dry weight basis using the percent moisture values determined earlier. Report all quality control data with the analytical results for the samples.

# lysis of Fish for Chlorinated Pesticides and Polychlorinated Biphenyls

1. Scope

1.1 The chlorinated pesticides and polychlorinated biphenyls (PCBs) listed in Table I are extracted from fish using either method A or B as described below. Method A employs a blender, whereas a Tissumizer or the equivalent is required for Method B. Either procedure results in an extract that can be incorporated directly into approved US EPA test procedures for pesticides or PCBs as cited in the Federal Register (3).

2. Special Apparatus and Materials

2.1 Method A Only

2.1.1 Blender, high speed - Waring Blender, Courdos, Omni-Mixer, or equivalent. Explosion proof model recommended. Quart container is suitable size for routine use.

2.1.2 Buchner funnel - porcelain, 12-cm.

2.1.3 Filter paper - 110-mm sharkskin circles.

2.1.4 Flask, vacuum filtration - 500-ml.

2.2 Method B Only

2.2.1 Tissumizer SDT-182EN (available from Tekmar Company, P.O. Box 37202, Cincinnati, Ohio 45222) or equivalent.

2.2.2 Centrifuge - capable of handling 100-ml centrifuge tubes.

2.3 Method A & B

2.3.1 Kuderna-Danish concentrator - 500-ml, with 10-ml graduated receiver and 3-ball Snyder column.

2.3.2 Chromatographic column - pyrex, 20-mm id x approximately 400-mm long, with coarse fritted plate on bottom.

3. Procedures

3.1 Method A

3.1.1 Weigh a 25 to 50-g portion of frozen, ground fish and add to a high speed blender. Add 100-g anhydrous $Na_2SO_4$ to combine with the water present and to disintegrate the sample. Alternately, blend and mix with a spatula until the sample and sodium sulfate are well mixed. Scrape down the sides of the blender jar and break up the caked material with the spatula. Add 150-ml of hexane and blend at high speed for 2 min.

3.1.2 Decant the hexane supernatant through a 12-cm Buchner filter with two sharkskin papers into a 500-ml suction flask. Scrape down the sides of the blender jar and break up the caked material with the spatula. Re-extract the residue in the blender jar with two 100-ml portions of hexane, blending 3 min each time. (After one min of blending, stop the blender, scrape the material from the sides of the blender jar, and break up the caked material between extractions.

3.1.3 Decant the hexane supernatants through the Buchner and combine with the first extract. After the last

blending, transfer the residue from the blender jar to the Buchner, rinsing the blender jar and material in the Buchner with three 25 to 50-ml portions of hexane. Immediately after the last rinse, press the residue in the Buchner with the bottom of a clean beaker to force out the remaining hexane.

3,1,4 Pour the combined extracts and rinses through a column of anhydrous $NA_2SO_4$ ., 20-mm x 100-mm, and collect the eluate in a 500-ml Kuderna-Danish concentrator. Wash the flask and then the column with small portions of hexane and concentrate the extract below 10-ml.

3.2 Method B

3.2.1 Weigh 20.0-g of frozen, ground fish and add to a 100-ml centrifuge tube. Add 20-ml of hexane and insert the Tissumizer into the sample. Turn on the Tissumizer and disperse the fish in the solvent for 1 min. Centrifuge and decant the solvent through a column of anhydrous $Na_2SO_4$ , 20-mm x 100-mm, and collect the eluate in a 500-ml Kuderna-Danish concentrator.

3.2.2 Repeat the dispersion twice using a 20-ml aliquot each time, combining all dried portions of solvent in the concentrator. Rinse the Tissumizer and the column with small portions of hexane and concentrate the extract below 10-ml.

3 Cleanup and Analysis

3.3.1 Unless prior experience would indicate the fish species fat content is low (less than 3 g/per extract), the hexaneacetonitrile clean-up procedures described in the reference methods should be followed. In all cases, Florisil column chromatography should be used to clean up the extracts before gas chromatography (a). An electron capture detector is used for final measurement, and results are calculated in ug/kg. Identifications can be confirmed by GC/MS techniques as described in the analytical protocol for wastewaters (4).

3.4 Quality Control

3.4.1 Standard quality assurance protocols should be employed, including blanks, duplicates, and dosed samples as described in the "Analytical Quality Control Handbook" (7).

3.4.2 Dose fish sample aliquots by injecting minimum amounts (<20-ul total) of concentrated pesticide or PCB solutions into the solid subsample 10 to 15 minutes before extraction.

Reporting of Data

4.1 Report results in ug/kg on a wet tissue basis. Report all quality control data with the analytical results for the samples.

# Analysis of Sediment for General Organics by Mechanical Dispersion Extraction

1. Scope

1.1 This method is designed to determine those "unambiguous priority pollutants" associated with the consent decree that are solvent extractable and amenable to gas chromatography. Tables I, II, and III are a summary of compounds that should be extracted at an 80-100% efficiency. It is a GC/MS method intended for qualitative and semi-quantitative determination of these compounds. Although this approach has not been sufficiently tested through extensive experimentation, it is based on laboratory experience and is presently considered to be a reasonable analytical approach for these organic materials in sediment.

1.2 This method is not applicable to those very volatile pollutants listed in Table IV.

2. Special Apparatus and Materials

2.1 Mechanical dispersion device.

2.2 Centrifuge - capable of handling 100-ml centrifuge tubes.

2.3 Separatory funnels - 2-liter with teflon stopcock.

2.4 Sieve, 20 mesh.

3. Procedure

3.1 Weigh 20.0-g of wet, well-mixed sediment into a 100-ml centrifuge tube. If the sediment contains grit larger than 20 mesh, it is necessary to extrude the sample through a

20 mesh sieve in order to prevent damage to the mechanical dispersion device. Add 20-ml acetonitrile and insert the dispersion device into the sample. Disperse the sediment into the solvent for 1 min. Centrifuge and decant the solvent into a 2-liter separatory funnel containing 1300-ml of a 2 percent aqueous solution of sodium sulfate previously adjusted to ph 11 with 6 N NaOH. Repeat the dispersion twice, using a 20-ml aliquot each time, and combine the acetonitrile washings in the separatory funnel. Caution! the dispersion should be carried out in a fume hood to avoid exposure to acetonitrile.

3.2 Extract the aqueous acetonitrile solution in the separatory funnel with 60-ml of hexane for 2 min. Drain the aqueous layer into a 2-liter Erlenmeyer flask and pour the hexane extract through a short column of anhydrous sodium sulfate prerinsed with hexane. Collect the dried extract in a 500-ml Kuderna-Danish (K-D) flask fitted with a 10-ml ampul. Repeat the extraction and drying steps twice, combining the extracts.

3.3 Evaporate the extract to 5 to 10-ml in a 500-ml K-D apparatus fitted with a 3-ball Snyder column and a 10-ml calibrated receiver tube. allow the K-D to cool to room temperature. Remove the receiver and adjust the volume to 10-ml. Label this as the base neutral fraction. If additional sensitivity is required, add fresh boiling chips, attach a two-ball micro-Snyder column, and carefully evaporate to 1.0-ml or when active distillation ceases.

3.4 Return the aqueous acetonitrile solution to the separatory funnel and adjust the ph with 6 N HCl to ph2 or less. Extract three times with 60-ml of hexane each time. Combine the extracts, dry, and concentrate as described above. Label this as the acid fraction.

3.5 Analyze both extracts according to "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants" (4). Should the acetonitrile partition used in this procedure not sufficiently remove interferences, florisil (2), alumina (5), and silica gel (2) column chromatographic clean-up and separation techniques can be employed. Sulfur can be removed by treatment with mercury (2).

3.6 Standard quality control assurance protocols should be employed, including blanks, duplicates and dosed samples, as described in the "Analytical Quality Control Handbook" (7). Dosing can be accomplished in injecting 1-20-ul of a standard solution into the homogenized sediment contained in the centrifuge tube.

4. Reporting of Data

4.1 Report results in ug/kg on a dry weight basis using the percent moisture values determined earlier. Report all quality control data with the analytical results for the samples.

Analysis of Fish for General Organics by Solvent Extraction

1. Scope

1.1 This method is designed to determine those "unambiguous priority pollutants" associated with the Consent Decree (4) that are solvent extractable and amenable to gas chromatography. These compounds are listed in Tables II and III. It is a GC/MS method intended for qualitative and semi-quantitative determination of these compounds. Although this approach has not been sufficiently tested through extensive experimentation, it is based on laboratory experience and is presently the best analytical approach for these organic materials in fish.

2. Special Apparatus and Materials

2.1 Tissumizer SDT-182EM (available from Tekmar Company, P.O. Box 37202, Cincinnati, Ohio 45222) or equivalent.

2.2 Centrifuge - capable of handling 100-ml centrifuge tubes

2.3 Separatory funnels - 2-liter with teflon stopcock.

2.4 Organic free water - prepared by passing distilled water through an activated carbon column.

3. Procedure

3.1 Weigh 20.0-g of ground, homogeneous fish and add to a 100-ml centrifuge tube. Add 20-ml of acetonitrile and insert the tissumizer into the sample. Turn on tissumizer and disperse the fish into the solvent for 1 min. Centrifuge and decant the solvent into a 2-liter separatory funnel containing 1300-ml of a 2 percent aqueous solution of

sodium sulfate. Repeat the dispersion twice, using a 20-ml aliquot each time, and combine the acetonitrile in the separatory funnel.

Caution: the dispersion should be carried out in a fume hood to avoid exposure to acetonitrile.

3.2 Adjust the ph of the sodium sulfate acetonitrile solution with 6N NaOH to ph 11 or greater. Use multirange ph paper for the measurement. Extract the aqueous acetonitrile solution with 60-ml of hexane. Shake the separatory funnel for 2 min. Drain the aqueous layer into a 2-liter erlenmeyer flask and pour the hexane extract through a short column of prerinsed anhydrous sodium sulfate. Collect the dried extract in a 500-ml Kuderna-Danish (K-D) flask fitted with a 10-ml ampul. repeat the extraction and drying steps twice, combining the extracts. Evaporate the extract to 5 to 10-ml in a 500-ml K-D apparatus fitted with a 2-ball Snyder column and a 10-ml. Analyze by GC/MS. If additional sensitivity is required, add fresh boiling chips, attach a two-ball micro-Snyder column, and carefully evaporate to 1.0-ml or when active distillation ceases.

3.3 Return the aqueous acetonitrile solution to the separatory funnel and adjust the pH with 6N HCl to pH 2 or less. Extract three times with 60-ml of hexane each time. Combine the extracts, dry, and concentrate as described above. Analyze by GC/MS.

NOTE: Should the partition used in this procedure not sufficiently remove the lipid material, gel permeation can

be employed. However, special expensive equipment is necessary for this procedure (6).

3.4 Standard quality assurance protocols should be employed, including blanks, duplicates, and dosed samples, as described in the "Analytical Quality Control Handbook" (7). Dosing can be accomplished by injecting 1 to 20-ul of a standard solution into the homogenized tissue contained in a centrifuge tube.

4. Reporting of Data

4.1 Report results in ug/kg on a wet tissue basis. Report all quality control data with the analytical results for the samples.

# Analysis of Sediment for Volatile Organics by Head Space Analyses

## 1. Scope

1.1 This method is designed to determine those "unambiguous priority pollutants," associated with the Consent Decree (4) that are amenable to head space analyses. These compounds are listed in Table IV. It is a GC/MS method intended for qualitative and semi-quantitative determination of these compounds. The head space analyses and the liquid-liquid extraction methods are complementary to one another. There is an overlap between the two: some compounds can be recovered by either method. The efficiency of recovery depends on the vapor pressure and water solubility of the compounds involved. Generally, the overlap involves compounds boiling between 130 and 150° C with a water solubility of approximately 2 percent. When compounds are efficiently recovered by both methods, the chromatography determines the method of choice. The GC conditions selected for the head space method are, generally, not suitable for the determination of compounds eluting later than chlorobenzene.

1.2 Under ideal conditions, the limit of detection for the various volatiles should be near 25-ug/kg. Actual detection limits will vary widely between samples because sediment surfaces, water content and non-volatile organic loading will influence the partition coefficients of the volatile organics.

1.3 Although the above approach has not been sufficiently tested through the extensive experimentation, it is based on laboratory experience and is presently considered to be the best analytical approach for volatile organic materials in sediments.

2. Special Apparatus and Materials

2.1 Gas-tight syringe - 5-cc.

2.2 Head space standard solutions - Prepare two standard methanol solutions of the compounds listed in Table IV at the 50-ng/ul and 250-ng/ul concentrations. The standard solutions should be stored at less than 0°C. Solutions should be allowed to warm to room temperature before dosing. Fresh standards should be prepared weekly. Procedures for preparing standards are outlined in the purge and trap section of Reference 4.

2.3 Vials, 125 ml "Hypo-Vials" (Pierce Chemical Co., #12995), or Equivalent.

2.4 Septa, "Tuf-Bond" (Pierce #12720), or equivalent.

2.5 Seals, aluminum, (Pierce #13214), or equivalent.

2.6 Crimper, hand, (Pierce #13212), or equivalent.

3. Procedures

3.1 Place 10.0-g each of the well-mixed, wet untreated sediment sample into five separate 125-ml septum seal vials.

3.2 Dose one sample vial through the septum with 10-ul of the 50-ng/ul standard methanol solution. Dose a second vial with 10-ul of the 250-ng/ul standard.

3.3 Place the two dosed sample vials and one non-dosed sample into a 90°C water bath for 1 hour. Store the two remaining samples near 4°C for possible future analyses.

3.4 While maintaining the sample at 90°C, withdraw 2.0-ml of the head gas with a gas tight syringe and analyze by injecting into a GC, operating under the conditions recommended in Reference (4).

3.5 Analyze all three samples in exactly the same manner. Subtract the peak areas of compounds found in the undosed sample from the corresponding compounds contained in the dosed samples. Construct a calibration curve from the corrected dosed data; quantify the unknown.

NOTE: If the calculated sample concentration is greater than the concentration of the dosed standard used in the dosing step, it is necessary to prepare additional standards in order to bracket the unknown.

3.6 Standard quality assurance protocols should be employed, including blanks, duplicates, and dosed samples, as described in the "Analytical Quality Control Handbook" (7).

4. Reporting of Data

4.1 Report all results in ug/kg on a dry weight basis using the percent moisture values determined earlier. Report all quality control data with the analytical results for the samples.

Analysis of Fish for Volatile Organics by Head Space Analyses

1. Scope

1.1 This method is designed to determine those "unambiguous priority pollutants" associated with the Consent Decree (4) that are amenable to head space analyses. These compounds are listed in Table IV. It is a GC-MS method intended for qualitative and semi-quantitative determination of these compounds. The head space analyses and the liquid-liquid extraction methods are complementary to one another. There is an overlap between the two: some compounds can be recovered by either method. The efficiency of recovery depends on the vapor pressure and water solubility of the compounds involved. Generally, the overlap involves compounds boiling between 130 and 150° C with a water solubility of approximately 2 percent. When compounds are efficiently recovered by both methods, the chromatography determines the method of choice. The GC conditions selected for the head space method are, generally, not suitable for the determination of compounds eluting later than chlorobenzene.

1.2 Although the above approach has not been sufficiently tested through extensive experimentation, it is based on laboratory experience and is presently considered to be the best analytical approach for volatile organic materials in fish.

2. Special Apparatus and Materials

2.1 Sonifier Cell Disrupter W-350 with microprobe (manufactured by Brawson Sonic Power Co., Danbury, Connecticut) or equivalent.

2.2 Gas-tight syringe - 5-cc.

2.3 Organic free water - Prepared by passing distilled water through an activated carbon column.

2.4 Head space standard solutions - Prepare three standard methanol solutions of the compounds listed in Table IV at the 50-ng/ul, 150-ng/ul, and 300-ng/ul concentrations. The standard solutions should be stored at less than 0°C. Solutions should be alloved to warm to room temperature before dosing. Fresh standards should be prepared weekly. Procedures for preparing standards are outlined in the purge and trap section of Reference (4).

3. Procedure

3.1 Renove four of the sample vials containing 10.0-g of homogenized fish from the freezer. Open the vials and add 10-ml of organic free water to each while the fish is still frozen. Sonify the fish for 30-sec at maximum probe power. Immediately reseal the vials.

3.2 Dose one sample vial through the septum below the water level with 10-ul of the 50-ng/ul standard methanol solution. Dose a second vial with 10-ul of the 150-ng/ul standard and a third vial with 10-ul of the 300-ng/ul standard.

3.3 Place all four sample vials into a 90°C water bath for 1 h.

3.4 While maintaining the sample at 90°C, withdraw 2.0-ml of the head gas with a gas tight syringe and analyze by injecting into a GC, operating under the conditions recommended in Reference (4).

NOTE: Specific GC detectors can be substituted for the ES.

3.5 Analyze the undosed sample first, followed by the 50-ng/ul dosed sample. If no compounds of interest are found in the undosed sample and the dosed sample produces peaks to indicate recovery of the protocol compounds, do not analyze the remaining samples. Calculate lower limits of detection based on the response obtained from the dosed sample. If compounds are observed in the undosed sample, analyze the two remaining dosed samples in exactly the same manner. Subtract the peak areas of compounds found in the undosed sample from the corresponding compounds contained in the dosed data; quantify the unknown.

NOTE: If the calculated sample concentration is greater than the concentration of of the dosed standard used in the dosing step, it is necessary to prepare additional standards in order to bracket the unknown. Utilize the remaining sample in the freezer for this purpose.

3.6 Standard quality assurance protocols should be employed, including blanks, duplicates, and dosed samples, as described in the "Analytical Quality Control Handbook" (7).

4. Reporting of Data

4.1 Report all results in ug/kg on a wet tissue basis. Report all quality control data with the analytical results for the samples.

# Analysis of Sediment for Cyanide

1. Scope and Application

1.1 This method is used for the determination of cyanide in sediments. Insoluble cyanide complexes are dissolved in 10% sodium hydroxide. The cyanide, as hydrocyanic acid (HCN), is released from the sample by means of a reflux-distillation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide in the absorbing solution is then determined by volumetric titration, colorimetrically or potentiometrically.

1.2 For cyanide levels exceeding 0.2 mg per 200-ml of absorbing liquid, the silver nitrate titrimetric method is used. For cyanide levels below this value, the colorimetric procedure is used. The probe method may be used for concentrations of 0.001 to 200-mg per 200-ml absorbing liquid.

2. Sample Preparation

2.1 Although a dry sample is preferred, a wet sample may also be taken for analysis. In either case the sediment samples must be well-mixed to insure a representative aliquot.

3. Interferences

3.1 Interferences are eliminated or lessened by using the distillation procedure.

3.2 Fatty acids will distill and form soap under the alkaline titration conditions. Therefore acidification and extraction with iso-octane, hexane, or chloroform is recommended.

3.3 Ammonia and thiosulfate interfer with the electrode method yielding higher measurements of cyanide ion activity than are actually present.

4. Preparation of Calibration Curve

4.1 The calibration curve is prepared as described in step 9.9 Reference 8.

4.2 The standards must contain the same concentration of NaOH (7.1) as the sample.

4.3 At least one standard should be treated as outlined below.

4.4 The calibration curve is prepared by plotting the absorbance or the mv reading versus the cyanide concentration.

5. Sample Procedure

5.1 Place a weighed portion of the well-mixed sediment (1 to 10-g) in an 800-ml beaker with 500-ml of 10% NaOH solution and stir for 1 hour.

5.2 Transfer the mixture to a 1 liter boiling flask. Rinse the beaker with several portions of deionized distilled water and add to the boiling flask.

5.3 Add 50-ml of 5% NaOH solution to the absorbing tube and dilute if necessary with deionized distilled water to obtain an adequate depth of liquid in the absorber. Connect the boiling flask, condenser, absorber, and trap in the distillation train as shown in Figure 1, Reference 8.

5.4 Add 50-ml of conc. $H_2SO_4$ slowly thru the air inlet tube. Rinse with distilled water. Add 20-ml of $MgCl_2 \cdot 6H_2O$ (510-g/l) solution thru the air inlet tube and

again rinse with distilled water. Continue with steps 8.4 and 8.5 (Reference 8). If the colorimetric method is used, continue thru 8.7 (Reference 8.)

5.5 Record the absorbance or mv reading and determine the cyanide concentration from the calibration curve.

6. Quality Assurance

6.1 Initially demonstrate quantitative recovery with each distillation digestion apparatus by comparing distilled aqueous standards to non-distilled aqueous standards. Each day, distill at least one standard to confirm distillation efficiency and purity of reagents.

6.2 At least 15% of the cyanide analyses should consist of duplicate and spiked samples. Quality control limits should be established and confirmed as described in Chapter 6 of the "Analytical Quality Control Handbook" Reference (7).

7. Reporting of Data

7.1 Report cyanide concentrations on a dry weight basis as follows: less than 1.0-mg/kg, to the nearest 0.01-mg/kg; 1.0-mg/kg and above, to two significant figures.

7.2 Report all quality control data with the analytical results for the samples.

# Analysis of Fish for Cyanide

1. Scope and Application

1.1 This method is used for the determination of cyanide in fish. All samples must be distilled prior to the analytical determination. For cyanide levels exceeding 0.2 mg/200-ml of absorbing liquid, the silver nitrate titrimetric method is used. For cyanide levels below this value, the colorimetric procedure is used.

2. Sample Preparation

2.1 A 5-g portion of the frozen, ground fish (see "Sample Handling") is used for the analysis. The sample should be thawed before the analysis begins.

3. Preparation of Calibration Curve

3.1 The calibration curve is prepared from values for portions of spiked fish tissue distilled in the manner used for the tissue sample being analyzed. For preparation of the calibration standards, choose and weigh a 50-g portion of fish and blend in a Waring blender (or equivalent) with 10-ml of 10% NaOH and sufficient deionized distilled water to bring the volume of the mixture to 500-ml.

3.2 Using a volumetric pipet which has had the tip removed, withdraw eight 50-ml portions and place in a series of 1 liter boiling flasks. Seven of the flasks should be spiked with progressively larger volumes of the cyanide standard as given in 8.3, Reference 8. Adjust the final volume of each flask to 500-ml with deionized distilled water.

3.3 Add 50-ml of 5% NaOH solution to the absorbing tube and dilute, if necessary, with deionized distilled water to obtain an adequate depth of liquid in the absorber. Connect the boiling flask, condenser, absorber, and trap in the train as shown in Figure 1, Reference 8.

3.4 Continue with step 8.2 through 8.7, Reference 8.

3.5 The calibration curve is prepared by plotting the absorbance versus the cyanide concentration. The blank absorbance value must be subtracted from each value before plotting the curve.

4. Sample Procedure

4.1 Place a weighed portion of the ground fish (approximately 5-g) in a blender with 100-ml of deionized distilled water and 1-ml of 5% NaOH solution.

4.2 Blend until a homogeneous mixture is obtained and transfer to a 1-liter boiling flask. Rinse the blender with several portions of deionized distilled water totaling 400-ml and add to the boiling flask.

4.3 Add 50-ml of 5% NaOH solution to the absorbing tube and dilute if necessary with deionized distilled water to obtain an adequate depth of liquid in the absorber. Connect the boiling flask, condenser, absorber, and trap in the distillation train as shown in Figure 1 and continue with step 8.2 through 8.7, Reference 8.

4.4 Read the absorbance and determine the cyanide concentration from the calibration curve.

5. Quality Assurance

Initially, demonstrate quantitative recovery with each distillation digestion apparatus by comparing distilled aqueous standards to non-distilled aqueous standards. Each day, distill at least one standard to confirm distillation efficiency and purity of reagents.

5.2 At least 15% of the cyanide analyses should consist of duplicate and spiked samples. Quality control limits should be established and confirmed as described in Chapter 6 of the "Analytical Quality Control Handbook" Reference (7).

6. Reporting of Data

6.1 Report cyanide concentrations as follows: less than 1.0-mg/kg, to the nearest 0.01-mg; 1.0-mg/kg and above, to two significant figures.

6.2 Report all quality control data with the analytical results for the samples.

# Analysis of Sediment for Phenols

1. Scope and Application

   1.1 This method is used for the determination of phenolics in sediments. All samples must be distilled prior to the determination of phenols, using the procedure given on page 576, Reference 9. Use method 510 B for samples that contain less than 1-mg phenol/kg and method 510 C for samples that contain more than 1-mg phenol/kg.

   1.2 The 4-amino-antipyrine method does not determine those parasubstituted phenols in which the substitution is an alkyl, aryl, nitro, benzoyl, nitroso, or aldehyde group.

2. Sample Preservation and Preparation

   2.1 Biological degradation is inibited by cooling the sample to 4°C. If the sample cannot be analyzed with 24 hours it should be frozen.

   2.2 A 5-g portion of the wet, or air dried sediment is used for the analysis. If the sample has been frozen it should be thawed before the analysis begins.

3. Preparation of Calibration Curve

   3.1 The calibration curve is prepared as described on p.579, 4.a.3 (Ref. 9) for samples containing less than 1-mg/kg and p. 581 for samples above 1-mg/kg.

   3.2 Record the absorbance of the standards and plot the values against micrograms of phenol.

4. Sample Procedure

4.1 Place a 5-g portion of the wet, or air dried sediment into a 200-ml beaker with 100-ml of distilled water. Mix well and lower the pH to 4.0 with (1 + 9) $H_3PO_4$ using a pH meter.

4.2 Add 5-ml of 10% $CuSO_4$ solution, mix and transfer to a 1-liter distilling flask.

4.3 Rinse the beaker with several portions of distilled water and add to the distilling flask. Adjust the volume in the flask to 500-ml.

4.4 Using a 500-ml graduated cylinder as a receiver, begin the distillation as described on p. 577, method 510 A; 4b, Reference 9.

4.5 Continue with the procedure using either the Chloroform Extraction Method 510 B, p. 577, Reference 9 or the Direct Photometric Method 510 C, p. 580, Reference 9.

4.6 Record the absorbance and determine the micrograms of phenol from the appropriate calibration curve.

5. Quality Assurance

5.1 Demonstrate quantitative recovery with each distillation apparatus by comparing aqueous distilled standards to non-distilled standards. Each day, distill at least one standard to confirm the distillation efficiency and purity of reagents.

5.2 At least 15% of the phenol analyses should consist of duplicae and spiked samples. Quality control limits should be established and confirmed as described in Reference 7.

6. Reporting of Data

6.1 Report phenol concentrations on a dry weight basis as follows:

Method 510 B, to the nearest ug/kg

Method 510 C, for less than 1.0-ug/kg to the nearest 0.01-ug and for 1.0-mg/kg and above to two siginificant figures.

6.2 Report all quality control data when reporting results of sample analysis.

# Analysis of Fish for Phenol

1. Scope and Application

1.1 This method is used for the determination of phenolics in fish. All samples must be distilled prior to the determination using the procedure given on page 576, Reference 9. Use method 510 B for samples that contain less than 1-mg phenol/kg and method 510 C for samples that contain more than 1-mg phenol/kg.

1.2 The 4-amino-antipyrine method does not determine those para-substituted phenols in which the substitution is an alkyl, aryl, nitro, benzoyl, nitroso, or aldehyde group.

2. Sample Preparation

2.1 A 5-g portion of the frozen, ground fish (see "Sample Handling") is used for the analysis. The sample should be thawed before the analysis begins.

3. Preparation of Calibration Curve

3.1 The calibration curve is prepared from values for portions of spiked fish tissue distilled in the manner used for as the tissue samples being analyzed. For preparation of the calibration standards, choose and weigh a 50-g portion of fish and blend in a Waring blender (or equivalent) with sufficient deionized distilled water to bring the total volume of the mixture to 500-ml.

3.2 Transfer a 50-ml portion of mixture to a beaker using a volumetric pipet which has had the tip removed and determine the volume of (1 + 9) $H_3PO_4$ required to

lower the pH to 4.0, using either methyl orange indicator or a pH meter. This volume of (1 + 9) $H_3PO_4$ is to be added to each 50-ml portion of fish mixture prior to the distillation step which follows.

3.3 Transfer 50-ml portions of the blended fish mixture to the distillation apparatus as shown in Figure 318:1, p. 241, Reference 9, adding the volume of $H_3PO_4$ (determined above) to lower the pH to 4.0. Add 5-ml of a 10% $CuSO_4$ solution and appropriate volumes of the standard phenol solution (Reference 9, p. 579, 3C), to each distillation flask. A blank and seven standards should be distilled for preparation of the calibration curve. Adjust the volume in the distillation flask to 500-ml. Use a 500-ml graduated cylinder as a receiver.

NOTE: The minimum detectable quantity is 1-ug/l phenol in a 500ml distillate.

3.4 Begin the distillation and continue until a distillate volume of 450-ml is obtained. Stop the distillation and add 50-ml deionized distilled water to the distillation flask after boiling has ceased. Continue the distillation until a total of 500-ml has been collected. If the distillate is turbid, acidify with (1 + 9) $H_3PO_4$ and repeat the distilation as described.

3.5 Continue with the procedure as given in the chloroform extraction method 510 B, p. 577, Reference 9. Read the absorbance of the standards against a reagent blank at a

wavelength of 460-nm. Plot absorbance against micrograms of phenol for the calibration curve.

3.6 Alternatively, follow the direct photometric method (510 C, p. 580, Reference 9), for those samples in which the phenol concentration exceeds 1-mg/kg.

4. Sample Procedure

4.1 Place a weighed portion of the ground fish (approximately 5-g) in a blender with 100-ml of distilled water. Blend until a homogeneous mixture is obtained and transfer to a 1-liter boiling flask.

4.2 Rinse the blender with several portions of distilled water and add to the distilling flask. Add a volume of (1 + 9) $H_3PO_4$ to bring the pH of the mixture to 4.0 (the same volume as that determined for preparation of the calibration standards can be used). Add 5-ml of 10% $CuSO_4$ solution and adjust the total volume to approximately 500-ml. Use a 500-ml graduated cylinder as a receiver.

4.3 Begin the distillation and continue as described in 3.4 through 3.6 above. Read the absorbance and determine the ug of phenol from the calibration curve.

5. Quality Assurance

5.1 Demonstrate quantitative recovery with each distillation apparatus by comparing aqueous distilled standards to non-distilled standards. Each day, distill at least one standard to confirm the distillation efficiency and purity of reagents.

5.2 At least 15% of the phenol analyses should consist of duplicate and spiked samples. Quality control limits should be established and confirmed as described in Reference (7).

Reporting of Data

6.1 Report phenol concentrations as follows:

Method 510 B, to the nearest ug/kg

Method 510 C, for less than 1.0-ug/kg to the nearest 0.01-ug and for 1.0-mg/kg and above to two significant figures.

6.2 Report all quality control data when reporting results of sample analysis.

# Analysis of Sediment for Mercury

1. Scope and Application

1.1 This method is used for the determination of total mercury (organic and inorganic) in sediment. A weighed portion of the sample is digested with aqua regia for 2 minutes at 95°C followed by oxidation with potassium permanganate. Mercury is subsequently measured by the cold vapor technique.

1.2 The range of the method is 0.2 to 5-ug/g but may be extended above or below the normal range by increasing or decreasing sample size or through instrument and recorder control.

1.3 For a complete description of the method the reader is referred to "Methods for Chemical Analysis of Water and Waste ", (8) pages 134-138.

2. Sample Preparation

2.1 Although a wet sample may be taken for analysis, a dry sample provides for ease of handling, better homogeniety, and better storage.

3. Preparation of Calibration Curve

3.1 The calibration curve is prepared using distilled water standards, treated in the same manner as the sediment samples being analyzed. The calibration procedure is described on p. 136, Reference 8.

3.2 The calibration curve is prepared by plotting the peak height versus the mercury concentration. The peak height of the blank is subtracted from each of the other values.

4. Sample Procedure

4.1 Weigh 0.2 to 0.3-g portions of the dry sample and place in the bottom of a BOD bottle. (If a wet sample is to be analyzed a proportionately larger sample must be taken.) Add 5-ml of distilled water and 5-ml of aqua regia and place the bottle in a water bath maintained at 95°C for 2 minutes.

4.2 Cool, add 50-ml distilled water, 15-ml of potassium permanganate solution and return the bottle to the water bath for an additional 30-minutes. Add additional $KMnO_4$, as necessary, to maintain oxidizing conditions.

4.3 Continue with the procedure as described in step 8.1, Reference 8.

5. Calculation

5.1 Measure the peak height of the unknown from the chart and read the mercury value from the standard curve.

5.2 Calculate the mercury concentration in the sample by the formula

$$\text{ug Hg/gram} = \frac{\text{ug Hg in aliquot}}{\text{wt. of aliquot in gms}}$$

5.3 Report mercury concentrations on a dry weight basis as follows:

Below 0.1-ug/gm, to nearest 0.01-ug; between 0.1 and 1-ug/gm, to nearest 0.01-ug; between 1 and 10-ug/gm, to nearest 0.1-ug; above 10-ug/gm, to nearest ug.

6. Quality Assurance

6.1 Standard quality assurance protocols should be employed, including blanks, duplicates and spiked samples, as described in the "Analytical Quality Control Handbook" (7).

7. Precision and Accuracy

7.1 The foloving standard deviations on replicate sediment samples were recorded by a single operator at the indicated levels: 0.29-ug/gm $\pm$0.02 and 0.82-ug/gm $\pm$0.03. Recovery of mercury at these levels, added as methyl mercuric chloride, was 97% and 94%, respectively.

# Analysis of Fish for Mercury

1. Scope and Application

1.1 This method is used for determination of total mercury (organic and inorganic) in fish. A weighed protion of the sample is digested with sulfuric and nitric acid at 58°C followed by overnight oxidation with potassium permanganate at room temperature. Mercury is subsequently measured by the conventional cold vapor technique.

1.2 The range of the method is 0.2 to 5-ug/g but may be extended above or below the normal instrument and recorder control.

2. Sample Preparation

2.1 The sample may be prepared as described under "Sample Handling" or the special metal procedure may be used. A 0.2 to 0.3-g portion should be taken for each analysis. The sample should not be allowed to thaw before weighing.

3. Preparation of Calibration Curve

3.1 The calibration curve is prepared from values for portions of spiked fish tissue treated in the manner used for the tissue samples being analyzed. For preparation of the calibration standards, choose a 5-g portion of fish and blend in a Waring blender.

3.2 Remove equal and accurately weighed portions (0.2-g) with a spatula and transfer to each of six dry BOD bottles. Add 4-ml of conc. H$d2$bSO$d4$b and 1-ml of conc. $HNO_3$ and

place in water bath at 58°C until the tissue is completely dissolved (30 to 60-min).

3.3 Cool, and transfer 0-, 0.5-, 1.0-, 2.0-, 5.0- and 10.0- ml aliquots of the working mercury solution containing 0 to 1.0-ug of mercury to the BOD bottles. Cool to 4°C in an ice bath and cautiously add 15-ml of potassium permanganate solution. Allow to stand overnight at room temperature under oxidizing conditions.

3.4 Add enough distilled water to bring the total volume to approximately 125-ml. Add 6-ml of sodium chloride-hydroxylamine sulfate solution to reduce the excess permangante.

3.5 Wait at least 30-sec after the addition of hydroxylamine. Treating each bottle individually, add 5-ml of the stannous sulfate solution and immediately attach the bottle to the aeration apparatus.

3.6 Continue with the procedure as given on page 121, Reference 8, The calibration curve is prepared by plotting the peak height versus the mercury concentration. The peak height of the blank is subtracted from each of the other values.

4. Sample Procedure

4.1 Weigh 0.2 to 0.3-portions of the sample and place in the botton of a dry BOD bottle. Care must be taken that none of the sample adheres to the side of the bottle. Add 4-ml of conc. $H_2SO_4$ and 1-ml of conc. $HNO_3$ and place in water bath maintained at 58°C until the tissue is completely dissolved (30 to 60-minutes).

4.2 Cool to 4°C in an ice bath and cautiously add 5-ml of potassium permanganate solution in 1-ml increments. Add an additional 10-ml or more of permangante, as necessary to maintain oxidizing conditions. Allow to stand overnight at room temperature (see NOTE). Continue as described under 3.4.

NOTE: As an alternate to the overnight digestion, the solublization of the tissue may be carried out in a water bath at 80°C for 30-min. The sample is then cooled and 15-ml of potassium permanganate solution added cautiously. At this point the sample is returned to the water bath and digested for an additional 90-min at 80°C. (10). If this method is followed, the calibration standards must also be treated in this manner. Continue as described under 3.4.

5. Calculation

5.1 Measure the peak height of the unknown from the chart and read the mercury value from the standard curve.

5.2 Calculate the mercury concentration in the sample by the formula

$$\text{ug Hg/gram} = \frac{\text{ug Hg in aliquot}}{\text{wt. of aliquot in gms}}$$

5.3 Report mercury concentrations as follows:

Below 0.1-ug/gm, <0.1-ug; between 0.1 and 1-ug/gm, to nearest 0.01-ug; between 1 and 10-ug/gm, to nearest 0.1-ug; above 10-ug/gm, to nearest ug.

6. Quality Assurance

6.1 Standard quality assurance protocols should be employed, including blanks, duplicates and spiked samples as described in the "Analytical Quality Control Handbook".(7)

6.2 Report all quality control data when reporting results of sample analyses.

7. Precision and Accuracy

7.1 The following standard deviations on replicate fish samples were recorded at the indicated levels: 0.19 ug/gm $\pm$0.02, 0.74-ug/gm $\pm$0.05, and 2.1-ug/gm $\pm$0.06. The coefficients of variation at these levels were 11.9%, 7.0%, and 3.6%, respectively. Recovery of mercury at these levels, added as methyl mercuric chloride, was 112%, 93%, and 86%, respectively.

# Analysis of Sediments for Metals

## (Sb, Be, Cd, Cr, Cu, Pb, Ni, Ag, Tl, & Zn)

1. Scope and Application

1.1 This method is designed to determine those priority pollutants in sediments as listed in the Consent Decree (4) that are classified as heavy metals or considered toxic as they exist in their elemental form and associated compounds. The pollutants include antimony, beryllium, cadmium, chromium, copper, lead, nickel, silver, thallium, and zinc.

2. Summary of Method

2.1 The sediment is prepared for analysis by drying and grinding the sample. A representative portion is subjected to a wet oxidation-digestion after which, atomic absorption - either direct aspiration, or a flameless technique-is used to measure the concentration of the pollutant.

2.2 For a discussion of basic principles, general operating parameters, preparation of standards and calibaration, and the method of standard addition, the reader is referred to "Methods for Chemical Analysis of Water and Wastes" (3) pages 73-91, and the individual analyses sheets as follow:

Page references to "Methods for Chemical Analysis of Water of Water and Wastes, 1974" (3)

| ELEMENT | Ag | Be | Cd | Cr | Cu | Ni | Pb | Sb | Tl | Zn |
|---|---|---|---|---|---|---|---|---|---|---|
| PAGE | 146 | 99 | 101 | 105 | 108 | 141 | 112 | 94 | 149 | 155 |

t+1

3. Preservation and Handling

3.1 The sample should be stored at 4° C if the analysis can be carried out within 7 days of collection. For longer periods the samples should be frozen. An alternative is to dry the sample as soon as possible, grind it with a mortar and pestle removing rocks, sticks, and other foreign objects and store the sediment in a vial or other suitable container.

3.2 Dust in the laboratory environment, impurities in reagents and impurities on laboratory apparatus which the sample contacts, are all sources of potential contamination. All glassware should be thoroughly washed with detergent and tap water, rinsed with 1:1 nitric acid, tap water, and finally deionized distilled water in that order. NOTE: Chromic acid may be useful to remove organic deposits from glassware; however, the analyst should be cautioned that the glassware must be thoroughly rinsed with water to remove the last trace of chromium. This is especially important if chromium is to be included in the analytical scheme. A commercial product--NOCHROMIX--available from

Codax Laboratories, 6 Yarick, New York, NY 10013, can be used in place of chromic acid.

4. Sample Preparation

4.1 Dry a representative portion of the well mixed sample (10 to 25-g) at 60°C until all moisture has been removed.

4.2 Grind the dry sample with a mortar and pestle, removing, sticks, stones, and other foreign material. Store the sample in glass or plastic vials removing aliquots as needed.

5. Procedure

5.1 Weigh 1.00-g of the well mixed sediment into a 250-ml Erlenmeyer flask and add 50-ml deionized water, 0.5-ml $HNO_3$ (sp. gr. 1.42) and 5-ml of HCl (sp. gr. 1.10) to each flask.

5.2 Heat the samples, blanks and standards on a hot plate maintained at approximately 95°C. until the volume has been reduced to 15 to 20-ml, making certain that the samples do not boil.

5.3 Cool and clarify the sample by centrifugation or by filtration through Whatman No. 42 filter papter or equivalent.

5.4 Dilute the sample to 100-ml or some appropriate volume based on the concentration present.

5.5 Proceed with the appropriate method for the atonic absorption analysis of the metals of interest using either direct aspiration or furnace techniques.

5.5.1 Because of the adequate sensivity for copper and zinc by direct aspiration AA and the probable concentration levels of those two metals in sediments, direct aspiration should be employed. The furnace technique is preferred for the determination of the remaining metals because of their expected low concentration. When using the furnace technique, the operating parameters and instructions as specified by the particular instrument manufacturer should be followed. If the concentration detected by the furnace procedure is above the working range of the standard curve, the sample should be either diluted and reanalyzed or analyzed by direct aspiration. The method for standard additions should be employed when needed.

6. Calculation

6.1 From the values read off the appropriate calibration curve, calculate the concentration of each metal pollutant in the sediment as follows:

$$\text{mg/kg} = \frac{\text{mg/l of constituent prepared sample} \times \text{volume of prepared sample in ml}}{\text{weight of dry sample in g}}$$

7. Quality Assurance

7.1 Standard quality assurance protocols should be employed, including blanks, duplicates, and does samples as described in the "Analytical Quality Control Handbook". (7)

7.2 Report all quality control data when reporting results of sample analyses.

# Analysis of Fish for Metals

1. Scope

1.1 This method is designed to determine in whole fish those priority pollutants listed in the Consent Decree (4) that are classified as heavy metals or considered toxic as they exist in their elemental form and associated compounds. The pollutants include antimony, arsenic, beryllium, cadmium, chromium, copper, lead, nickel, selenium, silver, thallium, and zinc.

2. Summary of Method

2.1 The fish is prepared for analysis by being chopped into small pieces, homogenized in a blender with dry ice, and solubilized by either dissolution after dry ashing or a wet oxidation digestion. After sample preparation, atomic absorption - either direct aspiration, gaseous hydride, or a flameless technique - is used to measure the concentration of the pollutant.

3. Preservation and Handling

3.1 Although an aliquot of the ground fish as prepared under "Sample Handling" may be used for the metals determination, it may be more desirable to prepare an individual fish to avoid possible metal contamination from the grinder. Dust in the laboratory environment, impurities in reagents, and impurities on laboratory apparatus that the sample contacts are all sources of potential contamination. All glassware should be thoroughly washed with detergent and tap water,

rinsed with 1:1 nitric acid, then tap water, and finally deionized distilled water.

NOTE: Chromic acid may be useful to remove organic deposits from glassware; however, the analyst should be cautioned that the glassware must be thoroughly rinsed with water to remove the last trace of chromium. This is especially important if chromium is to be included in the analytical scheme. A commercial product--NOCHROMIX--available from Godax Laboratories, 6 Varick Street, New York, NY 10013, can be used in place of chromic acid.

4. Sample Homogenization

4.1 If a fish sample other than that prepared under "Sample Handling" is to be used for metals analyses, unwrap and weigh the frozen fish at the time of processing. Select a fish that weighs between 50 and 300-g. If an analysis is required for a fish > 300-g, a 50-g representative portion must be taken from the sample after it has been pretreated as described in "Sample Handling" on page 1 of this document and proceed to step 4.3.

4.2 After weighing, the fish should be chopped into approximately 1-in. or smaller chunks with a meat cleaver of a knife and mallet (2 to 3-lb). Smaller pieces ensure efficient grinding.

4.3 Place crushed or pelleted dry ice into the blender container. The weight of dry ice should be equal to, or greater than, the weight of the fish.

4.4 Turn on the blender for 10-sec to pulverize the ice and chill the blender.

4.5 Add the pieces of fish and blend at high speed until the mixture is homogeneous. This usually requires 2 to 5-minutes. Add more dry ice if needed.

4.6 Pour the homogenate into a plastic bag and close the bag with a rubber band. Do not seal the bag tightly to allow CO$d2$b escape.

4.7 Place the bag in the freezer (-12°C for at least 16-h) until ready to proceed with the digestion step.

NOTE: If desired, the blender blades can be modified in order to have the leading edge of the blades (the sharpened edge) turned down so that, as it rotates, the blade will throw the material upwards. Stainless steel blades may be a possible source of nickel and chromium contamination and should be noted if detected. If a tantalum blade is available, it should be substituted for the stainless steel.

The hole in the blender lid should be enlarged sufficiently to allow the evolved gas to escape (1/2 in-quart-size, 1 in-gallon-size). Hold a cloth or labwipe over this hole when blending to prevent loss of the sample material. A glove should be worn to prevent possible freezing of the skin by escaping gas.

5. Reagents

5.1 Deionized distilled water: Prepare by passing distilled water through a mixed bed of cation and anion exchange

resins. Use deionized distilled water for the preparation of all reagents and calibration standards and as dilution water.

5.2 Nitric acid (conc.): If metal impurities are present, distill reagent grade nitric acid in a borosilicate glass distillation apparatus.

5.3 Sulfuric acid, ACS grade (95.5 % to 96.5%).

5.4 Sulfuric acid - 20% v/v solution. Carefully add 200-ml of concentrated $H_2SO_4$ to 500-ml of water. Cool and dilute to 1-liter with water.

5.5 Hydrochloric acid, ACS grade (37% to 38%).

5.6 Hydrogen Peroxide, 50% stabilized ACS grade.

5.7 Dry ice (frozen carbon dioxide), pellet form preferred.

6. Apparatus

6.1 Blender, Waring, two-speed, stainless steel blade or tantalum blade if available, glass container capacity 1000-ml, or equivalent equipment.

6.2 Drying oven - Controllable with the range of 100° to 150°C with less that ±5°C variation. Check calibration of oven temperature control to ensure accurate ashing temperatures. Furnace must be operated in suitable fume hood.

6.3 Hot plate, controllable within the range of 80° to 400°C. Hot plate must be operated in fume hood.

7. Procedure

Except for mercury, which requires a cold vapor technique, the pollutants can be divided into two groups for continued processing.

GROUP I: Be, Cd, Cr, Cu, Pb, Ni, Ag, Tl, and Zn

GROUP II: As and Se

Group I is digested by a dry ashing process (11) with the use of an ashing aid; Group II is prepared utilizing a wet ashing process.

7.1 Group I - Metals

7.1.1 Remove the homogenized sample from the freezer and weigh approximately 10-g into a tared, 100-ml tall, form, Pyrex beaker. Subtract the beaker weight from the total and record the wet sample weight.

7.1.2 Add 25-ml of 20% sulfuric acid. Mix each sample thoroughly with a glass stirring rod ensuring all sample material is wetted by the acid. Rinse the stirring rod with water into the ashing vessel and cover the sample with a ribbed watch glass.

7.1.3 Dry the samples in an oven or furnace at 110±5°C until a charred viscous sulfuric acid/sample residue remains. Usually 12 to 16-h (overnight) is sufficient. Transfer the ashing vessels containing the dried samples to a cold, clean muffle furnace which is provided with good external ventilation (fume hood), ensuring that the sample remains covered during the transfer. Initially set the furnace at 125°C and increase the temperature approximately every hour in 50° increments up to 275°C. Hold the temperature at 275°C for 3-h. Finally, increase the temperature to 450°C (at 50° per hour) and hold for

12 to 16-h (overnight). Remove the covered ashing vessels from the furnace and allow to cool to room temperature in a clean, draft-free area.

7.1.4 After initial overnight ashing, some residual carbon may remain in the samples. Treat each sample ash with 0.5-ml of water and 1-ml of concentrated nitric acid (whether or not they are already white). Evaporate carefully just to dryness on a warm hotplate (in a fume hood). Place the ashing vessels (covered with watch glasses) in a cool muffle furnace and raise the temperature to 300°C and hold for exactly 30-min. Remove each covered sample ash from the furnace and allow to cool as before. If residual carbon remains, repeat the nitric acid treatment until a carbon-free white ash is obtained. The covered ashing vessels containing the ash may be stored in a dessicator or in a laminar flow clean hood.

NOTE: Copious carbon residues (i.e., black ashes) after overnight ashing may indicate inefficient or uneven heating within the furnace. Routine calibration of the furnace is advised.

7.1.5 Add 0.5-ml of nitric acid and 10-ml of water to each cool ashing vessel, then warm gently on a hotplate at 80 to 90°C for 5 to 10-min to effect dissolution of the ash. A small amount of insoluble white siliceous-like residue may remain undissolved; do not

filter the residue because of the possibility of contamination. Quantitatively transfer the contents of each ashing vessel into a 100-ml volumetric flask, dilute to volume with water, and shake thoroughly. Allow any residue to settle to the bottom of the flask (about 2-hr). Do not shake the sample further before taking an aliquot. The sample is now ready for analysis.

NOTE: The presence of a precipitate other than the insoluble siliceous-like material may result in low or eratic results for Pb. Precipitate formation can result from heating the samples too long or at too high a temperature after nitric acid treatment of the ash. Precipitate formation must be avoided by maintenance of appropriate ashing temperatures.

7.1.6 The prepared sample should be analyzed by AA using either direct aspiration or furnace techniques. For a discussion of basic principles, the method of standard addition, the chelation/solvent extraction procedures, general instrumental operating parameters, and preparation of standards and calibration see the section on "Atomic Absorption Methods", pages 73-91, Reference 8, and the individual analyses sheets (on pages as listed below).

| ELEMENT | Ag | Be | Cd | Cr | Cu | Ni | Pb | Sb | Tl | Zn |
|---|---|---|---|---|---|---|---|---|---|---|
| PAGE | 146 | 99 | 101 | 105 | 108 | 141 | 112 | 94 | 149 | 155 |

7.1.7 Because of the adequate sensitivity by conventional flame AA and the expected concentration levels of cadmium, copper, and zinc in the sample, these three elements should be analyzed by direct aspiration. The furnace technique is preferred for the analysis of the other Group I metals because of their expected low concentrations. When using the furnace technique, the operating parameters and instructions as specified by the particular instrument manufacturer should be followed. If the concentration detected by the furnace procedure is beyond the working range of the standard curve, the sample should be either diluted and reanalyzed or analyzed by direct aspiration. The method of standard additions should be employed when needed. If the sample matrix is so complex that sample dilution followed by furnace analysis cannot be used, or if the use of the chelation/solvent extraction technique for concentration of Ag, Ni, Pb, and Tl is preferred, the procedure as described starting on page 30, Reference 3, should be utilized.

7.2 Group II - Metals

7.2.1 Remove the homogenized sample from the freezer and weigh approximately 5-g into a tared, 125-ml conical beaker. Subtract the beaker weight from the total and record the wet sample weight.

7.2.2 Add 5-ml of conc. $HNO_3$ . Then slowly add 6-ml of conc. $H_2SO_4$ and cover with a watch glass.

7.2.3 Place beaker on hot plate and warm slightly. Continue heating until the mixture becomes dark or a possible reducing condition is evident. Do not allow the mixture to char. Remove beaker from hotplate and allow to cool.

NOTE: Remove beaker if foaming becomes excessive.

7.2.4 Add an additional 5-ml of conc. $HNO_3$ ., cover with a watch glass, and return beaker to hot plate. Repeat step 7.2.3.

7.2.5 When mixture again turns brown, cool, and slowly add 5-ml of 50% hydrogen peroxide. Cover with watch glass and heat gently until the initial reaction has ceased. If the solution becomes dark, repeat the peroxide addition, several times if necessary, and heat to $SO_3$ fumes. If charring occurs, add further 1-ml portions of hydrogen peroxide until the fuming sulfuric acid remains colorless or very light yellow. (If at any stage it seems that the sulfuric acid may approach dryness, cool, add 2 to 3-ml of sulfuric acid, and continue).

7.2.6 Cool, add 40-ml of conc. HCl and dilute to 100-ml with deionized distilled water. The sample is now ready for analysis.

7.2.7 The Group II metals should be analyzed by AA using the gaseous hydride technique. The apparatus setup, standard preparation and calibration, and analysis procedure that is to be followed is given starting on page 159, Reference 9. From the prepared sample a 25-ml aliquot should be withdrawn and the analysis continued as described in section 3.d, page 162, Reference 9.

8. Calculation

8.1 Using the values from the appropriate calibration curve calculate the concentration of each metal pollutant in the fish as follows:

If the concentration of standards in the calibration curve is plotted as mg/l,

$$\text{ug/gram} = \frac{\text{mg/l of constituent in prepared sample} \times \text{volume of prepared sample in ml}}{\text{weight of wet sample in g}}$$

If the concentration of standards in the calibration curve is plotted as ug/l,

$$\text{ug/gram} = \frac{\frac{\text{ug/l of constituent in prepared sample}}{1000} \times \text{volume of prepared sample in ml}}{\text{weight of wet sample in g}}$$

Quality Assurance

9.1 Standard quality assurance protocols should be employed, including blanks, duplicates, and dosed samples, as described in the "Analytical Quality Control Handbook". (7)

9.2 Report all quality control data when reporting results of sample analyses.

Analysis of Sediment for Arsenic and Selenium

1. Scope and Application

1.1 This method is to be used for the determination of arsenic and selenium in sediment. A weighed portion of the wet, well-mixed sediment is digested with $HNO_3$ and $H_2SO_4$ followed by treatment with $H_2O_2$. Arsenic and selenium are subsequently determined by the gaseous hydride technique.

1.2 The range of the method is __ to __ ug/g but may be extended by varying the sample size.

2. Sample Preparation

2.1 The analysis should be performed on a wet, well-mixed sample.

3. Preparation of Calibration Curve

3.1 The calibration curve is prepared using distilled water standards, treated in the same manner as the samples being analyzed.

4. Procedure

4.1 Weigh approximately 5-grams of the wet, well-mixed, sediment into a tared 125-ml conical beaker.

4.2 Add 5-ml of conc. $HNO_3$. Then slowly add 6-ml conc. $H_2SO_4$ and cover with a watch glass.

4.3 Place the beaker on hot plate and warm slightly. (NOTE: Remove beaker if foaming becomes excessive.) Continue heating until the mixture becomes dark or a possible reducing condition is evident. Do not allow the mixture to

char. Remove the beaker from the hotplate and allow to cool.

4.4 Add an additional 5-ml of conc. $HNO_3$, cover with a watch glass, and return beaker to hot plate. Repeat step 4.3.

4.5 When mixture again turns brown, cool, and slowly add 5-ml of 50% hydrogen peroxide. Cover with watch glass and heat gently until the initial reaction has ceased. If the solution becomes dark, repeat the peroxide addition, several times if necessary, and heat to $SO_3$ fumes. If charring occurs, add additional 1-ml portions of hydrogen peroxide until the fuming sulfuric acid remains colorless or very light yellow. (If at any stage the sulfuric acid approaches dryness, cool, add 2 to 3-ml of additional sulfuric acid, and continue.)

4.6 Cool, add 40-ml of conc. HCl and dilute to 100-ml with deionized distilled water. The sample is now ready for analysis by the gaseous hydride technique.

4.7 The apparatus setup, standard preparation and calibration, and analytical procedure to be followed is given beginning on page 159, Reference 9. A 25-ml aliquot should be withdrawn from the prepared sample and the analysis continued as described in section 3.d, page 162, Reference 9.

5. Calibration

5.1 Calculate the concentration of arsenic and selenium present in mg/kg on a dry weight basis.

Quality Assurance

Standard quality assurance protocols should be employed, including blanks, duplicates, and spiked samples as described in the "Analytical Quality Control Handbook". (7)

## REFERENCES


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TABLE I

Priority Pollutants Analyzed by Soxhlet Extraction

Pesticides

| | | |
|---|---|---|
| Aldrin | DDD | Endosulfan sulfate |
| a-BHC | DDE | Endrin |
| b-BHC | DDT | Endrin aldehyde |
| d-BHC | Dieldrin | Heptachlor |
| g-BHC | a-Endosulfan | Heptachlor epoxide |
| Chlordane | b-Endosulfan | Toxaphene |

PCBs

| | | |
|---|---|---|
| Aroclor 1016 | Aroclor 1242 | Aroclor 1254 |
| Aroclor 1221 | Aroclor 1248 | Aroclor 1260 |
| Aroclor 1232 | | |

Non-polar Neutrals

| | | |
|---|---|---|
| Acenaphthylene | 1,3-dichlorobenzene | Bis (2-ethylhexyl) phthalate |
| Acenaphthene | 1,4-dichlorobenzene | Benzo (a) anthracene |
| Isophorone | Hexachlorethane | Benzo (b) fluoranthene |
| Fluroene | 1,2-dichlorobenzene | Benzo (k) fluroanthene |
| Phenanthrene | Hexachlorobutadiene | Benzo (a) pyrene |
| Anthracene | 1,2,4-trichlorobenzene | Indeno (1,2,3-cd) pyrene |
| Dimethylphthalate | 2,6-dinitrotoluene | Dibenzo (a,h) anthracene |
| Diethylphthalate | Hexachlorobenzene | Benzo (ghi) perylene |
| Fluoranthene | 4-bromophenyl phenyl ether | 4-chlorophenyl phenyl ether |
| Pyrene | Bis (2-chloroethoxy) methane | 2,3,7.8-tetrachlorodibenzo-p-dioxin |
| Naphthalene | 2-chloronaphthalene | Di-n-butylphthalate |
| Chrysene | | Butyl benzylphthalate |

# Table II Base-neutral Extractables

| Compound Name | RRT* (hexachloro-benzene) | Limit of Detection (ng) | Characteristic EI ions (Rel. Int.) | CI ions (Methane) |
|---|---|---|---|---|
| 1,3-dichlorobenzene | 0.35 | 40 | 146(100), 148(64), 113(12) | 146, 148, 150 |
| 1,4-dichlorobenzene | 0.36 | 40 | 146(100), 148(64), 113(11) | 146, 148, 150 |
| hexachloroethane | 0.38 | 40 | 117(100), 199(61), 201(99) | 199, 201, 203 |
| 1,2-dichlorobenzene | 0.39 | 40 | 146(100), 148(64), 113(11) | 146, 148, 150 |
| bis(2-chloroisopropyl) ether | 0.47 | 40 | 45(100), 77(19), 79(12) | 77, 135, 137 |
| hexachlorobutadiene | 0.55 | 40 | 225(100, 223(63), 227(65) | 223, 225, 227 |
| 1,2,4-trichlorobenzene | 0.55 | 40 | 74(100), 109(80), 145(52) | 181, 183, 209 |
| naphthalene | 0.57 | 40 | 128(100), 127(10), 129(11) | 129, 157, 169 |
| bis(2-chloroethyl)ether | 0.61 | 40 | 93(100), 63(99), 95(31) | 63, 107, 109 |
| hexachlorocyclopentadiene | 0.64 | 40 | 237(100), 235(63), 272(12) | 235, 237, 239 |
| nitrobenzene | 0.64 | 40 | 77(100), 123(50), 65(15) | 124, 152, 164 |
| bis(2-chloroethoxy)methane | 0.68 | 40 | 93(100), 95(32), 123(21) | 65, 107, 137 |
| 2-chloronaphthalene | 0.76 | 40 | 162(100), 164(32), 127(31) | 163, 191, 203 |
| acenaphthylene | 0.83 | 40 | 152(100), 153(16), 151(17) | 152, 153, 181 |
| acenaphthene | 0.86 | 40 | 154(100), 153(95), 152(53) | 154, 155, 183 |
| isophorone | 0.87 | 40 | 82(100), 95(14), 138(18) | 139, 167, 179 |
| fluorene | 0.91 | 40 | 166(100), 165(80), 167(14) | 166, 167, 195 |
| 2,6-dinitrotoluene | 0.93 | 40 | 165(100), 63(72), 121(23) | 183, 211, 223 |
| 1,2-diphenylhydrazine | 0.96 | 40** | 77(100), 93(58), 105(28) | 185, 213, 225 |
| 2,4-dinitrotoluene | 0.98 | 40 | 165(100), 63(72), 121(23) | 183, 211, 223 |
| N-nitrosodiphenylamine | 0.99 | 40** | 169(100), 168(71), 167(50) | 169, 170, 198 |
| hexachlorobenzene | 1.00 | 40 | 284(100), 142(30), 249(24) | 284, 286, 288 |
| 4-bromophenyl phenyl ether | 1.01 | 40 | 248(100), 250(99), 141(45) | 249, 251, 277 |
| phenanthrene | 1.09 | 40 | 178(100), 179(16), 176(15) | 178, 179, 207 |
| anthracene | 1.09 | 40 | 178(100), 179(16), 176(15) | 178, 179, 207 |
| dimethylphthalate | 1.10 | 40 | 163(100), 164(10), 194(11) | 151, 163, 164 |
| diethylphthalate | 1.15 | 40 | 149(100), 178(25), 150(10) | 177, 223, 251 |
| fluoranthene | 1.23 | 40 | 202(100), 101(23), 100(14) | 203, 231, 243 |
| pyrene | 1.30 | 40 | 202(100), 101(26), 100(17) | 203, 231, 243 |
| di-n-butylphthalate | 1.31 | 40 | 149(100), 150(27), 104(10) | 149, 205, 279 |
| benzidine | 1.38 | 40** | 184(100), 92(24), 185(13) | 185, 213, 225 |
| butyl benzylphthalate | 1.46 | 40 | 149(100), 91)50) | 149, 299, 327 |

* 1% SP-2250 on 100/120 mesh Supelcoport in a 6' x 2 mm id. glass column; He @ 30 ml/min; Program: 50° for 4 min, then 8°/min to 260° and hold for 15 min.

** Conditioning of column with base is required.

## Table II Base-neutral Extractables (Cont'd.)

| Compound Name | RRT* (hexachloro-benzene) | Limit of Detection (ng) | Characteristic EI ions (Rel. Int.) | CI ions (Methane) |
|---|---|---|---|---|
| chrysene | 1.46 | 40 | 228(100), 229(19), 226(23) | 228, 229, 257 |
| bis(2-ethylhexyl)phthalate | 1.50 | 40 | 149(100), 167(31), 279(26) | 149 |
| benzo(a)anthracene | 1.54 | 40 | 228(100), 229(19), 226(19) | 228, 229, 257 |
| benzo(b)fluoranthene | 1.66 | 40 | 252(100), 253(23), 125(15) | 252, 253, 281 |
| benzo(k)fluoranthene | 1.66 | 40 | 252(100), 253(23), 125(16) | 252, 253, 281 |
| benzo(a)pyrene | 1.73 | 40 | 252(100), 253(23), 125(21) | 252, 253, 281 |
| indeno(1,2,3-cd)pyrene | 2.07 | 100 | 276(100), 138(28), 277(27) | 276, 277, 305 |
| dibenzo(a,h)anthracene | 2.12 | 100 | 278(100), 139(24), 279(24) | 278, 279, 307 |
| benzo(g,h,i)perylene | 2.18 | 100 | 276(100), 138(37), 277(25) | 276, 277, 305 |
| N-nitrosodimethylamine | | | 42(100), 74(88), 44(21) | |
| N-nitrosodi-n-propylamine | | | 130(22), 42(64), 101(12) | |
| 4-chloro-phenyl phenyl ether | | | 204(100), 206(34), 141(29) | |
| endrin aldehyde | | | | |
| 3,3'-dichlorobenzidine | | | 252(100), 254(66), 126(16) | |
| 2,3,7,8-tetrachlorodibenzo-p-dioxin | | | 322(100), 320(90), 59(95) | |
| bis(chloromethyl)ether | | | 45(100), 49(14), 51(5) | |

* 1% SP-2250 on 100/120 mesh Supelcoport in a 6' x 2 mm id. glass column; He @ 30 ml/min; Program: 50° for 4 min, then 8°/min to 260° and hold for 15 min.

** Conditioning of column with base is required.

## Table III Acid Extractables

| Compound Name | RRT * (2-nitrophenol) | Limit of Detection (ng) | Characteristic EI ions (Rel. Int.) | CI ions (Methane) |
|---|---|---|---|---|
| 2-chlorophenol | 0.63 | 100 | 128(100), 64(54), 130(31) | 129, 131, 157 |
| phenol | 0.66 | 100 | 94(100), 65(17), 66(19) | 95, 123, 135 |
| 2,4-dichlorophenol | 0.96 | 100 | 162(100), 164(58), 98(61) | 163, 165, 167 |
| 2-nitrophenol | 1.00 | 100 | 139(100), 65(35), 109(8) | 140, 168, 122 |
| p-chloro-m-cresol | 1.05 | 100 | 142(100), 107(80), 144(32) | 143, 171, 183 |
| 2,4,6-trichlorophenol | 1.14 | 100 | 196(100), 198(92), 200(26) | 197, 199, 201 |
| 2,4-dimethylphenol | 1.32 | 100 | 122(100), 107(90), 121(55) | 123, 151, 163 |
| 2,4-dinitrophenol | 1.34 | 2 µg | 184(100), 63(59), 154(53) | 185, 213, 225 |
| 4,6-dinitro-o-cresol | 1.42 | 2 ug | 198(100), 182(35), 77(28) | 199, 227, 239 |
| 4-nitrophenol | 1.43 | 100 | 65(100), 139(45), 109(72) | 140, 168, 122 |
| pentachlorophenol | 1.64 | 100 | 266(100), 264(62), 268(63) | 267, 265, 269 |
| deuterated anthracene (d10) | 1.68 | 40 | 188(100), 94(19), 80(18) | 189, 217 |

* 1% SP-2250 on 100/120 mesh Supelcoport in a 6' x 2 mm id. glass column; He @ 30 ml/min; Program: 50° for 4 min, then 8°/min to 260° and hold for 15 min.

Table IV

Characteristic Ions of Volatile Organics

| Compound | EI Ions (Relative intensity) | Ion used to quantify |
|---|---|---|
| chloromethane | 50(100); 52(33) | 50 |
| dichlorodifluoromethane | 85(100); 87(33); 101(13); 103(9) | 101 |
| bromomethane | 94(100); 96(94) | 94 |
| vinyl chloride | 62(100); 64(33) | 62 |
| chloroethane | 64(100); 66(33) | 64 |
| methylene chloride | 49(100); 51(33); 84(86); 86(55) | 84 |
| trichlorofluoromethane | 101(100); 103(66) | 101 |
| 1,1-dichloroethylene | 61(100); 96(80); 98(53) | 96 |
| bromochloromethane(IS) | 49(100); 130(88); 128(70); 51(33) | 128 |
| 1,1-dichloroethane | 63(100); 65(33); 83(13); 85(8); 98(7); 100(4) | 63 |
| trans-1,2-dichloroethylene | 61(100); 96(90); 98(57) | 96 |
| chloroform | 83(100); 85(66) | 83 |
| 1,2-dichloroethane | 62(100); 64(33); 98(23); 100(15) | 98 |
| 1,1,1-trichloroethane | 98(100); 99(66); 117(17); 119(16) | 97 |
| carbon tetrachloride | 117(100); 119(96); 121(30) | 117 |
| bromodichloromethane | 83(100); 85(66); 127(13); 129(17) | 127 |
| bis-chloromethyl ether | 79(100); 81(33) | 79 |
| 1,2-dichloropropane | 63(100); 65(33); 112(4); 114(3) | 112 |
| trans-1,3-dichloropropene | 75(100): 77(33) | 75 |
| trichloroethylene | 95(100); 97(66); 130(90); 132(85) | 130 |
| dibromochloromethane | 129(100); 127(78); 208(13); 206(10) | 127 |
| cis-1,3-dichloropropene | 75(100); 77(33) | 75 |

TABLE IV (Continued)

| Compound | EI Ions (Relative intensity) | Ion used to quantify |
|---|---|---|
| 1,1,2-trichloroethane | 83(95); 85(60); 97(100); 99(63); 132(9); 134(8) | 97 |
| benzene | 78(100) | 78 |
| 2-chloroethylvinyl ether | 63(95); 65(32); 106(18) | 106 |
| 2-bromo-1-chloropropane(IS) | 77(100); 79(33); 156(5) | 77 |
| bromoform | 171(50); 173(100); 175(50); 250(4); 252(11); 254(11); 256(4) | 173 |
| 1,1,2,2-tetrachloroethene | 129(64); 131(62); 164(78); 166(100) | 164 |
| 1,1,2,2-tetrachloroethane | 83(100); 85(66); 131(7); 133(7); 166(5); 168(6) | 168 |
| 1,4-dichlorobutane(IS) | 55(100); 90(30); 92(10) | 55 |
| toluene | 91(100); 92(78) | 92 |
| chlorobenzene | 112(100); 114(33) | 112 |
| hylbenzene | 91(100); 106(33) | 106 |
| acrolein | 26(49); 27(100); 55(64); 56(83) | 56 |
| acrylonitrile | 26(100); 51(32); 52(75); 53(99) | 53 |