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Handbook of Methods for Acid Deposition Studies

Laboratory Analysis for Surface Water Chemistry

A Contribution to the
National Acid Precipitation Assessment Program

U.S. Environmental Protection Agency
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NOTICE

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This document is a contribution to the National Acid Precipitation Assessment Program. The methods contained in this document have been developed for use in the component programs of the Aquatic Effects Research Program. Previous publications from which these methods have been extracted, include:

Hillman, D. C., J. F. Potter, and S. J. Simon, 1986. National Surface Water Survey, Eastern Lake Survey - Phase I, Analytical Methods Manual. EPA-600/4-86/009. U.S. Environmental Protection Agency, Las Vegas, Nevada.

Kerfoot, H. B., and M. L. Faber, 1987. National Surface Water Survey, Western Lake Survey (Phase I - Synoptic Chemistry) Analytical Methods Manual. EPA-600/8-87/038. U.S. Environmental Protection Agency, Las Vegas, Nevada.

Hillman, D. C., S. H. Pia, and S. J. Simon, 1987. National Surface Water Survey, National Stream Survey (Phase I - Pilot, Mid-Atlantic Phase I, Southeast Screening, and Episodes Pilot) Analytical Methods Manual. EPA-600/8-87/005. U.S. Environmental Protection Agency, Las Vegas, Nevada.

Chaloud, D. J., L. J. Arent, B. B. Dickes, J. D. Nitterauer, M. O. Morison, and D. V. Peck, 1986. National Surface Water Survey (Eastern Lake Survey - Phase II, National Stream Survey - Phase I) Processing Laboratory Training and Operations Manual. Internal report. U.S. Environmental Protection Agency, Las Vegas, Nevada.

Kerfoot, H. B., T. E. Lewis, D. C. Hillman, and M. L. Faber, 1987. National Surface Water Survey, Eastern Lake Survey (Phase II - Temporal Variability) Analytical Methods Manual. EPA-600/X-87/008, U.S. Environmental Protection Agency, Las Vegas, Nevada.

ABSTRACT

The U.S. Environmental Protection Agency requires that data collection activities be based on a program which ensures that the resulting data are of known quality and are suitable for their intended purpose. In addition, it is necessary that the data obtained be consistent and comparable. For these reasons, the same reliable, detailed analytical methodology should be available to and should be used by all analysts participating in the study.

This handbook describes methods used to process and analyze surface water samples of low ionic strength. It is intended as a guidance document for groups involved in acidic deposition monitoring activities similar to those implemented by the Aquatic Effects Research Program of the National Acid Precipitation Assessment Program.

The chemical and physical parameters measured and the analytical methods used are listed below. Each parameter or activity marked by an asterisk is measured or performed at a processing laboratory before samples are shipped to an analytical laboratory.

Parameter or Procedure	Method
Acidity, Alkalinity, and pH	Titration with Gran plot
Aliquot preparation*	Filtration and preservation
Aluminum, total extractable*	Extraction with 8-hydroxyquinoline into methyl iso-butyl ketone
Aluminum, total and nonexchangeable PCV-reactive*	Flow injection analysis colorimetry (pyrocatechol violet)
Ammonium, dissolved	Automated colorimetry (phenate)
Ammonium, dissolved (alterate)	Flow injection analysis colorimetry
Chloride, Nitrate, and Sulfate	Ion chromatograhy
Chlorophyll a	Fluorometric and high performance liquid chromatographic analysis
Dissolved Inorganic Carbon*	Instrumental
Dissolved Inorganic Carbon and Dissolved Organic Carbon	Instrumental

Parameter or Procedure	Method
Fluoride, dissolved	Ion selective electrode and meter
Metals (Al, Ca, Fe, K, Mg, Mn, Na)	Atomic absorption spectroscopy
Metals (Ca, Fe, Mg, Mn) (alternate)	Inductively coupled plasma emission spectroscopy
Nitrogen, total	Flow injection analysis
pH, closed system*	pH electrode, meter, and subchamber
pH, open system*	pH electrode and meter
Phosphorus, total	Automated colorimetry (phosphomolybdate)
Silica, dissolved	Automated colorimetry (molybdate blue)
Specific conductance	Conductivity cell and meter
True Color*	Comparison to platinum-cobalt color standards
Turbidity*	Instrumental (nephelometer)

These methods were developed for use in component projects of the Aquatic Effects Research Program under the Acid Deposition and Atmospheric Research Division of the Office of Acid Deposition, Environmental Monitoring and Quality Assurance. This program addresses the following questions relating to the effects of acidic deposition of aquatic ecosystems:

1. The extent and magnitude of past change.

2. The change to be expected in the future under various deposition scenarios.

3. The maximum rates of deposition below which further change is not expected.

4. The rate of change or recovery of aquatic ecosystems if deposition rates are decreased.

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GLOSSARY

I. DEFINITIONS

Accuracy -- The degree of agreement of a measurement with an accepted or true value. As used here, accuracy is determined from the difference between recorded measurements and accepted true values of audit samples and calibration standards. Accuracy is generally expressed as percent bias.

Acid Neutralizing Capacity -- The buffering capacity of a carbonate system to acid inputs; specifically, the quantity of H^+ ions reacted over a given pH range during acid titration.

Aliquot -- A portion of sample treated (processed) in a specific way for a particular parameter or set of parameters.

Audit Sample -- A material with known characteristics which is used to determine the accuracy of the measurement system. In the AERP studies, natural lake samples, prepared matrices, and certified (purchased) audit samples are employed.

Base Neutralizing Capacity -- The buffering capacity of a carbonate system to alkali inputs; specifically, the quantity of OH^- ions reacted over a given pH range in a base titration.

Batch -- All samples, including routine, duplicate or replicate, blank, and audit samples, that are processed together at a single laboratory on a single day.

Bias -- A systemic difference between repeated measurements and an accepted or true value.

Blank sample -- Four different blank samples are referenced in these methods:

1. Calibration blank -- A 0 mg L^{-1} standard, containing only the matrix of the calibration standards. The measured concentration should be less than twice the instrumental detection limit.
2. Laboratory blank -- A deionized water sample prepared at the processing laboratory and treated as a regular sample. It serves as a check of processing laboratory-introduced contamination.
3. Reagent blank -- A reagent blank contains all of the reagents (in the same quantities) used in preparing a regular sample for analysis. It is processed in the same manner as a regular sample. The measured concentration should be less than twice the instrumental detection limit.

GLOSSARY (Continued)

4. System blank -- A deionized water sample collected by field personnel, then treated as a regular sample. It serves as a check of overall system contamination.

Calibration -- Establishment of a relationship between standards of known values and the recorded output of a measurement system. As used here, a calibration range is defined as the concentration range over which acceptable results of standards are obtained.

Confidence Interval -- A value interval that has a designated probability of including some defined parameter of the population.

Detection limit (also method detection limit or MDL) -- Three times the standard deviation of 10 nonconsecutive reagent or calibration blank analyses.

Dissolved -- Refers to all constituents which remain after filtration through a 0.45 µm filter.

Duplicate -- A second, independent determination of the same sample, performed by the same analyst, at essentially the same time and under the same conditions.

Matrix spike sample -- Addition of a known amount of analyte (spike) to a sample portion used to investigate chemical and matrix interferences. The spike should be a twice the endogenous level or ten times the detection limit, whichever is larger. See Section 16.1.4.

NBS-traceable -- A material or instrument which is certified against a National Bureau of Standards primary standard.

Percent relative standard deviation (%RSD) -- An expression of precision, calculated by:

$$\%RSD = \frac{s}{\overline{X}} \times 100$$

where:

s = standard deviation
$\overline{X}$ = mean of recorded measurements

GLOSSARY (Continued)

Precision -- The mutual agreement among individual measurements of the same property. As used here, precision is calculated from results of duplicate analyses and repetitive analyses of audit samples and quality control check solutions. Precision is generally expressed in terms of percent relative standard deviation.

Quality assurance -- The overall system used to ensure that the quality control system is performing.

Quality control -- The specific procedures and checks used to provide a quality product.

Quality control check sample (QCCS) -- A known sample containing the analyte of interest at a concentration in the low- to mid-calibration range. Whenever possible, the QCCS should be prepared from a source independent of that used to prepare calibration standards.

Standard additions -- A method of analysis in which equal volumes of a sample are added to a deionized water blank and to three standards containing different known amounts of the test element. Standard additions are used when matrix or chemical interferences are present. See Section 16.4.1.

Standard deviation -- The square root of the variance of a set of values.

Turbidity -- Organic and inorganic material suspended in the water column.

II. ACRONYMS AND ABBREVIATIONS

ACS = American Chemical Society
AERP = Aquatic Effects Research Program
ALK = alkalinity
ANC = acid neutralizing capacity
APHA = American Public Health Administration
ASTM = American Society of Testing and Materials
AW = acid-washed
BNC = base neutralizing capacity
BRC = Biologically Relevant Chemistry
% CD = percent conductivity difference
CDTA = 1,2 cyclohexylene dinitrilo tetraacetic acid
CE = column efficiency
CPR = cardio-plumonary resuscitation
DDRP = Direct/Delayed Response Project
DHEW = Department of Health, Education, and Welfare
DIC = dissolved inorganic carbon
DIW = deionized-water washed
DL = detection limit
DOC = dissolved organic carbon
EDTA = disodium ethylenediamine tetraacetate

GLOSSARY (Continued)

ELS-I = Eastern Lake Survey - Phase I
EMSL = Environmental Monitoring Systems Laboratory
EMSL-LV = Environmental Monitoring Systems Laboratory - Las Vegas
EPA = Environmental Protection Agency
ERP = Episodic Response Project
FIA = flow injection analysis
GFAA = graphite furnace atomic absorption
HPLC = high-performance liquid chromatography
IC = ion chromatography
ICP = inductively-coupled plasma
ID = identification
%ID = percent ion difference
IHHE = Indirect Human Health Effects
Inc. = Incorporated
IR = infrared
IS = internal standard
ISE = ion selective electrode
KHP = potassium hydrogen phthalate
MDL = method detection limit
MIBK = methyl isobutyl ketone
NAPAP = National Acid Precipitation Assessment Program
NBS = National Bureau of Standards
NED = N-(1-naphthyl)-ethylene diamine dihyrochloride
NIOSH = National Institute for Occupational Safety and Health
NLS-I and II = National Lake Survey - Phase I, Phase II
No. = Number
NSS-I = National Stream Survey - Phase I
NSWS = National Surface Water Survey
NTU = nephelometer turbidity units
OSHA = Occupational Safety and Health Administration
PCU = platinum-cobalt units
PCV = pyrocatechol violet
QA = quality assurance
QC = quality control
QCCS = quality control check solution
RF = radio frequency
%RSD = percent relative standard deviation
s or SD = standard deviation
TIME = Temporal Integrated Monitoring of Ecosystems
TISAB = total ionic strength buffer solution
U.S. = United States
WLS-I = Western Lake Survey - Phase I
WMP = Watershed Manipulation Project

III. MEASUREMENT SYMBOLS

aq = aqueous
°C = degrees, Centigrade
d = density
eq = equivalent
g = gram
°K = degrees, Kelvin
L = liter
m = meter
M = molarity
mA = milliAngstrom
mg = milligram, 10^{-3} g
min. = minute
mL = milliliter, 10^{-3} L
mm = millimeter, 10^{-3} m

GLOSSARY (Continued)

mV	=	millivolt, 10^{-3} V
N	=	normality
nm	=	nanometer, 10^{-9} m
ppm	=	parts per million
psi	=	pounds per square inch
sp. gr.	=	specific gravity
V	=	volts
v/v	=	volume to volume
w/v	=	weight to volume
w/w	=	weight to weight
<	=	less than
Σ	=	summation
μeq	=	microequivalent
μg	=	microgram, 10^{-6} g
μL	=	microliter, 10^{-6} L
μm	=	micron, 10^{-6} m
μmho	=	micromho
μSCm^{-1}	=	microSieman per centimeter
%	=	percent

CONTENTS

	Page	Rev	Effective Date
Abstract	iii	--	--
Glossary	1 of 5	0	8/87
Figures	1 of 2	0	8/87
Tables	1 of 2	0	8/87
1.0 Introduction to the Aquatic Effects Research Program	1 of 5	0	8/87
1.1 National Surface Water Survey (NSWS)	3 of 5	0	8/87
1.2 Direct/Delayed Response Project (DDRP)	3 of 5	0	8/87
1.3 Episodic Response Project (ERP)	4 of 5	0	8/87
1.4 Watershed Manipulation Project (WMP)	4 of 5	0	8/87
1.5 Temporal Integrated Monitoring of Ecosystems (TIME) Project	4 of 5	0	8/87
1.6 Biologically Relevant Chemistry (BRC) Project	4 of 5	0	8/87
1.7 Indirect Human Health Effects (IHHE) Project	4 of 5	0	8/87
2.0 Overview of AERP Handbooks	1 of 2	0	8/87
2.1 Purpose of Handbooks	1 of 2	0	8/87
2.1.1 Types of Handbooks	1 of 2	0	8/87
2.1.2 Structure of Volumes	1 of 2	0	8/87
2.1.3 Interrelationship of Volumes	1 of 2	0	8/87
2.2 Content of Laboratory Handbook	1 of 2	0	8/87
2.2.1 Laboratory Methods	2 of 2	0	8/87
2.2.2 Suitable Sample Types	2 of 2	0	8/87
3.0 Laboratory Facilities and Organization	1 of 7	0	8/87
3.1 Mobile Laboratories	1 of 7	0	8/87
3.2 Minimum Facility Requirements	2 of 7	0	8/87
3.3 Laboratory Staffing	3 of 7	0	8/87
3.4 Training	5 of 7	0	8/87
3.5 Contamination Avoidance	6 of 7	0	8/87
3.6 Safety	6 of 7	0	8/87
3.7 References	7 of 7	0	8/87
4.0 Sample Handling	1 of 9	0	8/87
4.1 Sample Types and Sample Containers	1 of 9	0	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
4.2 Sample Transport and Transfer	1 of 9	0	8/87
4.3 Sample Processing	2 of 9	0	8/87
4.4 Sample Analysis	6 of 9	0	8/87
4.5 Data Tracking and Recording	6 of 9	0	8/87
4.6 References	9 of 9	0	8/87
5.0 Determination of Acidity, Alkalinity, and pH	1 of 29	4	8/87
5.1 Overview	1 of 29	4	8/87
5.1.1 Scope and Application	1 of 29	4	8/87
5.1.2 Summary of Method	1 of 29	4	8/87
5.1.3 Interferences	1 of 29	4	8/87
5.1.4 Safety	2 of 29	4	8/87
5.2 Sample Collection, Preservation, and Storage	2 of 29	4	8/87
5.3 Equipment and Supplies	2 of 29	4	8/87
5.3.1 Equipment Specifications	2 of 29	4	8/87
5.3.2 Apparatus	2 of 29	4	8/87
5.3.3 Reagents and Consumable Materials	3 of 29	4	8/87
5.4 Preparation	4 of 29	4	8/87
5.4.1 Standardization of HCl Titrant	4 of 29	4	8/87
5.4.2 Initial Standardization of NaOH Titrant with KHP	6 of 29	4	8/87
5.4.3 NaOH-HCl Standardization Crosscheck	8 of 29	4	8/87
5.4.4 Daily NaOH Standardization with Standardized HCl	9 of 29	4	8/87
5.4.5 Rigorous Calibration and Characterization of Electrodes	11 of 29	4	8/87
5.4.6 Daily Calibration and Characterization of Electrodes	13 of 29	4	8/87
5.5 Procedure	14 of 29	4	8/87
5.5.1 Acid Titration	14 of 29	4	8/87
5.5.2 Base Titration	15 of 29	4	8/87
5.5.3 Air-Equilibrated pH Measurement	16 of 29	4	8/87
5.5.4 Calculations	16 of 29	4	8/87
5.6 Quality Assurance and Quality Control	25 of 29	4	8/87
5.6.1 Comparison of Initial Titration pH Values	25 of 29	4	8/87
5.6.2 Comparison of Calculated ANC and Measured ANC	25 of 29	4	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
5.6.3 Comparison of Calculated BNC and Measured BNC	27 of 29	4	8/87
5.6.4 Comparison of Calculated Total Carbonate and Measured Total Carbonate	27 of 29	4	8/87
5.6.5 Quality Control Checks	28 of 29	4	8/87
5.7 References	29 of 29	4	8/87
6.0 Preparation of Aliquots	1 of 10	10	8/87
6.1 Overview	1 of 10	10	8/87
6.1.1 Scope and Application	1 of 10	10	8/87
6.1.2 Summary of Method	1 of 10	10	8/87
6.1.3 Interferences	1 of 10	10	8/87
6.1.4 Safety	1 of 10	10	8/87
6.2 Sample Collection, Preservation, and Storage	1 of 10	10	8/87
6.3 Equipment and Supplies	3 of 10	10	8/87
6.3.1 Apparatus	3 of 10	10	8/87
6.3.2 Reagents and Consumable Supplies	3 of 10	10	8/87
6.4 Preparation	3 of 10	10	8/87
6.4.1 Filtration Unit Assembly	3 of 10	10	8/87
6.4.2 Maintenance	5 of 10	10	8/87
6.5 Procedure	5 of 10	10	8/87
6.5.1 Filter Rinsing	5 of 10	10	8/87
6.5.2 Sample Filtration	6 of 10	10	8/87
6.5.3 Between Sample Rinsing	7 of 10	10	8/87
6.5.4 Unfiltered Aliquots	8 of 10	10	8/87
6.5.5 Preservation	8 of 10	10	8/87
6.5.6 Shipping Instructions	9 of 10	10	8/87
6.6 Quality Assurance and Quality Control	10 of 10	10	8/87
6.7 References	10 of 10	10	8/87
7.0 Preparation of Total Extractable Aluminum Aliquot	1 of 9	10	8/87
7.1 Overview	1 of 9	10	8/87
7.1.1 Scope and Application	1 of 9	10	8/87
7.1.2 Summary of Method	1 of 9	10	8/87
7.1.3 Interferences	1 of 9	10	8/87
7.1.4 Safety	1 of 9	10	8/87
7.2 Sample Collection, Preservation, and Storage	2 of 9	10	8/87
7.3 Equipment and Supplies	2 of 9	10	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
7.3.1 Apparatus	2 of 9	10	8/87
7.3.2 Reagents and Consumable Materials	2 of 9	10	8/87
7.4 Preparation	5 of 9	10	8/87
7.4.1 Calibration and Standardization	5 of 9	10	8/87
7.4.2 Maintenance	5 of 9	10	8/87
7.5 Procedure	5 of 9	10	8/87
7.5.1 Filtration	5 of 9	10	8/87
7.5.2 Extraction	7 of 9	10	8/87
7.5.3 Cleanup	8 of 9	10	8/87
8.0 Fractionation and Determination of Aluminum Species	1 of 24	2	8/87
8.1 Overview	1 of 24	2	8/87
8.1.1 Scope and Application	1 of 24	2	8/87
8.1.2 Summary of Method	2 of 24	2	8/87
8.1.3 Interferences	2 of 24	2	8/87
8.1.4 Safety	3 of 24	2	8/87
8.2 Sample Collection, Preservation, and Storage	3 of 24	2	8/87
8.3 Equipment and Supplies	3 of 24	2	8/87
8.3.1 Equipment Specifications	3 of 24	2	8/87
8.3.2 Consumable Materials	3 of 24	2	8/87
8.3.3 Reagents	3 of 24	2	8/87
8.3.4 Amberlite Cation Exchange Resin	6 of 24	2	8/87
8.3.5 Aluminum Stock Solutions	8 of 24	2	8/87
8.3.6 Aluminum Calibration Standards	8 of 24	2	8/87
8.3.7 Quality Control Standards	10 of 24	2	8/87
8.3.8 Reagent Filtering/Degassing	11 of 24	2	8/87
8.4 Preparation	12 of 24	2	8/87
8.4.1 Precalibration Procedure	12 of 24	2	8/87
8.4.2 Calibration and Standardization	14 of 24	2	8/87
8.4.3 Maintenance	16 of 24	2	8/87
8.4.4 Column Packing Procedure	17 of 24	2	8/87
8.4.5 Troubleshooting	18 of 24	2	8/87
8.5 Procedure	19 of 24	2	8/87
8.5.1 Syringe Pump Setup	19 of 24	2	8/87
8.5.2 Sample Injection	19 of 24	2	8/87
8.5.3 Cleanup	20 of 24	2	8/87
8.6 Quality Assurance and Quality Control	21 of 24	2	8/87
8.6.1 Precision and Accuracy	21 of 24	2	8/87
8.6.2 Quality Control Checks	22 of 24	2	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
8.7 References	24 of 24	2	8/87
9.0 Determination of Ammonium	1 of 6	10	8/87
9.1 Overview	1 of 6	10	8/87
9.1.1 Scope and Application	1 of 6	10	8/87
9.1.2 Summary of Method	1 of 6	10	8/87
9.1.3 Interferences	1 of 6	10	8/87
9.1.4 Safety	1 of 6	10	8/87
9.2 Sample Collection, Preservation, and Storage	1 of 6	10	8/87
9.3 Equipment and Supplies	1 of 6	10	8/87
9.3.1 Apparatus and Equipment	1 of 6	10	8/87
9.3.2 Reagents and Consumable Materials	2 of 6	10	8/87
9.4 Preparation	3 of 6	10	8/87
9.4.1 Calibration and Standardization	3 of 6	10	8/87
9.5 Procedure	3 of 6	10	8/87
9.5.1 Standard Operating Procedure	3 of 6	10	8/87
9.5.2 Calculations	4 of 6	10	8/87
9.6 Quality Assurance and Quality Control	4 of 6	10	8/87
9.6.1 Precision and Accuracy	4 of 6	10	8/87
9.6.2 Quality Control Checks	4 of 6	10	8/87
9.7 References	6 of 6	10	8/87
10.0 Determination of Ammonium by Flow Injection Analysis	1 of 5	2	8/87
10.1 Overview	1 of 5	2	8/87
10.1.1 Scope and Application	1 of 5	2	8/87
10.1.2 Summary of Method	1 of 5	2	8/87
10.1.3 Interferences	1 of 5	2	8/87
10.1.4 Safety	1 of 5	2	8/87
10.2 Sample Collection, Preservation, and Storage	1 of 5	2	8/87
10.3 Equipment and Supplies	2 of 5	2	8/87
10.3.1 Equipment and Apparatus	2 of 5	2	8/87
10.3.2 Reagents and Consumable Materials	2 of 5	2	8/87
10.4 Preparation	3 of 5	2	8/87
10.4.1 Calibration and Standardization	3 of 5	2	8/87
10.5 Procedure	3 of 5	2	8/87
10.5.1 Standard Operating Procedure	3 of 5	2	8/87
10.5.2 Calculations	3 of 5	2	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
10.6 Quality Assurance and Quality Control	4 of 5	2	8/87
10.6.1 Precision and Accuracy	4 of 5	2	8/87
10.6.2 Quality Control Checks	4 of 5	2	8/87
10.7 References	5 of 5	2	8/87
11.0 Determination of Chloride, Nitrate, and Sulfate by Ion Chromatography	1 of 6	10	8/87
11.1 Overview	1 of 6	10	8/87
11.1.1 Scope and Application	1 of 6	10	8/87
11.1.2 Summary of Method	1 of 6	10	8/87
11.1.3 Interferences	2 of 6	10	8/87
11.1.4 Safety	2 of 6	10	8/87
11.2 Sample Collection, Preservation, and Storage	2 of 6	10	8/87
11.3 Equipment and Supplies	2 of 6	10	8/87
11.3.1 Equipment Specifications	2 of 6	10	8/87
11.3.2 Reagents and Consumable Materials	3 of 6	10	8/87
11.4 Preparation	4 of 6	10	8/87
11.4.1 Calibration and Standardization	4 of 6	10	8/87
11.5 Procedure	4 of 6	10	8/87
11.5.1 Standard Operating Procedure	4 of 6	10	8/87
11.5.2 Calculations	5 of 6	10	8/87
11.6 Quality Assurance and Quality Control	5 of 6	10	8/87
11.6.1 Precision and Accuracy	5 of 6	10	8/87
11.6.2 Quality Control Checks	5 of 6	10	8/87
11.7 References	6 of 6	10	8/87
12.0 Determination of Chlorophyll a	1 of 11	4	8/87
12.1 Overview	1 of 11	4	8/87
12.1.1 Scope and Application	1 of 11	4	8/87
12.1.2 Summary of Method	1 of 11	4	8/87
12.1.3 Interferences	1 of 11	4	8/87
12.1.4 Safety	1 of 11	4	8/87
12.2 Sample Collection, Preservation, and Storage	2 of 11	4	8/87
12.3 Equipment and Supplies	2 of 11	4	8/87
12.3.1 Equipment Specifications	2 of 11	4	8/87
12.3.2 Apparatus	3 of 11	4	8/87
12.3.3 Reagents and Consumable Materials	3 of 11	4	8/87
12.4 Preparation	4 of 11	4	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
12.4.1 HPLC Calibration	4 of 11	4	8/87
12.4.2 Fluorometry Calibration	6 of 11	4	8/87
12.5 Procedure	8 of 11	4	8/87
12.5.1 Sample Extraction	8 of 11	4	8/87
12.5.2 Analysis	8 of 11	4	8/87
12.5.3 Calculations	8 of 11	4	8/87
12.6 Quality Assurance and Quality Control	8 of 11	4	8/87
12.6.1 Precision and Accuracy	8 of 11	4	8/87
12.6.2 HPLC Analysis Quality Control Checks	9 of 11	4	8/87
12.6.3 Fluorometry Quality Control Checks	9 of 11	4	8/87
12.7 References	10 of 11	4	8/87
13.0 Determination of Dissolved Inorganic Carbon	1 of 17	10	8/87
13.1 Overview	1 of 17	10	8/87
13.1.1 Scope and Application	1 of 17	10	8/87
13.1.2 Summary of Method	1 of 17	10	8/87
13.1.3 Interferences	1 of 17	10	8/87
13.1.4 Safety	2 of 17	10	8/87
13.2 Sample Collection, Preservation, and Storage	2 of 17	10	8/87
13.3 Equipment and Supplies	2 of 17	10	8/87
13.3.1 Equipment Specifications	2 of 17	10	8/87
13.3.2 Apparatus	2 of 17	10	8/87
13.3.3 Reagents and Consumable Materials	2 of 17	10	8/87
13.4 Preparation	5 of 17	10	8/87
13.4.1 Instrument Setup	5 of 17	10	8/87
13.4.2 Initial Calibration	11 of 17	10	8/87
13.4.3 Linearity Check	13 of 17	10	8/87
13.4.4 Maintenance	14 of 17	10	8/87
13.5 Procedure	14 of 17	10	8/87
13.5.1 Sample Analysis	14 of 17	10	8/87
13.5.2 Data Reporting	15 of 17	10	8/87
13.5.3 Cleanup	15 of 17	10	8/87
13.6 Quality Assurance and Quality Control	16 of 17	10	8/87
13.6.1 Precision and Accuracy	16 of 17	10	8/87
13.6.2 Quality Control Checks	16 of 17	10	8/87
13.7 References	16 of 17	10	8/87
14.0 Determination of Dissolved Organic Carbon and Dissolved Inorganic Carbon	1 of 7	10	8/87

CONTENTS (Continued)

		Page	Rev	Effective Date
14.1	Overview	1 of 7	10	8/87
	14.1.1 Scope and Application	1 of 7	10	8/87
	14.1.2 Summary of Method	1 of 7	10	8/87
	14.1.3 Interferences	1 of 7	10	8/87
	14.1.4 Safety	1 of 7	10	8/87
14.2	Sample Collection, Preservation, and Storage	1 of 7	10	8/87
14.3	Equipment and Supplies	2 of 7	10	8/87
	14.3.1 Equipment Specifications	2 of 7	10	8/87
	14.3.2 Apparatus	2 of 7	10	8/87
	14.3.3 Reagents and Consumable Materials	2 of 7	10	8/87
14.4	Preparation	4 of 7	10	8/87
	14.4.1 Instrument Setup	4 of 7	10	8/87
	14.4.2 DOC Calibration	4 of 7	10	8/87
	14.4.3 DIC Calibration	6 of 7	10	8/87
14.5	Procedure	6 of 7	10	8/87
	14.5.1 DOC Standard Operating Procedure	6 of 7	10	8/87
	14.5.2 DIC Standard Operating Procedure	6 of 7	10	8/87
	14.5.3 Calculations	6 of 7	10	8/87
14.6	Quality Assurance and Quality Control	7 of 7	10	8/87
	14.6.1 Precision and Accuracy	7 of 7	10	8/87
	14.6.2 Quality Control Checks	7 of 7	10	8/87
14.7	References	7 of 7	10	8/87
15.0	Determination of Total Dissolved Fluoride by Ion-Selective Electrode	1 of 5	10	8/87
15.1	Overview	1 of 5	10	8/87
	15.1.1 Scope and Application	1 of 5	10	8/87
	15.1.2 Summary of Method	1 of 5	10	8/87
	15.1.3 Interferences	1 of 5	10	8/87
	15.1.4 Safety	2 of 5	10	8/87
15.2	Sample Collection, Preservation, and Storage	2 of 5	10	8/87
15.3	Equipment and Supplies	2 of 5	10	8/87
	15.3.1 Equipment and Apparatus	2 of 5	10	8/87
	15.3.2 Reagents and Consumable Materials	2 of 5	10	8/87
15.4	Preparation	3 of 5	10	8/87
	15.4.1 Calibration and Standardization	3 of 5	10	8/87
	15.4.2 Maintenance	4 of 5	10	8/87
15.5	Procedure	4 of 5	10	8/87
	15.5.1 Standard Operating Procedure	4 of 5	10	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
15.5.2 Calculations	5 of 5	10	8/87
15.6 Quality Assurance and Quality Control	5 of 5	10	8/87
15.6.1 Precision and Accuracy	5 of 5	10	8/87
15.6.2 Quality Control Checks	5 of 5	10	8/87
15.7 References	5 of 5	10	8/87
16.0 Determination of Metals (Al, Ca, Fe, K, Mg, Mn, Na) by Atomic Absorption Spectroscopy	1 of 23	10	8/87
16.1 Overview	1 of 23	10	8/87
16.1.1 Scope and Application	1 of 23	10	8/87
16.1.2 Summary of Method	1 of 23	10	8/87
16.1.3 Definitions	3 of 23	10	8/87
16.1.4 Interferences	3 of 23	10	8/87
16.1.5 Safety	5 of 23	10	8/87
16.2 Sample Collection, Preservation, and Storage	5 of 23	10	8/87
16.3 Equipment and Supplies	5 of 23	10	8/87
16.3.1 Equipment and Apparatus	5 of 23	10	8/87
16.3.2 Reagents and Consumable Materials	6 of 23	10	8/87
16.4 Preparation	7 of 23	10	8/87
16.4.1 Calibration and Standardization	7 of 23	10	8/87
16.5 Procedure	7 of 23	10	8/87
16.5.1 Flame Atomic Absorption Spectroscopy	7 of 23	10	8/87
16.5.2 Furnace Atomic Absorption Spectroscopy	8 of 23	10	8/87
16.5.3 Procedure for Determination of Total Aluminum	9 of 23	10	8/87
16.5.4 Procedure for Determination of Total Extractable Aluminum	11 of 23	10	8/87
16.5.5 Procedure for Determination of Dissolved Calcium	14 of 23	10	8/87
16.5.6 Procedure for Determination of Dissolved Iron	15 of 23	10	8/87
16.5.7 Procedure for Determination of Dissolved Magnesium	16 of 23	10	8/87
16.5.8 Procedure for Determination of Dissolved Manganese	17 of 23	10	8/87
16.5.9 Procedure for Determination of Dissolved Potassium	18 of 23	10	8/87
16.5.10 Procedure for Determination of Dissolved Sodium	20 of 23	10	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
16.5.11 Calculations	21 of 23	10	8/87
16.6 Quality Assurance and Quality Control	21 of 23	10	8/87
16.6.1 Precision and Accuracy	21 of 23	10	8/87
16.6.2 Quality Control Checks	23 of 23	10	8/87
16.7 References	23 of 23	10	8/87
17.0 Determination of Dissolved Metals (Ca, Fe, Mg, and Mn) by Inductively Coupled Plasma Emission Spectroscopy	1 of 10	10	8/87
17.1 Overview	1 of 10	10	8/87
17.1.1 Scope and Application	1 of 10	10	8/87
17.1.2 Summary of Method	1 of 10	10	8/87
17.1.3 Interferences	2 of 10	10	8/87
17.1.4 Interference Tests	4 of 10	10	8/87
17.1.5 Safety	6 of 10	10	8/87
17.2 Sample Collection, Preservation, and Storage	6 of 10	10	8/87
17.3 Equipment and Supplies	6 of 10	10	8/87
17.3.1 Equipment Specifications	6 of 10	10	8/87
17.3.2 Reagents and Consumable Materials	7 of 10	10	8/87
17.4 Preparation	8 of 10	10	8/87
17.4.1 Calibration and Standardization	8 of 10	10	8/87
17.5 Procedure	8 of 10	10	8/87
17.5.1 Standard Operating Procedure	8 of 10	10	8/87
17.5.2 Calculations	8 of 10	10	8/87
17.6 Quality Assurance and Quality Control	9 of 10	10	8/87
17.6.1 Precision and Accuracy	9 of 10	10	8/87
17.6.2 Quality Control Checks	9 of 10	10	8/87
17.7 References	9 of 10	10	8/87
18.0 Determination of Total Nitrogen	1 of 10	4	8/87
18.1 Overview	1 of 10	4	8/87
18.1.1 Scope and Application	1 of 10	4	8/87
18.1.2 Summary of Method	1 of 10	4	8/87
18.1.3 Definitions	1 of 10	4	8/87
18.1.4 Interferences	1 of 10	4	8/87
18.1.5 Safety	2 of 10	4	8/87
18.2 Sample Collection, Preservation, and Storage	2 of 10	4	8/87
18.3 Equipment and Supplies	2 of 10	4	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
18.3.1 Equipment and Apparatus	2 of 10	4	8/87
18.3.2 Reagents and Consumable Materials	2 of 10	4	8/87
18.3.3 Reduction Column and Reagents	4 of 10	4	8/87
18.3.4 Standard Solutions	4 of 10	4	8/87
18.3.5 Quality Control (QC) Standards	5 of 10	4	8/87
18.4 Preparation	6 of 10	4	8/87
18.4.1 Calibration and Standardization	6 of 10	4	8/87
18.4.2 Preparation of Reduction Column	6 of 10	4	8/87
18.5 Procedure	6 of 10	4	8/87
18.5.1 Standard Operating Procedure	6 of 10	4	8/87
18.5.2 Calculations	8 of 10	4	8/87
18.6 Quality Assurance and Quality Control	8 of 10	4	8/87
18.6.1 Precision and Accuracy	8 of 10	4	8/87
18.6.2 Quality Control Checks	8 of 10	4	8/87
18.6.3 Reduction Column Quality Control Checks	9 of 10	4	8/87
18.7 References	10 of 10	4	8/87
19.0 Determination of pH (Closed System)	1 of 16	10	8/87
19.1 Overview	1 of 16	10	8/87
19.1.1 Scope and Application	1 of 16	10	8/87
19.1.2 Summary of Method	1 of 16	10	8/87
19.1.3 Interferences	4 of 16	10	8/87
19.1.4 Safety	4 of 16	10	8/87
19.2 Sample Collection, Preservation, and Storage	4 of 16	10	8/87
19.3 Equipment and Supplies	4 of 16	10	8/87
19.3.1 Apparatus and Equipment	4 of 16	10	8/87
19.3.2 Reagents and Consumable Materials	4 of 16	10	8/87
19.4 Preparation	5 of 16	10	8/87
19.4.1 Instrument Preparation	5 of 16	10	8/87
19.4.2 Calibration and Standardization	6 of 16	10	8/87
19.4.3 Maintenance	6 of 16	10	8/87
19.4.4 pH Meter Electronic Checkout	7 of 16	10	8/87
19.4.5 Electrode Etching	8 of 16	10	8/87
19.5 Procedure	9 of 16	10	8/87
19.5.1 Sample Chamber Assembly	9 of 16	10	8/87
19.5.2 Initial QCCS Check	9 of 16	10	8/87
19.5.3 Sample Measurement	13 of 16	10	8/87
19.5.4 Additional Procedures Using Two pH Meters	14 of 16	10	8/87

CONTENTS (Continued)

		Page	Rev	Effective Date
	19.5.5 Cleanup	15 of 16	10	8/87
19.6	Quality Assurance and Quality Control	15 of 16	10	8/87
	19.6.1 Precision and Accuacy	15 of 16	10	8/87
	10.6.2 Quality Control Checks	15 of 16	10	8/87
19.7	References	16 of 16	10	8/87
20.0	Determination of pH (Open System)	1 of 7	2	8/87
20.1	Overview	1 of 7	2	8/87
	20.1.1 Scope and Application	1 of 7	2	8/87
	20.1.2 Summary of Method	1 of 7	2	8/87
	20.1.3 Interferences	1 of 7	2	8/87
	20.1.4 Safety	1 of 7	2	8/87
20.2	Sample Collection, Preservation, and Storage	2 of 7	2	8/87
20.3	Equipment and Supplies	2 of 7	2	8/87
	20.3.1 Apparatus and Equipment	2 of 7	2	8/87
	20.3.2 Reagents and Consumable Materials	2 of 7	2	8/87
20.4	Preparation	2 of 7	2	8/87
	20.4.1 Instrument Preparation	2 of 7	2	8/87
	20.4.2 Calibration and Standardization	2 of 7	2	8/87
	20.4.3 Maintenance	3 of 7	2	8/87
	20.4.4 pH Meter Electronic Checkout	3 of 7	2	8/87
	20.4.5 Electrode Etching Procedure	3 of 7	2	8/87
	20.4.6 Sample Preparation	3 of 7	2	8/87
20.5	Procedure	3 of 7	2	8/87
	20.5.1 Initial Quality Control Check	3 of 7	2	8/87
	20.5.2 Sample Measurement	6 of 7	2	8/87
	20.5.3 Routine Quality Control Check	6 of 7	2	8/87
	20.5.4 Cleanup	6 of 7	2	8/87
20.6	Quality Assurance and Quality Control	7 of 7	2	8/87
20.7	References	7 of 7	2	8/87
21.0	Determination of Total Phosphorus	1 of 7	10	8/87
21.1	Overview	1 of 7	10	8/87
	21.1.1 Scope and Application	1 of 7	10	8/87
	21.1.2 Summary of Method	1 of 7	10	8/87
	21.1.3 Interferences	1 of 7	10	8/87
	21.1.4 Safety	2 of 7	10	8/87
21.2	Sample Collection, Preservation, and Storage	2 of 7	10	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
21.3 Equipment and Supplies	2 of 7	10	8/87
21.3.1 Equipment Specifications	2 of 7	10	8/87
21.3.2 Apparatus	2 of 7	10	8/87
21.3.3 Reagents and Consumable Materials	2 of 7	10	8/87
21.4 Preparation	4 of 7	10	8/87
21.4.1 Calibration and Standardization	4 of 7	10	8/87
21.5 Procedure	4 of 7	10	8/87
21.5.1 Standard Operating Procedure	4 of 7	10	8/87
21.5.2 Calculations	5 of 7	10	8/87
21.6 Quality Assurance and Quality Control	5 of 7	10	8/87
21.6.1 Precision and Accuracy	5 of 7	10	8/87
21.6.2 Quality Control Checks	6 of 7	10	8/87
21.7 References	6 of 7	10	8/87
22.0 Determination of Dissolved Silica	1 of 7	10	8/87
22.1 Overview	1 of 7	10	8/87
22.1.1 Scope and Application	1 of 7	10	8/87
22.1.2 Summary of Method	1 of 7	10	8/87
22.1.3 Interferences	1 of 7	10	8/87
22.1.4 Safety	1 of 7	10	8/87
22.2 Sample Collection, Preservation, and Storage	1 of 7	10	8/87
22.3 Equipment and Supplies	2 of 7	10	8/87
22.3.1 Equipment Specifications	2 of 7	10	8/87
22.3.2 Reagents and Consumable Materials	2 of 7	10	8/87
22.4 Preparation	3 of 7	10	8/87
22.4.1 Calibration and Standardization	3 of 7	10	8/87
22.5 Procedure	4 of 7	10	8/87
22.5.1 Standard Operating Procedure	4 of 7	10	8/87
22.5.2 Calculations	6 of 7	10	8/87
22.6 Quality Assurance and Quality Control	6 of 7	10	8/87
22.6.1 Precision and Accuracy	6 of 7	10	8/87
22.6.2 Quality Control Checks	6 of 7	10	8/87
22.7 References	6 of 7	10	8/87
			8/87
23.0 Determination of Specific Conductance	1 of 10	4	8/87
23.1 Overview	1 of 10	4	8/87
23.1.1 Scope and Application	1 of 10	4	8/87
23.1.2 Summary of Method	1 of 10	4	8/87
23.1.3 Interferences	1 of 10	4	8/87

CONTENTS (Continued)

		Page	Rev	Effective Date
	23.1.4 Safety	1 of 10	4	8/87
23.2	Sample Collection, Preservation, and Storage	1 of 10	4	8/87
23.3	Equipment and Supplies	2 of 10	4	8/87
	23.3.1 Equipment Specifications	2 of 10	4	8/87
	23.3.2 Apparatus	2 of 10	4	8/87
	23.3.3 Reagents and Consumable Materials	2 of 10	4	8/87
23.4	Preparation	4 of 10	4	8/87
	23.4.1 Electronics Check	4 of 10	4	8/87
	23.4.2 Conductivity Cell Calibration Check	6 of 10	4	8/87
	23.4.3 Quality Control Check	7 of 10	4	8/87
	23.4.4 Maintenance	8 of 10	4	8/87
23.5	Procedure	9 of 10	4	8/87
23.6	Quality Assurance and Quality Control	9 of 10	4	8/87
	23.6.1 Precision and Accuracy	9 of 10	4	8/87
	23.6.2 Quality Control Checks	10 of 10	4	8/87
23.7	References	10 of 10	4	8/87
24.0	Determination of True Color	1 of 5	10	8/87
24.1	Overview	1 of 5	10	8/87
	24.1.1 Scope and Application	1 of 5	10	8/87
	24.1.2 Summary of Method	1 of 5	10	8/87
	24.1.3 Interferences	1 of 5	10	8/87
	24.1.4 Safety	1 of 5	10	8/87
24.2	Sample Collection, Preservation, and Storage	2 of 5	10	8/87
24.3	Equipment and Supplies	2 of 5	10	8/87
	24.3.1 Apparatus and Equipment	2 of 5	10	8/87
	24.3.2 Reagents and Consumable Materials	2 of 5	10	8/87
24.4	Preparation	2 of 5	10	8/87
	24.4.1 Sample Preparation	2 of 5	10	8/87
	24.4.2 Color Kit Preparation	3 of 5	10	8/87
24.5	Procedure	3 of 5	10	8/87
	24.5.1 Low Range Sample Color Determination	3 of 5	10	8/87
	24.5.2 High Range (100-500 PCU) Sample Color Determination	3 of 5	10	8/87
	24.5.3 High Range (500-1000 PCU) Sample Color Determination	4 of 5	10	8/87
	24.5.4 Cleanup	4 of 5	10	8/87
24.6	Quality Assurance and Quality Control	5 of 5	10	8/87
	24.6.1 Precision and Accuracy	5 of 5	10	8/87

CONTENTS (Continued)

	Page	Rev	Effective Date
24.6.2 Quality Control Checks	5 of 5	10	8/87
24.7 References	5 of 5	10	8/87
25.0 Determination of Turbidity	1 of 9	10	8/87
25.1 Overview	1 of 9	10	8/87
25.1.1 Scope and Application	1 of 9	10	8/87
25.1.2 Summary of Method	1 of 9	10	8/87
25.1.3 Interferences	1 of 9	10	8/87
25.1.4 Safety	1 of 9	10	8/87
25.2 Sample Collection, Preservation, and Storage	1 of 9	10	8/87
25.3 Equipment and Supplies	2 of 9	10	8/87
25.3.1 Equipment and Apparatus	2 of 9	10	8/87
25.3.2 Reagents and Consumable Materials	2 of 9	10	8/87
25.4 Preparation	3 of 9	10	8/87
25.4.1 Daily Calibration	3 of 9	10	8/87
25.4.2 Maintenance	4 of 9	10	8/87
25.5 Procedure	4 of 9	10	8/87
25.5.1 Low Turbidity Samples	4 of 9	10	8/87
25.5.2 High Turbidity Samples	6 of 9	10	8/87
25.5.3 Cleanup	7 of 9	10	8/87
25.5.4 General Precautionary Notes for Procedure	8 of 9	10	8/87
25.6 Quality Assurance and Quality Control	8 of 9	10	8/87
25.6.1 Precision and Accuracy	8 of 9	10	8/87
25.6.2 Quality Control Checks	8 of 9	10	8/87
25.7 References	9 of 9	10	8/87
Appendices			
Appendix A - National Surface Water Survey Mobile Laboratory Specifications	1 of 5	4	8/87
Appendix B - Processing Laboratory Equipment List	1 of 6	4	8/87
Appendix C - General Laboratory Procedures	1 of 6	4	8/87
Appendix D - National Surface Water Survey Blank Data Forms	1 of 21	4	8/87
Appendix E - Examples of Calculations Required for ANC and BNC Determinations	1 of 22	4	8/87
Appendix F - The Aid Photoionization Detector for Use as an MIBK Detection System	1 of 1	4	8/87
Appendix G - Internal Quality Control Requirements	1 of 10	10	8/87

FIGURES

		Page	Rev	Effective Date
1-1	Aquatic Effects Research Program component projects.	2 of 5	0	8/87
1-2	Regions sampled during National Surface Water Survey.	3 of 5	0	8/87
6-1	Filtration apparatus.	4 of 10	10	8/87
7-1	Aluminum extraction flowchart	6 of 9	10	8/87
8-1	Schematic of flow injection system for aluminum speciation.	13 of 24	2	8/87
9-1	Ammonia manifold AAI.	5 of 6	10	8/87
9-2	Ammonia manifold AAII	5 of 6	10	8/87
12-1	Example high performance liquid chromatography chromatogram.	5 of 11	4	8/87
13-1	Flowchart for dissolved inorganic carbon analysis	6 of 17	10	8/87
13-2	Troubleshooting flowchart for dissolved inorganic carbon analysis	7 of 17	10	8/87
13-3	Diagram of the Dohrmann carbon analyzer	8 of 17	10	8/87
13-4	External plumbing of the Dohrmann carbon analyzer	9 of 17	10	8/87
13-5	Internal connections of the Dohrmann carbon analyzer.	10 of 17	10	8/87
16-1	Standard addition plot.	8 of 23	10	8/87
18-1	Schematic of flow injection system for determination of total nitrogen.	7 of 10	4	8/87
19-1	Schematic of pH measurement system.	2 of 16	10	8/87
19-2	pH sample chamber	3 of 16	10	8/87
19-3	Flowchart for pH determination.	10 of 16	10	8/87
19-4	Troubleshooting flowchart for pH determination.	11 of 16	10	8/87
19-5	pH logbook and example page; organization of raw data.	12 of 16	10	8/87
20-1	Flowchart for pH determination.	4 of 7	2	8/87
20-2	Troubleshooting flowchart for pH determination.	5 of 7	2	8/87
21-1	Total phosphorus manifold	5 of 7	10	8/87
22-1	Silica manifold	5 of 7	10	8/87
23-1	Flowchart for specific conductance measurement.	5 of 10	4	8/87
25-1	Flowchart for turbidity	5 of 9	10	8/87
A-1	Schematic drawing of laboratory trailer and features, curbside view	2 of 5	4	8/87
A-2	Schematic drawing of laboratory trailer and features, roadside view	3 of 5	4	8/87

FIGURES (Continued)

		Page	Rev	Effective Date
E-1	Plot of F_{1b} versus V for HCl standardization. . . .	2 of 22	4	8/87
E-2	Plot of F_{3b} versus V for initial NaOH standardization with KHP.	5 of 22	4	8/87
E-3	Plot of F_1 versus V for NaOH-HCl standardization cross-check	7 of 22	4	8/87
E-4	Plot of F_1 versus V for daily NaOH standardization.	9 of 22	4	8/87
E-5	Plot of pH* versus pH for electrode calibration . .	12 of 22	4	8/87
E-6	Plot of F_{1a} versus V for ANC determination of blank.	13 of 22	4	8/87
E-7	Plot of F_{1a} versus V_a for initial determination of V_1	16 of 22	4	8/87
E-8	Plot of F_{1c} versus V_a for V_1 determination.	18 of 22	4	8/87
E-9	Plot of F_{2c} versus V_b for V_2 determination.	19 of 22	4	8/87

TABLES

		Page	Rev	Effective Date
4-1	Aliquots, Containers, Preservatives, and Corresponding Parameters for the National Surface Water Survey.	3 of 9	0	8/87
4-2	Samples for Special Studies Conducted During the National Surface Water Survey	4 of 9	0	8/87
4-3	National Surface Water Survey Standard Analyses . .	7 of 9	0	8/87
4-4	National Surface Water Survey Experimental or Special Study Analyses.	8 of 9	0	8/87
5-1	Calculation Procedures for Combinations of Initial V_1 and pH*.	18 of 29	4	8/87
5-2	Constants and Variable Descriptions	19 of 29	4	8/87
6-1	Aliquots, Containers, Preservatives, and Corresponding Parameters for the National Surface Water Survey.	2 of 10	10	8/87
8-1	Volume of Aluminum Stock Standards Required to Prepare Daily Standards	10 of 24	2	8/87
8-2	Volume of Aluminum Stock Standards Required for High Range Calibration Standards.	10 of 24	2	8/87
8-3	Precision and Accuracy for Single Operator and Single Laboratory Analysis of Inorganic Monomeric Aluminum by Flow Injection/Pyrocatechol Violet Method	22 of 24	2	8/87
8-4	Precision and Accuracy for Single Operator and Single Laboratory Analysis of Inorganic Monomeric Aluminum by Flow Injection/Pyrocatechol Violet Method	22 of 24	2	8/87
8-5	Percent Recovery of Monomeric Al From Two Spiked Natural Surface Water Samples Analyzed by the Flow Injection/Pyrocatechol Violet Method	23 of 24	2	8/87
11-1	Suggested Concentration of Dilute Calibration Standards	4 of 6	10	8/87
11-2	Typical Ion Chromatograph Operating Conditions. . .	5 of 6	10	8/87
11-3	Single Operator Accuracy and Precision.	6 of 6	10	8/87
12-1	Dilutions of Chlorophyll a Stock Standard to Make Working Standards	7 of 11	4	8/87
16-1	Atomic Absorption Concentrations Ranges	2 of 23	10	8/87
17-1	Recommended Wavelengths and Estimated Instrumental Detection Limits.	2 of 10	10	8/87
17-2	Analyte Concentation Equivalents (mg L^{-1}) Arising from Interferences at the 100-mg L^{-1} Level. . . .	3 of 10	10	8/87

TABLES (Continued)

		Page	Rev	Effective Date
17-3	Interference and Analyte Elemental Concentrations Used for Interference Measurements in Table 17-2.	5 of 10	10	8/87
17-4	Inductively Coupled Plasma Emission Spectroscopy Precision and Accuracy Data.	10 of 10	10	8/87
19-1	pH Values of Buffers at Various Temperatures. . . .	7 of 16	10	8/87
21-1	Percent Recovery of Total Phosphorus in the Presence of Silica.	1 of 7	10	8/87
21-2	Precision and Accuracy of the Phosphorus Method for Natural Water Samples	6 of 7	10	8/87
21-3	Precision and Accuracy of the Phosphorus Method for Analyst-Prepared Standards.	6 of 7	10	8/87
23-1	Temperature Correction Factors to Compute Specific Conductance Values at 25.0 °C	8 of 10	4	8/87
E-1	Acid Titration.	10 of 22	4	8/87
E-2	Base Titration.	11 of 22	4	8/87
G-1	Summary of Internal Method Quality Control Checks .	2 of 10	10	8/87
G-2	Maximum Control Limits for Quality Control Samples	3 of 10	10	8/87
G-3	Required Minimum Analytical Detection Limits, Expected Ranges, and Intralaboratory Relative Precision	5 of 10	10	8/87
G-4	Factors to Convert mg L^{-1} to μeq L^{-1}.	8 of 10	10	8/87
G-5	Chemical Reanalysis Criteria.	9 of 10	10	8/87

1.0 INTRODUCTION TO THE AQUATIC EFFECTS RESEARCH PROGRAM

Concern over the effects of acidic deposition on the nation's surface water resources led the U.S. Environmental Protection Agency (EPA) to initiate research in the field in the late 1970s. Early research, focusing on a diversity of potential effects, provided insight into those research areas which were considered central to key policy questions. Recognizing the need for an integrated, stepwise approach to resolve the issues, EPA implemented the Aquatic Effects Research Program (AERP) in 1983 with its present structure, focus, and approach. The Program, a part of EPA's Office of Research and Development, is administered by the Acid Deposition and Atmospheric Research Division in the Office of Acid Deposition, Environmental Monitoring, and Quality Assurance. The AERP is also a major component of the National Acid Precipitation Assessment Program's (NAPAP) Aquatic Effects Research Task Group 6, a cooperative effort of nine federal agencies tasked with addressing important policy and assessment questions relating to the acidic deposition phenomenon and its effects.

Initially, AERP studies focused on process-oriented research at a few sites to generate hypotheses for further testing and to identify key parameters associated with the effects of acidic deposition on aquatic ecosystems. In 1983, after it was determined that regional assessments of the effects of acidic deposition could not be made with confidence on the basis of available historical data, the AERP redirected its focus to provide the required information. Weaknesses of available data included possible inconsistencies in the selection of study sites, lack of data for certain important parameters, inconsistent sampling and analytical methods, and little or no information on quality assurance.

The AERP addresses four major policy questions relating to the effects of acidic deposition on aquatic ecosystems:

1. The extent and magnitude of past change.

2. The change to be expected in the future under various deposition scenarios.

3. The maximum rates of deposition below which further change is not expected.

4. The rate of change or recovery of aquatic ecosystems if deposition rates are decreased.

An integrated, stepwise approach is used within the AERP to provide the necessary data for assessment and policy decisions related to effects of acidic deposition on aquatic resources. The approach employs statistically based site selection, standardized sampling procedures and analytical methods, and rigorous quality assurance protocols. At present, the AERP includes five major research component projects that have been initiated or are being planned: the National Surface Water Survey (NSWS), the Direct/Delayed Response Project (DDRP), the Episodic Response Project (ERP), the Watershed Manipulation Project (WMP), and the Temporal Integrated Monitoring of Ecosystems (TIME) Project. Two additional projects, Biologically Relevant Chemistry (BRC) and Indirect Human Health Effects (IHHE), have been incorporated into the AERP research design. The AERP projects form an integrated program to quantify the chemical status of surface waters, to predict the response of biologically relevant water chemistry to variable rates of acidic deposition, and to verify and validate the predictions (Figure 1-1).

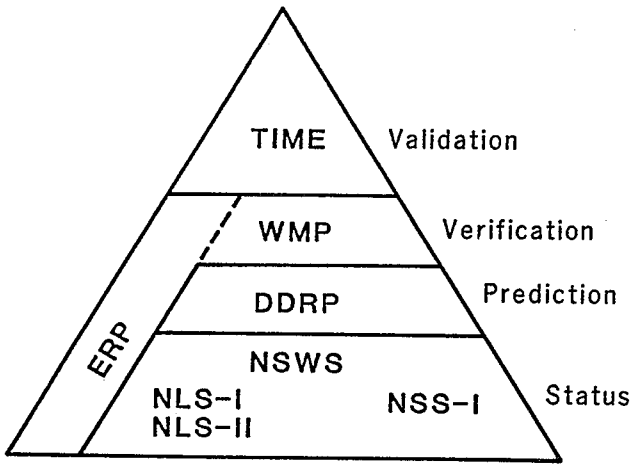

Figure 1-1. Aquatic Effects Research Program component projects.

The AERP projects are concerned primarily with assessing chronic, or long-term, acidification of surface waters as affected by sulfur deposition. The Episodic Response Project will assess the importance of acute, or short-term, acidification and nitrate deposition. Components of the Biologically Relevant Chemistry Project address issues of both chronic and acute acidification.

1.1 NATIONAL SURFACE WATER SURVEY (NSWS)

The NSWS is divided into two components: the National Lake Survey (NLS) and the National Stream Survey (NSS). Figure 1-2 shows the various regions sampled during the NSWS.

Phase I activities of the NSWS provide information to determine the current chemical status of lakes and streams. Phase II activities of these surveys describe seasonal variability in regional surface water chemistry. Because of the statistical basis of the sampling design, data from the NSWS can be used to classify lakes and streams so that selected subsets can be identified for more detailed studies during other components of the AERP. Results of these more detailed studies can be interpreted then at a regional scale with greater confidence.

1.2 DIRECT/DELAYED RESPONSE PROJECT (DDRP)

The DDRP provides data on watersheds and soils to complement the surface water data of the NSWS. These data are used in three watershed acidification models to predict the time scales over which surface waters are expected to become chronically acidic, given different levels of acidic inputs.

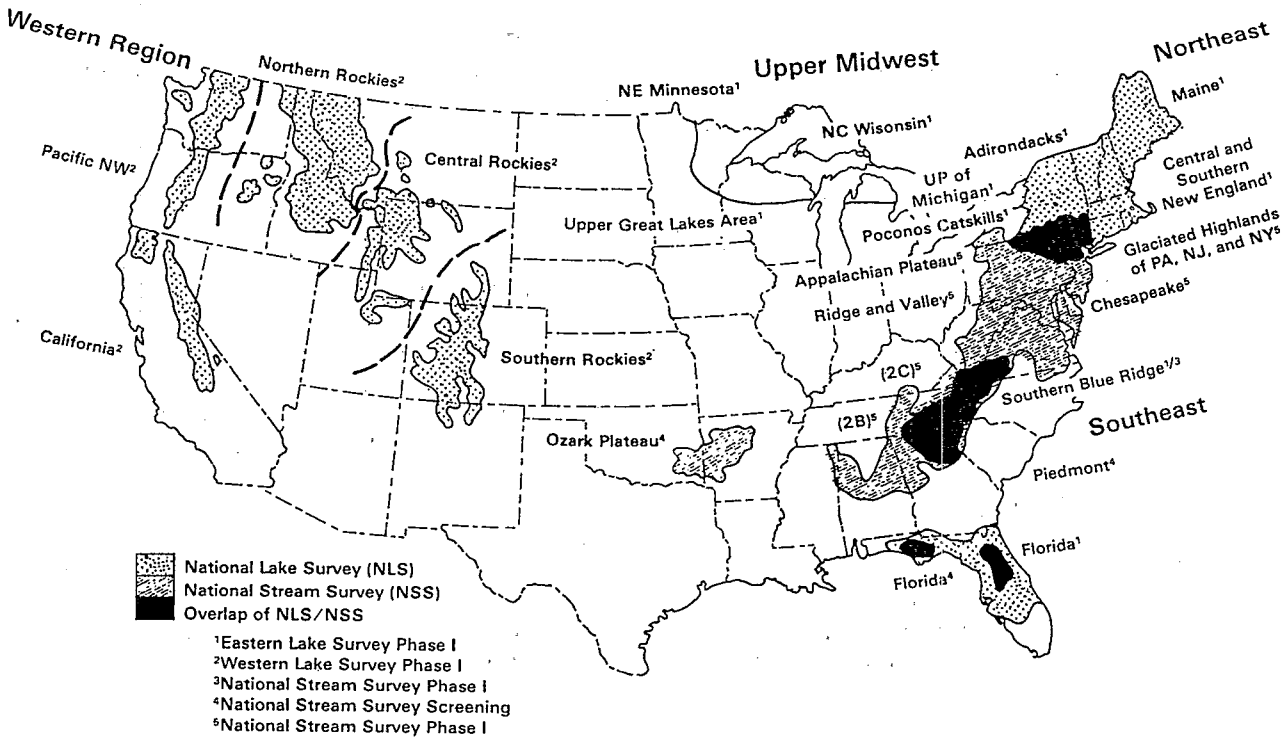

Figure 1-2. Regions sampled during the National Surface Water Survey.

1.3 EPISODIC RESPONSE PROJECT (ERP)

The ERP has objectives similar to those of the NSWS, but focuses on the magnitude, frequency, and duration of episodic acidification and the effect of episodes on regional water chemistry and watershed processes. The ERP is conducted at a small number of watersheds believed to represent the range of conditions found within a region, based on the results of the NSWS and the DDRP. Empirical and conceptual models are developed from these site-specific studies to address the regional extent of episodes, using the NSWS statistical frame.

1.4 WATERSHED MANIPULATION PROJECT (WMP)

The WMP, involving process-oriented research at a small number of watersheds, is designed to assess the quantitative and qualitative response of watershed soils and surface waters to altered deposition. Designed primarily to verify the models used for prediction in the DDRP, the WMP also determines the interactions among biogeochemical mechanisms controlling the response of surface waters to acidic inputs at various scales within watersheds, ranging from plot to whole ecosystem studies.

1.5 TEMPORAL INTEGRATED MONITORING OF ECOSYSTEMS (TIME) PROJECT

The TIME Project, a long-term monitoring activity, evolves from the existing projects within EPA and NAPAP. TIME sites are selected by evaluating data from currently monitored systems and from the NSWS results. These sites, which will be established throughout the United States by 1990, are monitored to quantify the rate, direction, and magnitude of changes in surface water chemistry due to increased and decreased levels of acidic deposition. The TIME sites also provide information on surface water chemistry that can be used to validate the conclusions of the DDRP, the ERP, and the WMP.

1.6 BIOLOGICALLY RELEVANT CHEMISTRY (BRC) PROJECT

The BRC Project provides data that can be used to assess the risk that acidic deposition poses to aquatic biota. Several complementary studies are incorporated as components of the BRC. One study determines the present status of fish populations in a subset of lakes sampled during the eastern component of the NLS and quantifies the chemical characteristics of these lakes. Another study, planned in conjunction with the ERP, determines the effects of episodic acidification on fish populations.

1.7 INDIRECT HUMAN HEALTH EFFECTS (IHHE) PROJECT

The IHHE Project targets two areas: (1) the alteration of drinking water supplies in response to acidic inputs and (2) the accumulation of mercury and other potentially toxic metals in the muscle tissues of edible fish. Emphasizing precipitation-dominated surface water systems, drinking water studies

include the examination of existing data to determine the potential modification of drinking water quality by acidic deposition. In addition, existing process-oriented and survey data are examined to evaluate the relationship between mercury bioaccumulation in sport fish and surface water chemistry in areas receiving high levels of acidic deposition.

2.0 OVERVIEW OF AERP HANDBOOKS

2.1 PURPOSE OF HANDBOOKS

Numerous private, state, and federal groups have initiated research projects similar to those developed as components of the AERP. Existing AERP field and laboratory manuals and quality assurance plans were not written for an overall methods application or for general use. Developed for specific survey requirements, available operational documents do not provide general guidelines and procedures that can be adapted readily by different research groups. AERP handbooks are designed to fill this gap. As guidance documents for groups involved in acidic deposition monitoring activities, the handbooks enable researchers to avoid duplication of efforts and to make maximum use of tested methods.

2.1.1 Types of Handbooks

AERP handbooks focus on surface water chemistry, based on documents written for the NSWS, and on soil chemistry, based on the DDRP manuals. The handbooks contain procedures for field operations, laboratory operations, and quality assurance criteria for water and soil monitoring activities. Surface water chemistry and soil chemistry are discussed in separate three-volume sets.

2.1.2 Structure of Volumes

Because the AERP is a dynamic program, inclusion of additional methods is anticipated. Each document is contained in a three-ring binder to facilitate insertion of revisions by handbook subscribers. Each document contains an independent Table of Contents with titles, revision numbers, and effective dates for revisions; a complete, updated Table of Contents will accompany dissemination of each revision. The availability of each volume or revision will be announced in the AERP status.

2.1.3 Interrelationship of Volumes

Each volume of a particular handbook set represents one aspect of an acidic deposition monitoring activity. Collectively, the field, laboratory, and quality assurance handbooks offer a comprehensive guide to surface water chemistry or soil chemistry monitoring.

2.2 CONTENT OF LABORATORY HANDBOOK

This handbook describes methods used to process and analyze surface water samples.

2.2.1 Laboratory Methods

The chemical and physical parameters measured and the analytical methods used are listed below:

Section	Parameter	Analytical Method
5.0	Acidity, Alkalinity, and pH	Titration with Gran plot
6.0	Aliquot preparation[a]	Filtration and preservation
7.0	Aluminum, total extractable[a]	Extraction with 8-hydroxyquinoline into methyl isobutyl ketone
8.0	Aluminum, total and non-exchangeable PCV-reactive[a]	Flow injection analysis colorimetry (pyrocatechol violet)
9.0	Ammonium, dissolved	Automated colorimetry (phenate)
10.0	Ammonium, dissolved (alternate)	Flow injection analysis colorimetry
11.0	Chloride, Nitrate, and Sulfate	Ion chromatograhy
12.0	Chlorophyll a	Fluorometric and high performance liquid chromatographic analysis
13.0	Dissolved Inorganic Carbon[a]	Instrumental
14.0	Dissolved Inorganic Carbon and Dissolved Organic Carbon	Instrumental
15.0	Fluoride, dissolved	Ion selective electrode and meter
16.0	Metals (Al, Ca, Fe, K, Mg, (Mn, Na)	Atomic absorption spectroscopy
17.0	Metals (Ca, Fe, Mg, Mn) (alternate)	Inductively coupled plasma emission spectroscopy
18.0	Nitrogen, total	Flow injection analysis
19.0	pH, closed system[a]	pH electrode, meter, and subchamber
20.0	pH, open system[a]	pH electrode and meter
21.0	Phosphorus, total	Automated colorimetry (phosphomolybdate)
22.0	Silica, dissolved	Automated colorimetry (molybdate blue)
23.0	Specific conductance	Conductivity cell and meter
24.0	True Color[a]	Comparison to platinum-cobalt color standards
25.0	Turbidity[a]	Instrumental (nephelometer)

[a]Parameters measured or activities performed at the processing laboratory before the samples are transmitted to the analytical laboratory.

2.2.2 Suitable Sample Types

The processing and analytical methods described in this handbook have been used for surface water samples of low ionic strength and snowpack samples. These methods can be altered slightly to apply to precipitation samples.

3.0 LABORATORY FACILITIES AND ORGANIZATION

Many biological and chemical parameters are subject to rapid change or degradation following sample collection. The period of time between sample collection and sample analysis is defined as the holding time; the recommended holding time is the maximum sample storage time before significant changes in the parameter of interest can be expected to occur. Sample processing procedures stabilize samples, thereby increasing the holding time. Sample processing includes refrigeration, filtration, extraction, and chemical preservation. In general, sample processing procedures do not produce quantitative results. Procedures which do produce quantitative results are termed sample analyses.

3.1 MOBILE LABORATORIES

Studies assessing the water quality of lakes or streams often involve sampling in remote locations over a large geographic area. During large-scale studies, a central, analytical laboratory cannot always perform all analyses within the recommended holding times. Using mobile laboratories and multiple analytical laboratories provides alternatives to the single-laboratory approach.

For the NSWS, a mobile field laboratory was designed to provide sample processing facilities. The mobile laboratory also was used to perform some analyses, including pH, dissolved inorganic carbon (DIC), true color, and turbidity. Six mobile laboratories were deployed during the ELS-I and WLS-I; however, they were located centrally in Las Vegas during later surveys. The decision processes followed by the NSWS can be used in similar situations to determine whether mobile laboratories may be beneficial and what activities are to be performed in the mobile or central processing laboratory. This decision process is outlined below:

1. Determine parameters to be measured, recommended holding times for those parameters, and processing alternatives to lengthen the holding time. For processing techniques, determine holding time of raw sample prior to processing.

2. Determine sampling locations and available shipping services (i.e., can samples be collected, shipped, received, and analyzed at a laboratory within the recommended holding times).

3. Determine the sample load and the length of time required to perform processing or analysis. Determine the number of personnel and the units of equipment necessary to complete the activity within the holding time. Determine the space required.

4. If more than one option is feasible, determine the costs of all viable options.

During Phase I of the NLS, six mobile laboratories were used. In later surveys, it was determined that additional mobile laboratories would be required, or existing units would require relocation every three to four days, with a downtime of at least two days during moves. Both options were prohibitively expensive. In addition, an analytical method for aluminum determination was added to the processing laboratory-performed analyses, placing limitations on trailer space. Because overnight courier services were available within reasonable distances of sampling points, the final decision was to provide a central processing laboratory for these later surveys.

3.2 MINIMUM FACILITY REQUIREMENTS

Whether mobile laboratories or a central processing laboratory are used, certain specifications should be considered. Specifications of the mobile laboratory designed for the NSWS are provided in Appendix A; equipment used in the processing laboratory is listed in Appendix B.

A clean air station is highly recommended to provide a contamination-free work area for sample filtration and for possible use as a fume hood. The clean air station shoud contain a high efficiency purification apparatus capable of delivering Class 100 air (Federal Standard 209B, 1978). For sample filtration, the clean air station should be under slight positive pressure. If the clean air station is also to be used as a fume hood, it should have adjustable flow vents to allow conversion from positive pressure to negative pressure.

A supply of Type I reagent grade water (ASTM, 1984) also is recommended for reagent preparation, glassware washing, and preparation of blank samples. A reverse osmosis and deionization system is effective in providing Type I water in the quantities needed. A specific conductance test should be performed weekly to ensure that the system is meeting the Type I specification of less than 1 $\mu S\ cm^{-1}$ specific conductance. An analysis of the feed water, which is available from the local water district, is valuable in determining cartridge arrangement and expected cartridge life expectancy.

Samples are generally shipped and stored at 4 °C prior to processing; processed aliquots are also stored and shipped at 4 °C. Refrigerator space should be adequate to provide storage, and temperature should be checked daily. Freeze-gel packs and insulated coolers provide refrigeration during shipment. Adequate freezer space is needed to freeze the gel packs. Due to the freezer space needed for a study of even moderate size, stand-alone freezers are recommended. Additionally, some biological samples may require storage at -20 °C; only freezers that meet this specification should be used.

A number of logistical concerns should be considered, including electric power, telephones, laboratory counter and storage space, solid and liquid waste

disposal, water, shipping access, and chemical storage. Local building and fire safety codes must be considered. Electric power should be adequate to power the clean air station, the deionized water system, interior lights, refrigerators and freezers, and the heating and air conditioning systems. A large number of outlets is needed for the equipment. Fluctuations in power can severely damage sensitive instruments; a constant voltage regulator and surge protectors are recommended. Some instruments, particularly those interfaced to computers, may lose memory or calibration during power outages; a battery back-up system capable of maintaining power for at least 6 hours may be necessary.

3.3 LABORATORY STAFFING

The mobile laboratories used in the NSWS were designed for a staff of five people--the coordinator, the laboratory supervisor, and three analysts. These five positions were standard during NLS-I; a sixth position, to perform pyrocatechol violet (PCV) aluminum analysis, was added in later surveys. Each of these positions is described below:

The laboratory coordinator has the following responsibilities:

1. Serves as overall laboratory administrator.
2. Oversees the laboratory installation and operations.
3. Serves as the liaison between management, the processing laboratory, and the Quality Assurance manager.
4. Receives samples from the field; organizes the daily batch formation and the insertion of associated quality assurance (QA) and quality control (QC) samples.
5. Coordinates the shipment of processed aliquots to the analytical laboratories.
6. Coordinates purchases and audit sample receipt.
7. Checks and transfers data from the laboratory to the data base manager via the quality assurance manager.
8. Coordinates sample and batch tracking from the field to the laboratory and then to the analytical laboratories.
9. Serves as a reserve laboratory analyst.

The laboratory supervisor has the following responsibilities:

1. Oversees daily laboratory operations, including maintenance and troubleshooting of all instrumentation and equipment.

2. Ensures that samples are processed in accordance with approved methodologies and the quality assurance program.

3. Assists the laboratory coordinator in transcribing data from DIC, pH, turbidity, conductivity, PCV-aluminum, and true color determinations.

4. Supervises the preparation of sample aliquots for shipment.

5. Ensures laboratory safety, cleanliness, and security.

6. Tracks the field and laboratory supply inventory.

7. Serves, along with the laboratory coordinator, as facility safety compliance officer.

8. Serves as the acting laboratory coordinator in the coordinator's absence.

9. Serves as the reserve laboratory analyst.

In addition, the laboratory supervisor, or, alternately, a separate analyst:

1. Performs pH determinations and equipment maintenance.

2. Performs DIC determinations and carbon analyzer maintenance.

Analyst 1 has the following responsibilities:

1. Operates and maintains the flow injection analyzer to conduct PCV-aluminum determinations.

Analyst 2 has the following responsibilities:

1. Prepares an aliquot for the analysis of extractable aluminum using MIBK.

2. Prepares MIBK aliquots for shipping.

3. Disposes of solid and liquid MIBK waste.

Analyst 3 has the following responsibilities:

1. Assists the laboratory coordinator with sample batching.
2. Filters aliquots from routine, duplicate, blank, and audit samples.
3. Prepares aliquot bottles and labels.
4. Assists in preparing preserved aliquots for shipping.

Analyst 4 has the following responsibilities:

1. Preserves aliquots as they are filtered.
2. Performs turbidity and true color determinations.
3. Performs specific conductance measurements.

Additionally, Analyst 4, or a separate analyst, has the following responsibilities:

1. Prepares and ships reagents and supplies to the field sites.
2. Maintains an ample stock of shipping containers and frozen gel packs.
3. Assists in preparing preserved aliquots for shipping.
4. Maintains the organization and cleanliness of the laboratory.

3.4 TRAINING

Recommended qualifications for processing laboratory personnel include a knowledge of basic chemistry, laboratory experience, and a high level of work neatness and precision. A college degree in one of the physical sciences is recommended, but is not absolutely necessary. The nature of processing laboratory work demands close attention to detail and the ability to perform at a consistently high quality level.

Laboratory training programs should include thorough coverage of each procedure and hands-on practice sessions. Theory and rationale for each laboratory rule and procedure should be covered in detail. A written laboratory manual containing detailed, step-by-step procedures is invaluable during training. At least one week should be devoted to training. A typical training schedule should include one day of orientation (including explanation of laboratory rules and an overview of operations). Another day should be devoted to each method or procedure, including general laboratory procedures (Appendix C). This training should include a lecture, an observation period, a question and

answer period, and hands-on practice. At the conclusion of training, written testing is recommended with follow-up, supervised practice in any areas of weakness. Each analyst should be fully trained in all areas to provide coverage for absences and potential for rotation of duties.

3.5 CONTAMINATION AVOIDANCE

Particular emphasis should be placed on avoiding sample contamination throughout training and operation. Lake and stream samples in the AERP studies are generally of low ionic strength; even a single fingerprint on the inside edge of a sample container can contaminate the entire sample. For this reason, disposable sterile gloves and labcoats should be worn when handling samples. As much as possible, all sample handling should be done inside the clean air station. Eating and smoking are forbidden inside the laboratory and all personnel should wash their hands after breaks. Personnel should not wear makeup or perfume, including men's cologne. All glassware and plasticware should be washed in Type I reagent grade water and rinsed with a portion of the sample. When adding reagents to samples, the pipet tip should not contact the sample. Acid-washed and deionized water-washed apparatus and glassware should be stored separately and both types should be labeled clearly. Counter areas should be covered in Benchkote, which should be changed frequently. The laboratory floors and counters should be washed and swept daily. Only water should be used for washing; no soap should be used. Additional precautions regarding contamination avoidance are described in the methods relating to sample processing.

3.6 SAFETY

Safety is also a primary consideration in the laboratory. In most locales, inspection and certification by the local fire department is required. Smoke detectors and class A-B fire extinguishers are recommended and may be required.

Fire escape routes should be clearly marked and each person should be aware of at least two escape routes from their work area. A circuit breaker with a mainswitch shutoff should be located on the outside of each mobile laboratory to avert electrical problems. Chemicals should be stored in approved containers and cabinets; bases and acids should be stored in separate places. Chemical spill kits should be located in each work area. Disaster plans should be developed and included in training so that each person knows exactly what to do in the event of an accident. Emergency numbers should be posted next to each telephone.

Personnel safety includes proper laboratory clothing (e.g., lab coat, fitted safety glasses, and protective footwear). If MIBK is used, personnel should be fitted for half-mask respirators with organics cartridges. Personnel should be certified in cardio-pulmonary resuscitation (CPR) and first aid; local Red Cross or American Heart Association groups can provide classes for a

nominal fee. A medical surveillance program, including testing before and after sample handling, is recommended if hazardous materials are used. A complete physical, including blood testing, is recommended prior to initiation of laboratory activities.

3.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

4.0 SAMPLE HANDLING

The sections below describe some alternatives for sample handling. These alternatives provide guidelines of the types of questions one should consider in planning sample handling. The methods used for the NSWS are described for illustration purposes.

4.1 SAMPLE TYPES AND SAMPLE CONTAINERS

The "basic" water sample consists of water collected in an appropriately sized sample container. The quantity should be sufficient to prepare all necessary aliquots, perform all analyses, and provide extra volume for rinses. Generally, the sample container should not be washed with acid and should be filled totally to minimize atmospheric contact. Filled containers are stored at 4 °C in the dark until sample processing. Polyethylene containers are recommended because of their durability, light weight, and low contamination potential. Containers used for the NSWS include 4-L Cubitainers, 20-L Cubitainers, and 500-mL bottles. Either dark or clear containers may be used. Clear containers were used in the NSWS.

The containers described above are not impervious to CO_2 exchange. Therefore, for measurements in which CO_2 is a factor (pH, DIC, aluminum fractions), the raw sample should be collected without atmospheric contact into containers which limit gas exchange. For the AERP studies, 60-mL sterile syringes were used with Leur Lok syringe valves. The valve attached to a port on the sample collection device, allowing the syringe to be filled without atmospheric exposure. Syringes then were sealed and stored at 4 °C in the dark until analysis. Syringes also were used to collect samples from the oxygen-depleted hypolimnion.

Containers for processed aliquots are specific to the type of aliquot. For the NSWS, the majority of aliquot containers were made of amber-colored, high-density polyethylene. Clear polyallomer or polycarbonate centrifuge tubes were used for MIBK extractions. The bottle size should be of sufficient volume to perform required analyses and rinses. Extra volume is desirable to permit replicate analyses. Bottles are acid-washed for acid-preserved aliquots and deionized water-washed for aliquots not containing acid. Complete filling to eliminate headspace is required only for unpreserved aliquots. Storage at 4 °C in the dark is desirable for all aliquots, but is necessary only for certain types. If aliquots are refrigerated, the lids should be retightened when bottles have cooled because polyethylene tends to shrink, which causes leaks. Specific aliquot types are discussed in Section 4.3.

4.2 SAMPLE TRANSPORT AND TRANSFER

Field-collected samples are transferred to a laboratory; if mobile or processing laboratories are used, processed aliquots are transferred to analytical

laboratories. Means of transportation depend on the distance between points and the sample holding time. In all cases, careful packaging and shipment tracking is needed. In many studies, particularly if results may be used for litigation purposes, formal chain-of-custody procedures may be required.

Raw samples should be processed as soon as possible, usually within 24 hours of collection. In the NLS-I, helicopters were used as sampling platforms and were used to deliver samples to the mobile laboratory immediately after collection. In later surveys, samples were shipped by overnight courier service to the processing laboratory in Las Vegas. Samples were packed in insulated styrofoam or plastic ice chests with frozen gel packs. Documentation accompanied each shipment to identify samples and collection teams.

After processing, aliquots were shipped by overnight courier service to analytical laboratories. Packing was similar to that described above for raw samples. Overnight service was necessary because some aliquots had a seven-day holding time. Documentation in each shipment included two copies of a four-copy shipping form (see Appendix D). The shipping form provided the necessary chain-of-custody documentation.

A central Communications Center tracked all shipments and provided coordination among field teams, processing laboratory(ies), analytical laboratories, quality assurance, and management. Airbill numbers, sample identification, and the number of containers were called in to Communications immediately after shipping. Communications notified the recipient and, the next day, verified arrival and sample condition. In this way, problems were noted and resolved quickly.

4.3 SAMPLE PROCESSING

The purpose of sample processing is to stabilize the chemical parameter of interest. Processing may include refrigeration or freezing, filtration, chemical preservation, or more complex procedures. The aliquots prepared for the NSWS are shown in Table 4-1.

Of the seven standard NSWS aliquots, three are prepared by filtration with (1) sulfuric acid preservation, (2) nitric acid preservation, and (3) no preservative. Three are not filtered, with the same three treatments. The remaining aliquot is an MIBK-extraction. The only variation of these seven standard aliquots is Aliquot 6*, prepared for stream samples, which is unfiltered for measurement of total phosphorus.

Additional aliquots or split samples were prepared for special studies during one or more of the NSWS surveys. These aliquots are described in Table 4-2.

TABLE 4-1. ALIQUOTS, CONTAINERS, PRESERVATIVES, AND CORRESPONDING PARAMETERS FOR THE NATIONAL SURFACE WATER SURVEY

Aliquot/(Container)[a]	Processing and Preservation[b]	Parameters
1 (250 mL, AW)	Filtered pH < 2 with HNO_3[c]	Ca, Mg, K, Na, Mn, Fe
2 (15 mL, AW)	Filtered MIBK Extract	Total Extractable Al
3 (250 mL, DIW)	Filtered No Preservative	Cl^- F^- SO_4^{-2} NO_3 SiO_2
4 (125 mL, AW)	Filtered pH <2 with H_2SO_4[d]	Dissolved organic carbon NH_4^+
5 (500 mL, DIW)	Unfiltered No Preservative	pH Alkalinity Acidity Conductance Dissolved inorganic carbon
6 (125 mL, AW)	Filtered pH <2 with H_2SO_4[d]	Dissolved P
6* (125 mL, AW)	Unfiltered pH <2 with H_2SO_4[d]	Total P
7 (125 mL, AW)	Unfiltered pH <2 with HNO_3[c]	Total Al

[a]AW = acid-washed container DIW = deionized water-washed container.
[b]Aliquots 2, 3, 4, 5, and 6 are stored at 4 °C in the dark.
[c]12M nitric acid, Baker Ultrex grade or equivalent.
[d]18M sulfuric acid, Baker Ultrex grade or equivalent.

TABLE 4-2. SAMPLES FOR SPECIAL STUDIES CONDUCTED DURING THE NATIONAL SURFACE WATER SURVEY

Destination	Container[a]	Number	Processing; Analytes
EPA-Corvallis (Oregon)	125 mL, AW	All samples	Filtered, pH <2 w/HNO_3[b]; Aliquot 1 cations
Norwegian Institute for Water Research (Norway)	500 mL, DIW	90	Raw unfiltered sample; PCV aluminum, ANC, Ca, Cl, specific conductance, K, Mg, Na, Nitrate, pH, sulfate
	2x500 mL, DIW	25	
Ontario Ministry of the Environment (Canada)	250 mL, AW	115	Raw unfiltered sample; pH < 2 w/HNO_3[b]; P, F, Al, Fe, Mn
Canada Centre for Inland Waters	250 mL, DIW	115	Raw unfiltered sample; pH, Cl, NO_3, Ca, Si, DOC, DIC, K, Na, Mg, SO_4, ANC, specific conductance
	125 mL, DIW	115	Raw unfiltered sample; same as above
Indiana University	10-15 mL, AW	All samples	Filtered, pH <2 w/HNO_3[b]; Pb, Cd, Ni, Mn, and Cu
Indiana University	10-15 mL, AW	Anoxics study samples	Syringe-filtered, pH <2 w/HNO_3[b]; Pb, Cd, Ni, Mn, and Cu
Freshwater Institute (Canada)	c	Chlorophyll routine, duplicate, audit, and referee samples	250 mL unpreserved sample filtered, filter stored at -20°C in dark; chlorophyll a
Oak Ridge National Laboratory (Tennessee)	c	Chlorophyll audit and referee samples only	250 mL unpreserved sample filtered, filter stored at -20°C in dark; chlorophyll a

(continued)

See footnotes at end of table.

TABLE 4-2. (Continued)

Destination	Container[a]	Number	Processing; Analytes
Academy of Natural Sciences of Philadelphia (Pennsylvania)	3x250 mL, DIW Glass	Zooplankton samples (3 tows per lake) no true duplicates, no blanks or audits	Filtered (net tow), preserved with 4 percent formalin/sucrose; zooplankton
Environmental Monitoring Sytems Laboratory - Las Vegas (EMSL-LV) (Nevada)	125 mL (in HCl-washed bottle)	All samples	Unfiltered, pH < 2 w/H_2SO_4[d]; total N and total P
EMSL-LV	60-125 mL, AW	Anoxics study sample	Syringe-filtered, pH <2 w/HNO_3[b]; Fe and Mn
EMSL-LV	30 mL, AW clear polyethene	maximum 10 per batch	Filtered, pH <2 w/HNO_3[b]; 32 elements

[a]AW = acid-washed container DI = deionized water-washed container.
[b]12M nitric acid, Baker Ultrex grade or equivalent.
[c]Container not applicable. Chlorphyll samples are contained on a polycarbonate filter.
[d]18M sulfuric acid, Baker Ultrex grade or equivalent.

Three notes on aliquots are necessary. First, although Aliquot 1 was always preserved with nitric acid during the NSWS, many researchers have found that preservation is not absolutely necessary if analyses are completed within 30 days of sample collection. Second, comparisons of PCV aluminum and Aliquot 2 data indicate the FIA method may replace the aliquot as the preferred measurement technique (Lewis et al., 1986). Third, in-house studies have indicated that the holding time for nitrate and sulfate may be extended to three weeks if the aliquot is preserved with mercuric chloride (Suarez et al., 1986).

4.4 SAMPLE ANALYSIS

The analyses performed for the NSWS are listed in Table 4-3. Of these, closed system pH, DIC, true color, and turbidity were performed in the mobile laboratory; PCV aluminum and specific conductance were added later to the mobile laboratory analyses. The remaining analyses were performed in analytical laboratories or by special investigators.

In addition to the analyses listed in Table 4-3, other analyses were conducted in conjunction with a single survey or as special interest studies. These are listed in Table 4-4. As these additional methods are tested and proven reliable, they will be included in the methods sections of this handbook.

4.5 DATA TRACKING AND RECORDING

Each form used in the NSWS assisted in data tracking or creation of the final data base. Additional records were maintained to provide a complete history of the survey and of laboratory operations. While the documentation scheme should be tailored to the needs of an individual project or data user, the basics addressed here should be considered. Copies of the forms used in the NSWS are presented in Appendix D.

Sample tracking or chain-of-custody is needed to ensure the integrity of the sample, as well as to provide a means of tracing lost or damaged samples. Tracking is critically important if more than one organization is involved in sample handling, or if results may be used for litigation purposes. In the latter case, formal chain-of-custody documents are necessary. In the former case, multicopy shipping or data forms with provision for inclusion of information by both the originator and the recipient may be sufficient. Commercial carriers generally require additional documentation, including a packing list, bill of lading, or airbill, copies of which also should be kept.

Samples must be identified uniquely. An identification system can become quite complex if more than one point in a single water body is sampled, temporally separate samples are taken from the same location, or multiple samples are collected from a single location. Additionally, it is important in a quality assurance program that QA and QC samples be, as far as possible, indistinguishable from routine samples to the analyst, yet recognizable in the final data base. In the NSWS, a batch system was used, whereby all samples processed on a given day were assigned a sequential batch number and a unique, randomly-selected sample number. Batches were prepared in the processing laboratory and included routine, duplicate or replicate, blank, and audit samples. The assigned number was recorded on the sample label and field data form, in addition to the primary processing laboratory data form (Form 2, see Appendix D).

TABLE 4-3. NATIONAL SURFACE WATER SURVEY STANDARD ANALYSES

Parameter	Method
Acidity, alkalinity, and pH	Titration with Gran plot
Aluminum, total extractable	Extraction with 8-hydroxyquinoline into MIBK followed by atomic absorption spectroscopy (AAS)
Aluminum, total and non-exchangeable PCV reactive	Flow injection analysis colorimetry (pyrocatechol violet)
Ammonium, dissolved	Automated colorimetry (phenate)
Chloride, Nitrate, and Sulfate	Ion chromatography
Dissolved Inorganic Carbon and Dissolved Organic Carbon	Instrumental
Fluoride, dissolved	Ion selective electrode and meter
Metals (Al, Ca, Fe, K, Mg, Mn, Na)	Atomic absorption spectroscopy
Metals (Ca, Fe, Mg, Mn) (alternate)	Inductively coupled plasma emission spectroscopy
pH, closed system	pH electrode, meter, and subchamber
pH, open system	pH electrode and meter
Phosphorus, total	Automated colorimetry (phosphomolybdate)
Silica, dissolved	Automated colorimetry (molybdate blue)
Specific Conductance	Conductivity cell and meter
True Color	Comparison to platinum-cobalt color standards
Turbidity	Instrumental (nephelometer)

TABLE 4-4. NATIONAL SURFACE WATER SURVEY EXPERIMENTAL OR SPECIAL STUDY ANALYSES

Parameter	Method
Ammonium, dissolved[a]	Flow injection analysis colorimetry
Chlorophyll a[a]	Fluorimetric and high performance liquid chromatographic analysis
Mercury, total	Atomic absorption spectroscopy (cold vapor)
Mercury, total organic	Gas chromatography
Methyl Mercury	Gas chromatography/Mass spectroscopy
Nitrogen, total[a]	Flow injection analysis

[a]Indicates method included in this handbook.

Logbooks should be used to maintain complete records of each laboratory activity. At a minimum, the logbook should contain: batch and sample identification (ID) instrumentation ID, data, analyst name or ID, calibration information, all raw data, and complete narrative descriptions of any unusual circumstances or observations. Logbooks should be checked after completion of the day's activity, both by the analyst recording the information and by the laboratory supervisor or QA personnel.

Data are transcribed onto Form 2. Transcribed values should be checked against logbook entries by the person transcribing the data and by the laboratory supervisor or QA personnel. Data also may be entered into a computer-based data system. Double entry is recommended to guard against transcription errors.

Verification and validation of the raw data base are covered in detail in the Handbook of Methods for Acid Deposition Studies, Quality Assurance for Surface Water Chemistry. Within the laboratory, the following quality control records should be maintained:

1. Records of audit samples used, tabulation of audit sample analysis values, and periodic calculation of mean and range (standard deviation) for each analyzed parameter.

2. Control charts of QC checks for each instrument and periodic computation of mean, warning limits, and control limits.

3. Periodic determination of detection limits for each instrument.

4. Calculation of percent difference between duplicate analyses of the same sample and, if samples are identifiable, percent differences between analyses of the field routine and any replicates.

5. Records of pipet and balance calibrations (see Appendix C).

6. Records of refrigeraor and freezer temperatures.

7. Records of deionized water system performance and specific conductance.

In addition, it is recommended that the laboratory coordinator or supervisor maintain a detailed, daily log of all laboratory activities. At a minimum, this log should identify the analysts performing each procedure, describe any observed problems or successes, and describe any deviations from the prescribed protocols. Changes to protocols should be documented and the protocol revised after the change has been substantiated as an improvement. For this reason, all the methods contained in this handbook are identified with a revision number. Updates to this handbook will be provided as protocols are revised or new protocols are developed.

4.6 REFERENCES

Lewis, T. E., J. M. Henshaw, and E. M. Heithmar, 1986. A comparison of PCV-reactive and 8-hydroxyquinoline-extractable aluminum in lake and stream waters. Presented at the North American Lake Management Society Annual Meeting. November 3-7, Portland, Oregon.

Suarez, F. X., D. C. Hillman, and E. M. Heithmar, 1986. Stability of nitrate in preserved and unpreserved natural surface waters. Presented at the Rocky Mountain Conference on Analytical Chemistry, August 3-5, Denver, Colorado.

5.0 DETERMINATION OF ACIDITY, ALKALINITY, AND pH

5.1 OVERVIEW

This procedure is a slight modification of the one used during Phase I of the National Lake Survey. In this procedure, pH is determined prior to sample titration rather than during sample titration.

5.1.1 Scope and Application

This procedure is applicable to the determination of pH, acid neutralizing capacity (ANC), and base neutralizing capacity (BNC) in weakly buffered natural waters of low ionic strength. The terms ANC and BNC refer to the alkalinity and acidity of systems which are based on the carbonate ion system. The soluble reacting species are H_2CO_3, HCO_3^-, and CO_3^{-2}. For calculation purposes, it is assumed that surface waters are represented by a carbonate ion system; hence, the ANC and BNC definitions are made in relation to the carbonate ion species (Kramer, 1982; Butler, 1982).

This method is applicable to ANC in the range of 10 to 150 μeq L^{-1}, BNC in the range of -100 to 1,000 μeq L^{-1} and pH in the range of 3-8 pH units.

5.1.2 Summary of Method

The pH is determined prior to the start of sample titration. The same electrode used during titration is used to measure initial pH (U.S. EPA, 1983; McQuaker et al., 1983; NBS, 1982). While pH is monitored and recorded, samples are titrated with standardized acid or base.

ANC and BNC are determined by analyzing the titration data using a modified Gran analysis technique (Kramer, 1982; Butler, 1982; Kramer, 1984; Gran, 1952). The Gran analysis technique defines the Gran functions F_1 and F_3, which are calculated from sample volume, acid (base) volume added, and constants. The Gran function is calculated for several titration data pairs (volume of titrant added, resulting pH) on either side of the equivalence point. When the Gran function is plotted versus volume of titrant added, a linear curve is obtained. The equivalent point can be interpolated from where the line crosses the volume axis.

The air-equilibrated pH is determined after equilibrating the sample with 300 ppm CO_2 in air. Air equilibration is expected to normalize pH values by factoring out the day-to-day and seasonal fluctuations in dissolved CO_2 concentrations.

5.1.3 Interferences

No interferences are known.

5.1.4 Safety

The standards, sample types, and most reagents pose no hazard to the analyst. Protective clothing (lab coat, gloves, and safety glasses) should be used when handling concentrated acids and bases.

Gas cylinders should be secured in an upright position.

5.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

The sample for which ANC, BNC, and pH are to be determined is raw sample (not filtered or chemically preserved) stored in a 500-mL amber polyethylene bottle. The bottle should be completely filled to eliminate headspace. Only deionized water-washed containers should be used to collect and store the sample. Store at 4 °C and minimize atmosphere exposure.

5.3 EQUIPMENT AND SUPPLIES

5.3.1 Equipment Specifications

1. pH/mV Meter--A digital pH/mV meter capable of measuring pH to ±0.01 pH unit, potential to ±1 mV, and temperature to ±0.5 °C is required. The meter should also have automatic temperature compensation capability.

2. pH Electrodes--High-quality, low-sodium glass pH and reference electrodes should be used. (Gel-type reference electrodes should not be used.) A combination electrode is recommended (such as the Orion Ross combination pH electrode or equivalent). This procedure is written assuming a combination electrode is used.

3. Buret--A microburet capable of precisely and accurately delivering 10 to 50 µL is required (relative error and standard deviation less than 0.5 percent).

5.3.2 Apparatus

1. Teflon-coated stir bars.
2. Variable-speed magnetic stirrer.
3. Plastic gas dispersion tube.

NOTE: A glass dispersion tube should not be used because it can add ANC to a sample. Plastic dispersion tubes are available in most aquarium supply stores.

4. Titration System--A commercial titration instrument may be used in place of the pH/mV meter, pH electrode, and buret if the instrument meets all required specifications.

5.3.3 Reagents and Consumable Materials

1. Carbon Dioxide Gas (300 ppm CO_2 in Air)--Certified standard grade or better.

2. Hydrochloric Acid Titrant (0.01N HCl)--Add 0.8 mL concentrated hydrochloric acid (HCl, 12N, ACS reagent grade or equivalent) to 500 mL water, then dilute to 1.00 L with water. Standardize as described in Section 5.4.1. Store in a clean polyethylene bottle. Although stable, it should be restandardized monthly.

3. Nitrogen Gas (N_2)--CO_2-free.

4. Potassium Chloride Solution (0.10M KCl)--Dissolve 75 g KCl (Alfa Ultrapure or equivalent) in water, then dilute to 1.00 L with water.

5. Potassium Hydrogen Phthalate (KHP)--Dry 5 to 10 g KHP (ACS-certified primary standard grade or equivalent) at 110 °C for 2 hours, then store in a desiccator.

6. pH Calibration Buffers (pH 4, 7, and 10)--NBS-traceable pH buffers at pH values of 4, 7, and 10.

7. pH 4 QC sample--Dilute 1.00 mL standardized 0.01N HCl titrant to 100.00 mL with water (prepare daily). The theoretical pH is calculated by:

$$pH = -\log\left(\frac{N_{HCl}}{100}\right)$$

8. pH 10 QC sample--Dilute 1.00 mL standardized 0.01N NaOH titrant to 100.00 mL with water (prepare daily). The theoretical pH is calculated by:

$$pH = -\log\left(\frac{N_{NaOH}}{100}\right)$$

9. Sodium Carbonate (Na_2CO_3)--Dry 5 to 10 g Na_2CO_3 (ACS-certified primary standard grade or equivalent) at 110 °C for 2 hours, then store in a desiccator.

10. Sodium Hydroxide Stock Solution (50 percent w/v NaOH)--Dissolve 100 g NaOH (ACS reagent grade or equivalent) in 100 mL water. After cooling solution and allowing precipitate to settle (may be hastened by centrifugation), transfer the supernatant to a linear polyethylene or Teflon container. Store bottle tightly capped and avoid exposing solution to the atmosphere.

11. Sodium Hydroxide Titrant (0.01N NaOH)--Dilute 0.6 to 0.7 mL 50 percent NaOH to 1.0 L with water. Standardize as described in Section 5.4.2. Store in linear polyethylene or Teflon container with a CO_2-free atmosphere (e.g., under CO_2-free air, nitrogen, or argon).

12. Water--At the point of use, water used to prepare reagents and standards should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).

5.4 PREPARATION

5.4.1 Standardization of HCl Titrant

1. Weigh about 1 g anhydrous Na_2CO_3 to the nearest 0.1 mg, dissolve in water, then dilute to 1.000 L. Calculate the concentration by:

$$N_{Na_2CO_3} = \frac{\text{wt. } Na_2CO_3}{\frac{106.00\text{ g}}{\text{mole}} \times \frac{1\text{ mole}}{2\text{ eq}}} \times \frac{1}{1\text{ L}}$$

NOTE: Fresh Na_2CO_3 solution is to be prepared just before use.

2. Calibrate the pH meter and electrode as recommended by the manufacturer.

3. Pipet 1.00 mL standard Na_2CO_3, 4.00 mL 1.0M KCl, plus 36.00 mL CO_2-free deionized water into a clean, dry titration vessel. Add a Teflon stir bar and stir at medium speed (no visible vortex).

4. Immerse the pH electrode and record the pH when a stable reading is obtained.

5. Add a known volume of the HCl titrant and record the pH when a stable reading is obtained. Use the following table as a guide to the volume of titrant that should be added for each pH range:

pH	Maximum Volume Increment of HCl Titrant (mL)
>7.5	0.2
4 to 7.5	0.1
<4	0.2

Continue the titration until the pH is less than 4. Obtain at least seven data points in the range pH 4 to 7.

6. Calculate F_{1b} for each data pair (volume acid added, pH) with pH in the range 4 to 7:

$$F_{1b} = (V_s + V)\left[\frac{V_s C}{(V_s + V)}\left(\frac{[H^+]K_1 + 2\,K_1\,K_2}{[H^+]^2 + [H^+]K_1 + K_1\,K_2}\right) + \frac{K_w}{[H^+]} - [H^+]\right]$$

where

F_{1b} = Gran function
V_s = initial sample volume (40.00 mL)
V = volume of HCl added (mL)
C = $N_{Na_2CO_3}$/(2 x dilution factor)
$[H^+]$ = 10^{-pH}
K_1 = 7.079×10^{-7} (25 °C, 0.1 ionic strength, Butler, 1982)
K_2 = 1.202×10^{-10} (25 °C, 0.1 ionic strength, Butler, 1982)
K_w = 1.660×10^{-14} (25 °C, 0.1 ionic strength, Butler, 1982)

7. Plot F_{1b} versus V. Using the points on the linear portion of the plot, perform a linear regression of F_{1b} on V to obtain the coefficients of the line $F_{1b} = a + bV$. The correlation coefficient should exceed 0.999. If it does not, reexamine the plot to make sure only points on the linear portion are used in the linear regression.

8. Calculate the equivalence volume, V_1, by:

$$V_1 = -a/b$$

Then calculate the HCl normality by:

$$N_{HCl} = \frac{N_{Na_2CO_3} \times V_{Na_2CO_3}}{V_1}$$

9. Perform steps 5 through 8 two more times. Calculate an average N_{HCl} and standard deviation. The percent relative standard deviation (%RSD) should be less than 2 percent. If it is not, the entire standardization should be repeated until the %RSD is less than 2 percent.

   The concentration of each new batch of HCl titrant should be crosschecked using the procedure described in Section 5.4.3.

NOTE: An example of an HCl standardization is given in Appendix E, Section 1.0.

5.4.2 Initial Standardization of NaOH Titrant with KHP

Every batch of NaOH titrant is initially standardized against KHP (see below) and the standardization is crosschecked against standardized HCl titrant (Section 5.4.3). Thereafter, it is restandardized daily against the HCl titrant (Section 5.4.4).

1. Weigh about 0.2 g KHP to the nearest 0.1 mg, dissolve in water, then dilute to 1.000 L. Calculate the normality of the solution by:

$$N_{KHP} = \frac{\text{wt. KHP g}}{\frac{204.22\ \text{g}}{\text{eq}}} \times \frac{1}{1\ \text{L}}$$

2. Calibrate the pH electrode and meter as recommended by the manufacturer.

3. Purge the titration vessel with CO_2-free nitrogen, then pipet 5.00 mL standard KHP solution, 2.00 mL 1.0M KCl, and 18.00 mL CO_2-free water into the vessel. Maintain a CO_2-free atmosphere above the sample throughout the titration. Add a Teflon stir bar and stir at medium speed (no visible vortex).

4. Immerse the pH electrode and record the reading when it stabilizes.

5. Titrate with the 0.01N NaOH using the increments specified in the table below. Record the volume and pH (when stable) between additions. Continue the titration until the pH is greater than 10. Obtain at least four data points in the pH range 5 to 7 and four data points in the pH range 7 to 10.

pH	Maximum Volume Increment of NaOH Titrant (mL)
<5	0.10
5 to 9	0.05
>9	0.2

6. Calculate F_{3b} for each data pair (volume acid added, pH) that has a pH greater than 9 by:

$$F_{3b} = (V_s + V)\left[\frac{V_s C}{(V_s + V)}\left(\frac{[H^+]K_1 + 2\,[H^+]^2}{[H^+]^2 + [H^+]K_1 + K_1\,K_2}\right) + [H+] - \frac{K_w}{[H^+]}\right]$$

where

F_{3b} = Gran function
V_s = initial sample volume (20.00 mL)
V = volume NaOH added (mL)
C = N_{KHP} corrected for initial dilution = $N_{KHP}/5$
$[H^+]$ = 10^{-pH}
K_1 = 1.3×10^{-3}
K_2 = 3.9×10^{-6}
K_w 1.660×10^{-14}

7. Plot F_{3b} versus V. Using the points on the linear portion of the plot, perform a linear regression of F_{3b} on V to obtain the coefficients of the line $F_{3b} = a + bV$. The correlation coefficient should exceed 0.999. If it does not, examine the plot to ensure that only points on the linear portion are used in the linear regression.

8. Calculate the equivalence volume, V_3, by:

$$V_3 = -a/b$$

Then calculate the NaOH normality by:

$$N_{NaOH} = \frac{N_{KHP} \times V_{KHP}}{V_3}$$

9. Perform steps 5 through 8 two more times. Calculate an average N_{NaOH} and standard deviation. The %RSD should be less than 2 percent. If it is not, the entire standardization should be repeated until the %RSD is less than 2 percent.

NOTE: An example of an NaOH standardization is given in Appendix E, Section 2.0.

5.4.3 NaOH-HCl Standardization Crosscheck

1. Purge a titration vessel with CO_2-free nitrogen, then pipet 0.500 mL 0.01N NaOH, 2.50 mL 1.0M KCl, and 22.00 mL CO_2-free water into the vessel. Maintain a CO_2-free atmosphere above the sample. Add a Teflon stir bar and stir at medium speed.

2. Immerse the pH electrode and record the reading when it stabilizes.

3. Titrate with the standardized 0.01N HCl using the increments specified in the table below. Record the volume and pH (when stable) between additions. Continue the titration until the pH is less than 3.5. Obtain at least seven data points in the pH range 4 to 10.

pH	Maximum Volume Increment of HCl Titrant (mL)
>10	0.2
4 to 10	0.05
<4	0.2

4. Calculate F_1 for each data pair (V, pH) that has a pH in the range 4 to 10 by:

$$F_1 = (V_s + V)\left(\frac{K_w}{[H^+]} - [H^+]\right)$$

where

F_1 = Gran function
V_s = initial sample volume (25.5 mL)
V = volume of HCl added (mL)
K_w = 1.660×10^{-14}
$[H^+]$ = 10^{-pH}

5. Plot F_1 versus V. Using the points on the linear portion of the plot, perform a linear regression of F_1 on V to obtain the coefficients of the line $F_1 = a + bV$. The correlation coefficient should exceed 0.999. If it does not, reexamine the plot to ensure that only points on the linear portion are used in the linear regression.

6. Calculate the equivalence volume, V_1, by:

$$V_1 = -a/b$$

Then calculate the HCl normality (designated as N'_{HCl}) by:

$$N'_{HCl} = \frac{N_{NaOH} \times V_{NaOH}}{V_1}$$

where

$$V_{NaOH} = 0.500$$

7. Calculate the absolute relative percent difference (RPD) between N'_{HCl} and N_{HCl} by:

$$RPD = \left| \frac{N'_{HCl} - N_{HCl}}{0.5\ (N'_{HCl} + N_{HCl})} \right| \times 100$$

The absolute RPD should be less than 5 percent. If it is not, a problem exists in the acid or the base standardization or both (bad reagents, out-of-calibration burets). The problem should be identified and both procedures (standardization of HCl titrant and standardization of NaOH titrant) should be repeated until the calculated RPD is less than 5 percent.

NOTE: An example of an NaOH-HCl standardization crosscheck is given in Appendix E, Section 3.0.

5.4.4 <u>Daily NaOH Standardization with Standardized HCl</u>

1. Calibrate the pH meter and electrode as recommended by the manufacturer.

2. Purge the titration vessel with CO_2-free nitrogen, then pipet 0.500 mL NaOH titrant, 2.50 mL 1.0M KCl, and 22.00 mL CO_2-free deionized water into the vessel. Maintain a CO_2-free nitrogen atmosphere above the sample. (Smaller or larger volumes of NaOH may be used. A known volume of CO_2-free water should be added to bring solution to 25.00 mL). Add a Teflon stir bar and stir at medium speed.

3. Immerse the pH electrode and record the reading when it stabilizes.

4. Titrate with the standardized HCl titrant using the increments specified in the table below. Record the volume and pH between additions. Continue the titration until the pH is less than 4. Obtain at least seven data points in the pH range 4 to 10.

pH	Maximum Volume Increment of HCl Titrant (mL)
>10	0.2
4 to 10	0.05
<4	0.2

5. Calculate F_1 for each data pair (volume acid added, pH) in the pH range 4 to 10 by:

$$F_1 = (V_s + V)\left(\frac{K_w}{[H^+]} - [H^+]\right)$$

where

F_1 = Gran function
V_s = initial sample volume (25.00 mL)
V = volume of HCl added (mL)
K_w = 1.660×10^{-14}
$[H^+]$ = 10^{-pH}

6. Plot F_1 versus V. Using the points on the linear portion of the plot, perform a linear regression of F_1 on V to obtain the coefficients of the line $F_1 = a + bV$. The correlation coefficient should exceed 0.999. If it does not, reexamine the plot to make sure that only points on the linear portion are used in the linear regression.

7. Calculate the equivalence volume, V_1, by:

$$V_1 = -a/b$$

Then calculate the NaOH normality by:

$$N_{NaOH} = \frac{N_{HCl} \times V_1}{V_{NaOH}}$$

8. Perform steps 4 through 7 two more times. Calculate an average N_{NaOH} and standard deviation. The %RSD should be less than 2 percent. If it is not, the entire standardization should be repeated until the RSD is less than 2 percent.

Because the NaOH titrant can readily deteriorate through exposure to the air, every effort should be made to prevent its exposure to the air at all times. Furthermore, it should be standardized daily or before every major work shift. Store in a linear polyethylene or Teflon container with a CO_2-free atmosphere (e.g., under CO_2-free air, nitrogen, or argon).

NOTE: An example of daily NaOH standardization is given in Appendix E, Section 4.0.

5.4.5 Rigorous Calibration and Characterization of Electrodes

Separate electrodes should be used for the acid and base titration. Each new electrode pair should be rigorously evaluated for Nernstian response, using the rigorous calibration procedure described below, prior to analyzing samples. Also, it familiarizes the analyst with the electrode's characteristic response time. After the initial electrode evaluation, the electrodes are calibrated daily using the procedure in the daily calibration procedure described in Section 5.4.6.

1. Following the manufacturer's instructions, calibrate the electrode and meter used for acid titrations with pH 7 and 4 buffer solutions, and calibrate the electrode used for base titrations with pH 7 and 10 buffer solutions.

2. Prepare a blank solution by pipetting 45.00 mL CO_2-free water and 5.0 mL 1.0M KCl into a titration vessel. Add a Teflon stir bar and stir at medium speed using a magnetic stirrer.

3. Titrate the blank with standardized 0.01N HCl using the increments specified below. Continue the titration until the pH is in the range 3.3 to 3.5. Record the pH between each addition, noting the time required for stabilization. Obtain at least seven data points that have a pH less than 4.

pH	Maximum Volume Increment of HCl Titrant (mL)
>4	0.050
<4	0.3

4. Prepare a fresh aliquot of water and 1.0M KCl as in step 2.

5. Under a CO_2-free atmosphere, titrate the blank with standardized 0.01N NaOH using the increments specified below:

pH	Maximum Volume Increment of NaOH Titrant (mL)
<10	0.10
>10	0.20

6. Continue the titration until the pH is in the range 10.5 to 11. Record the pH between each addition. Obtain at least 10 data points between pH 9 and 10.5.

7. For each titration, calculate the pH for each data point by:

$$pH = -\log [H^+]$$

where, for acid titration:

$$[H^+] = \frac{V_A \; C_A}{V_s + V_A}$$

and for base titration:

$$[H^+] = \frac{K_w}{\left(\frac{V_B \; C_B}{V_s + V_B}\right)}$$

and where

V_A = acid volume (mL)
C_A = HCl concentration (eq L^{-1})
V_s = sample volume (50.0 mL)
K_w = 1.660×10^{-14}
V_B = base volume (mL)
C_B = NaOH concentration (eq L^{-1})

8. For each titration, plot the measured pH versus the calculated pH (designated as pH*). Perform a linear regression on each plot to obtain the coefficients of the line pH = a + b(pH*). The plots should be linear with b = 1.00 ± 0.05 and r > 0.999. Typically, some

nonlinearity exists in the pH region 6 to 8. This is most likely due to small errors in titrant standardization, impure salt solutions, or atmospheric CO_2 contamination. The nonlinear points should not be used in the linear regression.

If the plots are not linear and do not meet the specifications above, the electrode should be considered suspect. The electrode characterization should then be repeated. If results are still unacceptable, the electrode should be replaced.

9. Combine the data from both titrations and perform a linear least-squares analysis on the combined data to obtain new estimates for the coefficients of pH = a + b(pH*). The electrodes are now calibrated. Do not move any controls on the meter.

   The plots for both titrations should be coincident. If the two plots are not coincident (i.e., the coefficients a and b do not overlap), the characterization should be repeated. If the plots are still not coincident, the electrode should be replaced.

5.4.6 Daily Calibration and Characterization of Electrodes

Generally, the calibration curve prepared during the rigorous calibration procedure is stable from day to day. This daily calibration is designed to verify the calibration on a day-to-day basis.

1. Copiously rinse the electrode with water. Immerse the electrode in 20 mL pH 7 buffer and stir for 1 to 2 minutes. Discard the buffer and replace with 40 mL pH 7 buffer. While the solution is stirred gently, measure the pH. Adjust the pH meter calibration knob until the pH is equal to the theoretical pH of the buffer. Record the theoretical pH and the final, measured pH reading. The two values should be identical.

2. Copiously rinse the electrode with water. Immerse it in 20-mL pH 4 QC sample and stir for 1 to 2 minutes. Discard the sample and replace with 40-mL pH 4 QC sample. While the solution is stirred, measure and record the pH. From the calibration curve of pH versus pH*, determine the pH* for the observed pH. Compare pH* to the theoretical pH of the QC sample. The two values should agree within ±0.05 pH unit. If the two values do not agree, the rigorous calibration procedure should be performed (Section 5.4.5) prior to sample analysis.

3. Repeat step 2 with the pH 10 QC sample. This sample should be kept under a CO_2-free atmosphere when in use, or acceptable results may not be obtained.

The electrode calibration procedure is described in detail in Section 5.0 of Appendix E.

5.5 PROCEDURE

An acid titration (Section 5.5.1) and a base titration (Section 5.5.2) are necessary to determine the BNC and ANC of a sample. As part of each titration, the sample pH is determined. The air-equilibrated pH is determined in a separate sample portion (Section 5.5.3).

5.5.1 Acid Titration

1. Allow a sealed sample to reach ambient temperature.

2. Copiously rinse the electrode with deionized water, then immerse in 10 to 20 mL of sample. Stir for 30 to 60 seconds.

3. Pipet 36.00 mL of sample into a clean, dry titration flask. Add a clean Teflon stir bar and place flask on a magnetic stirrer. Stir at medium speed (no visible vortex).

4. Immerse the pH electrode and read pH. Record pH on forms similar to Forms 11 and 13 (Appendix D) when the reading stabilizes (1 to 2 minutes). This is the initial measured pH at $V_{titrant} = 0$.

5. Add 4.00 mL 1.0M KCl. Read pH and record the value on Form 13. This is the initial measured pH at $V_{titrant} = 0$ after addition of KCl spike.

6. Add increments of 0.01N HCl as specified in the table below. Record the volume of HCl added, and record the pH when a stable reading is obtained. Adjust the volume increment of titrant so that readings can be taken at pH values of 4.5 and 4.2. Continue the titration until the pH is between 3.3 and 3.5. Obtain at least six data points between a pH of 4.5 and 5.5 and at least six that have a pH less than 4.

pH	Maximum Volume Increment of HCl Titrant (mL)
>9	0.1
7.0 to 9.0	0.025
5.5 to 7.0	0.1
4.5 to 5.5	0.05
4.50 to 3.75	0.1
<3.75	0.3

5.5.2 Base Titration

1. Take a portion of the sample for dissolved inorganic carbon (DIC) determination. If the DIC is not determined immediately, the sample should be kept sealed from the atmosphere and stored at 4 °C. A simple way to do this is to withdraw the sample for DIC using a syringe equipped with a syringe valve. By closing the valve, the sample is sealed from the atmosphere (syringe valves that fit standard Luer-Lok syringes are available from most chromatography supply companies).

2. Purge the titration vessel with CO_2-free air, nitrogen, or argon.

3. Copiously rinse the electrode with deionized water, then immerse it in 10 to 20 mL of sample for 30 to 60 seconds.

4. Pipet 36.00 mL of sample into the CO_2-free titration vessel. Maintain a CO_2-free atmosphere above the sample. Do not bubble the nitrogen (or other CO_2-free gas) through the sample. Add a clean Teflon stir bar and place on a magnetic stirrer. Stir at medium speed (no visible vortex).

5. Immerse the pH electrode, read pH, and record pH on forms similar to Forms 11 and 13 (Appendix D) when the pH stabilizes. This is the initial measured pH at $V_{titrant} = 0$.

6. Add 4.0 mL 1.0M KCl. Read pH, and record pH on Form 13.

7. Add 0.025 mL of 0.01N NaOH. Record the NaOH volume and pH when it stabilizes. Continue the titration by adding increments of NaOH as specified below until the pH is greater than 11. Record the volume of NaOH added and the pH after each addition. Obtain at least 6 data points in the pH region 7 to 9 and at least 6 with a pH greater than 10. If the initial sample pH is less than 7, obtain at least five data points below pH 8.

pH	Maximum Volume Increment of NaOH Titrant (mL)
<5	0.025
5 to 7	0.050
7 to 9	0.025
9 to 10	0.10
10 to 10.5	0.30
>10.5	1.00

5.5.3 Air-Equilibrated pH Measurement

1. Allow the sealed sample to reach ambient temperature.

2. Copiously rinse the electrode with deionized water, then immerse in 10 to 20 mL of sample. Stir for 30 to 60 seconds.

3. Pipet 20 to 40 mL of sample into a clean, dry titration flask. Add a clean Teflon stir bar and place flask on a magnetic stirrer. Stir at a medium speed.

4. Bubble standard gas containing 300 ppm CO_2 through the sample for 20 minutes. Raise gas tube above the liquid surface to maintain atmosphere above sample. Measure and record the pH.

5. While maintaining 300 ppm CO_2 atmosphere, take a subsample for DIC determination. The subsample should be kept sealed from the atmosphere prior to analysis. The DIC should be measured as soon as possible (see Section 14.5.2).

5.5.4 Calculations

During the titrations, any substance which reacts with the acid or base is titrated. However, for calculations, it is assumed that the samples represent carbonate systems and that the only reacting species are H^+, OH^-, H_2CO_3, HCO_3^-, and CO_3^{-2}. Using this assumption, the two parameters "ANC" (ANC) and "CO_2-BNC" (BNC) are calculated. The validity of the assumption is checked as described in previous sections.

The theory behind the calculations is available elsewhere (Kramer, 1982; Butler, 1982; Kramer, 1984). Examples of the calculations are given in Appendix E.

1. Initial Calculations--

   a. From the calibration curve of measured pH versus calculated pH (pH*), determine pH* for each pH value obtained during both the acid and base titrations. Next, convert all pH* values to hydrogen ion concentrations by:

$$[H^+] = 10^{-pH^*}$$

   b. Using the acid titration data, calculate the Gran function F_{1a} for each data pair (V_a, pH*) in which pH* is less than 4 by:

$$F_{1a} = (V_s + V_a)\,[H^+]$$

where

V_s = total initial sample volume (36.00 + 4.00) mL
V_a = cumulative volume of acid titrant added

c. Plot F_{1a} versus V_a. The data should be on a straight line with the equation $F_{1a} = a + bV$.

d. Perform a linear regression of F_{1a} on V_a to determine the correlation coefficient (r) and the coefficients a and b. The coefficient r should exceed 0.999. If it does not, examine the data to ensure that only data on the linear portion of the plot were used in the regression. If any outliers are detected, repeat the regression analysis. Calculate an initial estimate of the equivalence volume (V_1) by:

$$V_1 = -a/b$$

Further calculations are based on this initial estimate of V_1 and the initial sample pH*. Table 5-1 lists the appropriate calculation procedure for the different combinations of V_1 and initial sample pH*. These calculation procedures are given in steps 2 through 4.

NOTE: For blank analyses, calculate ANC by:

$$ANC = V_1 \left(\frac{Ca}{V_{sa}} \right)$$

where

C_a = concentration of acid titrant
V_{sa} = original sample volume (acid titration)

Further calculations are not necessary.

Throughout the calculations, Equations 5-1 and 5-2 (given in step 2), as well as the constants listed in Table 5-2, are used frequently.

2. Calculation Procedure A (Initial V_1 <0)--

a. From the base titration data, determine which data set (V, pH*) has the pH* nearest (but not exceeding) pH_{e2} (calculate using Equation 5-4). As an initial estimate, set the equivalence volume (V_2) equal to the volume recorded for this data set.

TABLE 5-1. CALCULATION PROCEDURES FOR COMBINATIONS OF INITIAL V_1 AND pH*

Sample Description		Calculation Procedure	Step Number
Initial V_1	Initial pH*[a]		
<0		A	2
>0	$\leq pH_{e2}$	B	3
>0	$> pH_{e2}$	C	4

[a] pH_{e2} is calculated using Equation 5-4.

$$F_{1c} = (V_s + V)\left[\frac{C([H^+]K_1 + 2\,K_1K_2)}{[H^+]^2 + [H^+]K_1 + K_1K_2} + \frac{K_w}{[H^+]} - [H^+]\right] \quad (5\text{-}1)$$

$$F_{2c} = (V_s + V)\left[\frac{C([H^+]^2 - K_1K_2)}{[H^+]^2 + [H^+]K_1 + K_1K_2} + [H^+] - \frac{K_w}{[H^+]}\right] \quad (5\text{-}2)$$

$$pH_{e1} = -\log(H_{e1}) \quad (5\text{-}3)$$

$$H_{e1} = \left[\frac{(DIC)K_1}{12{,}011}\right]^{1/2} - \frac{K_1}{2}$$

$$pH_{e2} = -\log(H_{e2}) \quad (5\text{-}4)$$

$$H_{e2} = \left[K_1K_w\left(1 + \frac{12{,}011}{DIC}\right)\right]^{1/2} \quad (5\text{-}5)$$

TABLE 5-2. CONSTANTS AND VARIABLE DESCRIPTIONS

V_s	=	total initial sample volume
V	=	cumulative volume of titrant added
C	=	total carbonate expressed in moles L^{-1}
$[H^+]$	=	hydrogen ion concentration
K_1	=	7.079×10^{-7} at 25 °C and 0.1M ionic strength (Butler, 1982)
K_2	=	1.202×10^{-10} at 25 °C and 0.1M ionic strength
K_w	=	1.660×10^{-14} at 25 °C and 0.1M ionic strength

b. Calculate initial estimates of ANC, BNC, and C by:

$$ANC = \frac{V_1 C_a}{V_{sa}}$$

$$BNC = \frac{V_2 C_b}{V_{sb}}$$

$$C = ANC + BNC$$

where

C_a = concentration of acid titrant
V_{sa} = original sample volume (acid titration)
C_b = concentration of base titrant
V_{sb} = original sample volume (base titration)

c. Estimate the equivalence point pH_{e1} using Equation 5-3. Calculate the Gran function F_{1c} for seven to eight points of the base titration with pH* spanning pH_{e1} using Equation 5-1. Plot F_{1c} versus V_b. Perform a linear regression with the points lying on the linear portion of the plot. Determine the coefficients of the line $F_{1c} = a + bV$. The coefficient r should exceed 0.999.

If it does not, examine the plot to assure that only points on the linear portion are used. From the coefficients, calculate a new estimate of V_1 by:

$$V_1 = -a/b$$

d. Calculate the Gran function F_{2c} (Equation 5-2) for data from the base titration across the current estimate of V_2. (Use the first four to six sets that have a volume less than V_2 and the first six to eight sets greater than V_2.) Plot F_{2c} versus V_b. The data should lie on a straight line with the equation $F_{2c} = a + bV$. Perform a linear regression of F_{2c} on V_b and determine the coefficients of the line. If r does not exceed 0.999, reexamine the data to assure that only points on the linear portion were used in the regression. Calculate a new estimate of V_2 by:

$$V_2 = -a/b$$

e. Calculate new estimates of ANC, BNC, and C using the new estimates of V_1 and V_2 (an asterisk indicates a new value) by:

$$ANC^* = \frac{-V_1 C_b}{V_{sb}}$$

$$BNC^* = \frac{V_2 C_b}{V_{sb}}$$

$$C^* = ANC + BNC$$

If $C^* < 0$, then set $C^* = 0$.

f. Compare the latest two values for total carbonate. If:

$$\left|\frac{C - C^*}{C + C^*}\right| > 0.001$$

then calculate a new estimate for C by:

$$C(new) = (C + C^*)/2$$

g. Using the new value for C, repeat the calculations as above. Continue repeating the calculations until the relative difference between C and C* is less than 0.001.

h. When the expression is less than 0.001, convert the final values for ANC, BNC, and C to μeq L^{-1} by:

$$ANC\ (\mu eq\ L^{-1}) = ANC\ (eq\ L^{-1}) \times 10^6$$

$$BNC\ (\mu eq\ L^{-1}) = BNC\ (eq\ L^{-1}) \times 10^6$$

$$C\ (\mu eq\ L^{-1}) = C\ (eq\ L^{-1}) \times 10^6$$

3. Calculation Procedure B (Initial $V_1 > 0$, Initial pH* $\leq pH_{e2}$)--

a. From the base titration data, determine which data set (V, pH*) has the pH* nearest, but not exceeding, pH_{e2} (calculate using Equation 5-4). As an initial estimate, set the equivalence volume V_2 equal to the volume recorded for this data set. Next calculate initial estimates of ANC, BNC, and C by:

$$ANC = \frac{V_1 C_a}{V_{sa}}$$

$$BNC = \frac{V_2 C_b}{V_{sb}}$$

$$C = ANC + BNC$$

b. Calculate the Gran function F_{1c} (Equation 5-1) for data sets from the acid titration with volumes across the current estimate of V_1 (use the first four to six sets with volumes less than V_1 and the first six to eight sets with volumes greater than V_1). Plot F_{1c} versus V_a. The data should lie on a straight line with the equation $F_{1c} = a + bV$. Perform a linear regression of F_{1c} on V_a and determine the coefficients of the line. If r does not exceed 0.999, reexamine the data to assure that no outliers were used in the regression. Calculate a new estimate for V_1 by:

$$V_1 = -a/b$$

c. Calculate the Gran function F_{2c} (Equation 5-2) for data sets from the base titration with volumes across the current estimate of V_2. (Use the first four to six sets with volumes less than V_2 and the first six to eight sets with volumes greater than V_2). Plot F_{2c} versus V_b. The data should lie on a straight line with the equation:

$$F_{2c} = a + bV.$$

Perform a linear regression of F_{2c} on V_b and determine the coefficients of the line. If r does not exceed 0.999, reexamine the data to assure that only data on the linear portion were included in the regression. Calculate a new estimate for V_2 by:

$$V_2 = -a/b$$

d. Calculate new estimates of ANC, BNC, and C using the latest estimates of V_1 and V_2 by:

$$ANC^* = \frac{V_1 C_a}{V_{sa}}$$

$$BNC^* = \frac{V_2 C_b}{V_{sb}}$$

$$C^* = ANC + BNC$$

e. Compare the latest two values for total carbonate. If:

$$\left| \frac{C - C^*}{C + C^*} \right| > 0.001$$

then calculate a new estimate of C by:

$$C(new) = (C + C^*)/2$$

f. Using the new value of C, repeat the calculations as above. Continue repeating the calculations until the above expression is less than 0.001.

g. When the expression is less than 0.001, convert the final values for ANC, BNC, and C to $\mu eq\ L^{-1}$ by:

$$ANC\ (\mu eq\ L^{-1}) = ANC\ (eq\ L^{-1}) \times 10^6$$

$$BNC\ (\mu eq\ L^{-1}) = BNC\ (eq\ L^{-1}) \times 10^6$$

$$C\ (\mu eq\ L^{-1}) = C\ (eq\ L^{-1}) \times 10^6$$

4. Calculation Procedure C (Initial $V_1 > 0$, Initial pH* > pH_e2)--

   a. Using data sets from the acid titration with pH* values above and below pH 7 (use four to six sets with a pH* ≤ 7 and four to six sets with a pH* ≥ 7), calculate the Gran function F_{2a} by:

$$F_{2a} = (V_1 - V_a)H$$

   b. Plot F_{2a} versus V_a. The data should lie on a straight line with the equation $F_{2a} = a + bV$. Perform a linear regression of F_{2a} on V_a. The coefficient r should exceed 0.999. If it does not, reexamine the plot to assure that only data on the linear portion were used in the calculation. Calculate an estimate for V_2 by:

$$V_2 = -a/b$$

   c. Calculate estimates of ANC, BNC, and C by:

$$ANC = \frac{V_1 C_a}{V_{sa}}$$

$$BNC = \frac{-V_2 C_a}{V_{sa}}$$

$$C = ANC + BNC$$

   d. Calculate the Gran function F_{1c} (Equation 5-1) for data sets from the acid titration with volumes across the current estimate of V_1 (use the first four to six sets with volume less than V_1 and the first six to eight sets with volumes greater than V_1). Plot F_{1c} versus V_a. The data should lie on a straight line with the equation $F_{1c} = a + bV$. Perform a linear regression of F_{1c} on V_a and determine the coefficients of the line. The coefficient r should exceed 0.999. If it does not, reexamine the plot to assure that only data on the linear portion were included in the regression. Calculate a new estimate for V_1 by:

$$V_1 = -a/b.$$

   e. Calculate the Gran function F_{2c} (Equation 5-2) for data sets from the acid titration with volumes across the current estimate of V_2 (use the first four to six sets with volumes less than V_2 and the first six to eight sets with volumes greater than V_2). Plot F_{2c} versus V_a. The data should lie on a straight line with the equation $F_{2c} = a + bV$. Perform a linear regression of F_{2c} on V_a and determine the coefficients of the line. The coefficient r should

exceed 0.999. If it does not, reexamine the plot to assure that only data on the linear portion were included in the regression. Calculate a new estimate of V_2 by:

$$V_2 = -a/b.$$

If $V_2 < 0$, use calculation procedure B (step 3).

f. Calculate new estimates of ANC, BNC, and C using the latest estimates of V_1 and V_2:

$$ANC^* = \frac{V_1 C_a}{V_{sa}}$$

$$BNC^* = \frac{-V_2 C_a}{V_{sa}}$$

$$C^* = ANC + BNC$$

g. Compare the latest two values for total carbonate. If:

$$\left| \frac{C - C^*}{C + C^*} \right| > 0.001$$

then calculate a new estimate of C by:

$$C(new) = (C + C^*)/2$$

h. Using this new value of C, repeat the calculations in step 4, b through d. Continue repeating the calculations until the above expression is less than 0.001.

i. When the expression is less than 0.001, convert the final values for ANC, BNC, and C to $\mu eq\ L^{-1}$ by:

$$ANC\ (\mu eq\ L^{-1}) = ANC\ (eq\ L^{-1}) \times 10^6$$

$$BNC\ (\mu eq\ L^{-1}) = BNC\ (eq\ L^{-1}) \times 10^6$$

$$C\ (\mu eq\ L^{-1}) = C\ (eq\ L^{-1}) \times 10^6$$

$$C^* = ANC + BNC$$

5.6 QUALITY ASSURANCE AND QUALITY CONTROL

5.6.1 Comparison of Initial Titration pH Values

The values for measured pH at $V_{titrant} = 0$ (before KCl spike) of the acid and base titrations should be within ±0.1 pH unit. If they are not, check operation to ensure that cross-contamination is not occurring.

For a sample with ANC $\leq$ -15 μeq L^{-1}, calculate a value for ANC as follows:

$$[ANC]_{CO} = 10^6 \times 10^{-pH*} \text{ (pH at V=0)}$$

(The pH at $V_{titrant} = 0$ is taken from the acid titration.) If ANC differs from $[ANC]_{CO}$ by more than 10 μeq L^{-1}, check the electrode operation and calibration.

5.6.2 Comparison of Calculated ANC and Measured ANC

A value for ANC can be calculated from a sample's DIC concentration and pH. Two sets of pH and DIC values are obtained in the lab: (1) pH* at V=0 of the base titration and the associated DIC concentration, and (2) pH of the air-equilibrated sample and the associated DIC concentration. Each set can be used to calculate a value for ANC. Since ANC is a conservative parameter (i.e., constant with changing dissolved CO_2 concentrations), the two values should be equal. The calculated values for ANC can also be compared to the measured value of ANC. The comparisons are useful in checking both the validity of assuming a carbonate system and the possibility of analytical error. ANC is calculated from pH and DIC as follows:

$[ANC]_{C1}$ = calculated ANC from initial pH and DIC at time of base titration

$[ANC]_{C2}$ = calculated ANC from air-equilibrated pH and DIC

$$[ANC]_C \text{ (μeq } L^{-1}) = \left[\frac{DIC}{12{,}011} \left(\frac{[H^+]K_1 + 2\,K_1K_2}{[H^+]^2 + [H^+]K_1 + K_1K_2} \right) + \frac{K_w}{[H^+]} - [H^+] \right] \times 10^6$$

where

DIC = DIC in mg L^{-1} (the factor 12,011 converts mg L^{-1} to moles L^{-1})
$[H^+]$ = 10^{-pH}
K_1 = 7.0795×10^{-7} at 25 °C, 0.1M ionic strength
K_2 = 1.2023×10^{-10} at 25 °C, 0.1M ionic strength
K_w = 1.6596×10^{-14} at 25 °C, 0.1M ionic strength

$[ANC]_{C1}$ and $[ANC]_{C2}$ are compared as follows:

For $[ANC]_{C1} \leq 100\ \mu eq\ L^{-1}$, the following condition applies:

$$\left| [ANC]_{C1} - [ANC]_{C2} \right| \leq 15\ \mu eq\ L^{-1}$$

For $[ANC]_{C1} > 100\ \mu eq\ L^{-1}$, the following condition applies:

$$\left| \frac{[ANC]_{C1} - [ANC]_{C2}}{([ANC]_{C1} + [ANC]_{C2})/2} \times 100 \right| \leq 10\%$$

If either condition is violated, a problem is indicated in either the pH and/or the DIC determination. In such cases, the problem should be found, corrected, and the samples reanalyzed.

It is very important that the pH and DIC be measured as closely together in time as possible. If they are not measured closely in time, acceptable agreement between $[ANC]_{C1}$ and $[ANC]_{C2}$ may not be obtained.

When acceptable values for $[ANC]_{C1}$ and $[ANC]_{C2}$ are obtained, their average is compared to the measured ANC as described below. For $[ANC]_{C\text{-}avg} \leq 100\ \mu eq\ L^{-1}$ the difference "D" and the acceptance window "w" are:

$$D = [ANC]_{C\text{-}avg} - ANC \quad \text{and,}$$

$$w = 15\ \mu eq\ L^{-1}$$

For $[ANC]_{C\text{-}avg} > 100\ \mu eq\ L^{-1}$:

$$D = \frac{[ANC]_{C\text{-}avg} - ANC}{[ANC]_{C\text{-}avg}} \times 100 \quad \text{and,}$$

$$w = 10\%$$

If $|D| \leq w$, it is valid to assume a carbonate system. If $D < -w$, then the assumption of a pure carbonate system is not valid and the sample contains noncarbonate protolytes (soluble reacting species), such as organic species. If $D > w$, an analytical problem exists in the pH determination, DIC determination, Gran analysis calculation, or acid titration (such as titrant concentration). In this case, the problem should be identified and the sample should be reanalyzed.

5.6.3 Comparison of Calculated BNC and Measured BNC

Just as for ANC, pH and DIC values can be used to calculate a BNC value. Because the BNC of a sample changes with changing dissolved CO_2 concentration, only the initial pH and DIC values measured at the beginning of the base titration are used to calculate a BNC value. This calculated BNC is then compared to the measured BNC value. BNC is calculated by:

$$[BNC]_C \ (\mu eq\ L^{-1}) = \left[\frac{DIC}{12{,}011} \left(\frac{[H^+]^2 - K_1K_2}{[H^+]^2 + [H^+]K_1 + K_1K_2} \right) + [H^+] - \frac{K_w}{[H^+]} \right] \times 10^6$$

$[BNC]_C$ is compared to BNC as described below.

For $[BNC]_C \leq 100\ \mu eq\ L^{-1}$:

$$D = [BNC]_C - BNC \quad \text{and,}$$
$$w = 10\ \mu eq\ L^{-1}$$

For $[BNC]_C > 100\ \mu eq\ L^{-1}$:

$$D = \frac{[BNC]_C - BNC}{[BNC]_C} \times 100 \quad \text{and,}$$
$$w = 10\%$$

If $|D| \leq w$, then it is valid to assume a carbonate system. If $D < -w$, the assumption of a pure carbonate system is not valid, and the sample contains noncarbonate protolytes, such as organic species. If $D > w$, an analytical problem exists in the pH determination, DIC determination, Gran analysis calculation, or base titration (such as titrant concentration). In this case, the problem should be identified and the sample should be reanalyzed.

5.6.4 Comparison of Calculated Total Carbonate and Measured Total Carbonate

If the assumption of a carbonate system is valid, the sum of ANC plus BNC is equal to the total carbonate. This assumption can be checked by calculating the total carbonate from the sum of $[ANC]_C$ and $[BNC]_C$, then comparing the calculated total carbonate to the measured estimate of total carbonate (the sum of ANC plus BNC). The total carbonate is calculated by:

$$C_C \ (\mu mole\ L^{-1}) = [ANC]_{C\text{-}avg} + [BNC]_C$$

C_C is compared to (ANC + BNC) as follows:

For $C_C \leq 100$ µmole L^{-1}:

$$D = C_C - (ANC + BNC) \text{ and } w = 10 \text{ µmole } L^{-1}$$

For $C_C > 100$ µmole L^{-1}:

$$D = \frac{C_C - (ANC + BNC)}{C_C} \times 100 \text{ and,}$$

$$w = 10\%$$

If $|D| \leq w$, the assumption of a carbonate system is valid. If $D < -w$, the assumption is not valid and the sample contains noncarbonate protolytes. If $D > w$, an analytical problem exists. It should be identified and the sample should be reanalyzed.

5.6.5 Quality Control Checks

1. Duplicate Analysis--Analyze one sample per batch in duplicate. The duplicate precision (expressed as %RSD for ANC and BNC and standard deviation (SD) for pH) should be less than or equal to 10 percent for ANC and BNC and 0.05 for pH. If the duplicate precision is unacceptable (%RSD >10 percent, SD >0.05), then a problem exists in the experimental technique. Determine and eliminate the cause of the poor precision prior to continuing sample analysis.

2. Blank Analysis--Determine the ANC in one blank per batch. The absolute value of the ANC should be less than or equal to 10 µeq L^{-1}. If it is not, contamination is indicated. Determine and eliminate the contamination source (often the source will be the water or the KCl) prior to continuing sample analysis. A detailed procedure for the determination of ANC in a blank solution is presented in Appendix E, Section 6.0.

3. pH QC check--Prior to analysis of the first sample in a shift and every five samples thereafter, or at intervals recommended by the QA program, the appropriate pH QC sample (pH 4 QC sample for acid titrations and pH 10 QC sample for base titrations) should be analyzed using the following procedure:

   a. Copiously rinse the electrode with deionized water. Immerse it in 20 mL of QC sample and stir it for 30 to 60 seconds. Discard the sample and replace with an additional 40 mL of QC sample. While the solution is gently stirred, measure and record the pH.

   b. From the calibration curve of pH versus pH*, determine the pH*. If the pH* and theoretical pH of the QC sample differ by more

than 0.05 pH unit, stop the analysis and repeat the rigorous calibration procedure in Section 5.4.5.

c. Previously analyzed samples (up to the last acceptable QC sample) should be reanalyzed. Acceptable values of pH* are reported on a form similar to NSWS Form 20 (see Appendix D).

5.7 REFERENCES

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6.0 PREPARATION OF ALIQUOTS

6.1 OVERVIEW

Changes in chemical parameters may occur in the time interval between sample collection and analysis. These changes can be minimized by preparing sample aliquots using a variety of techniques, including filtration and preservation. The particular parameter being measured dictates the type of preparation and preservation necessary to ensure sample stability until analysis is complete.

6.1.1 Scope and Application

These procedures are specific to filtration and preservation methods employed in the NSWS for the parameters listed in Table 6-1. Preparation of an extractable aluminum aliquot is described in Section 7.0.

6.1.2 Summary of Method

Samples may be filtered to remove the biotic and abiotic particles which exceed 0.45 µm in size. Preservation may include adjustment of aliquot pH to less than 2 pH units with concentrated acids or storage at 4 °C. Samples should be processed within 24 to 48 hours from the time of sample collection.

6.1.3 Interferences

The known interferences specific to the parameter being measured are detailed in the sections containing anlaysis procedures for that parameter. In preparing aliquots, all interferences are collectively termed contamination. Measures taken to minimize contamination include processing inside a clean air station, separation of acid-washed and deionized water-washed apparatus, use of ultrapure acids, use of Type I reagent grade water (ASTM, 1984), and strict adherence to laboratory cleanliness rules (see Section 3.5).

6.1.4 Safety

The sample types and most reagents used in preparing aliquots pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses should be worn when handling concentrated acids.

6.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples should be collected in deionized water-washed containers of sufficient volume to prepare all necessary aliquots. For the NSWS, 4-L polyethylene Cubitainers are generally used. Containers should be filled completely to minimize atmospheric contact and stored at 4 °C in the dark until processed.

TABLE 6-1. ALIQUOTS, CONTAINERS, PRESERVATIVES, AND CORRESPONDING PARAMETERS FOR THE NATIONAL SURFACE WATER SURVEY

Aliquot/(Container)[a]	Processing and Preservation[b]	Parameters
1 (250 mL, AW)	Filtered pH < 2 with HNO_3[c]	Ca Na Mg Mn K Fe
2 (15 mL, AW)	Filtered MIBK Extract	Total Extractable Al
3 (250 mL, DIW)	Filtered No Preservative	Cl^- F^- SO_4^{-2} NO_3 SiO_2
4 (125 mL, AW)	Filtered pH <2 with H_2SO_4[d]	Dissolved organic carbon NH_4^+
5 (500 mL, DIW)	Unfiltered No Preservative	pH BNC ANC Conductance DIC
6 (125 mL, AW)	Filtered pH <2 with H_2SO_4[d]	Dissolved P
6* (125 mL, AW)	Unfiltered pH <2 with H_2SO_4[d]	Total P
7 (125 mL, AW)	Unfiltered pH <2 with HNO_3[c]	Total Al

[a]AW = acid-washed container, DIW = deionized water-washed container.
[b]Aliquots 2, 3, 4, 5, and 6 should be stored at 4 °C in the dark.
[c]12M nitric acid, Baker Ultrex grade or equivalent.
[d]18M sulfuric acid, Baker Ultrex grade or equivalent.

6.3 EQUIPMENT AND SUPPLIES

6.3.1 Apparatus

Filtration apparatus includes filter holder, vacuum chamber, and vacuum pump (Figure 6-1).

6.3.2 Reagents and Consumable Supplies

1. Nitric Acid (HNO_3, 12M, Baker Ultrex grade or equivalent).

2. 5% Nitric acid wash--Carefully add 50 mL of concentrated HNO_3 to 500 mL water, then dilute to 1 L.

3. Sulfuric Acid (H_2SO_4, 18M, Baker Ultrex grade or equivalent).

4. Water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).

5. Aliquot Bottles--Clean aliquot bottles are required for the aliquots prepared from each sample (see Table 6-1 for the sizes used in the NSWS).

6. Indicating pH Paper (Range 1 to 3).

7. Membrane Filters (0.45-µm pore size).

8. Capillary tubes.

6.4 PREPARATION

6.4.1 Filtration Unit Assembly

NOTE: A slight positive (blowing into the laboratory) air flow should be maintained in the clean air station. Check for positive air flow by taping a Kimwipe strip to the bottom of the glass window.

1. Up to four filtration units may be connected to a single vacuum pump. Acid-washed and deionized water-washed units may be connected in the same series. Each type should be clearly labelled. When arranged in close proximity, a plexiglass barrier should be constructed around deionized water-washed units to minimize potential contamination from nitric acid aerosols.

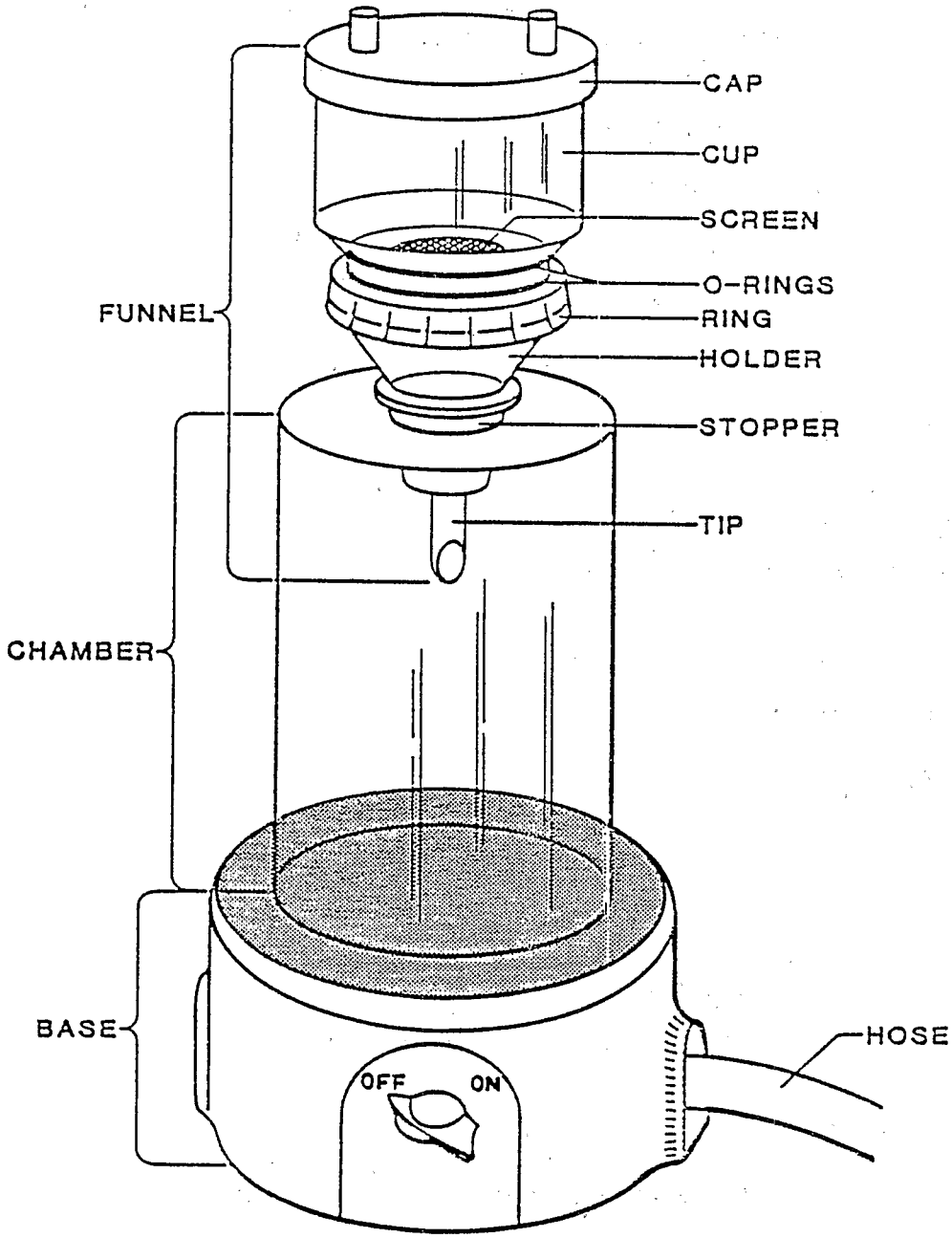


Figure 6-1. Filtration apparatus.

2. Attach the vacuum line from the vacuum pump via a waste filter flask to the outlet on the first filtration base. Turn on the vacuum. Adjust the vacuum pump to 10-12 inches mercury (Hg).

CAUTION: Do not exceed 12 inches Hg under any circumstances.

Be sure the waste flask remains upright and is emptied on a regular basis.

3. Two sets of Teflon forceps are needed. One set is acid-rinsed. Label one pair of this set "ACID-CLEAN" and the other "ACID-DIRTY". One set is deionized water-rinsed. Label one pair of this set "NONACID-CLEAN", the other "NONACID-DIRTY". Different color tapes, such as red for acid and blue for not acid, provide easy identification.

6.4.2 Maintenance

The filter holder and vacuum chamber require periodic cleaning. Acid-washed apparatus should be soaked in 5 percent nitric acid wash for 24 hours or longer. Deionized water-washed apparatus should be soaked in deionized water for a minimum of 48 hours.

The rubber gaskets on the filtration base and the vacuum lines require periodic replacement due to wear.

6.5 PROCEDURE

6.5.1 Filter Rinsing

NOTE 1: The 0.45-µm membrane filter should be replaced before a new sample is to be filtered. Be sure the filter is centered and lies smoothly on the filter screen with no tears.

NOTE 2: Make sure the blue filter separators are removed before placing the filter on the screen. Do not touch the filter to any object other than clean Teflon forceps or the filter screen. If the filter does touch another object, discard the filter and obtain a new one.

NOTE 3: Empty the waste beaker under the filtration apparatus into a second waste beaker to be dumped outside the hood. Never remove the apparatus waste beaker from the clean air station.

NOTE 4: If any part of the deionized water-washed apparatus is contaminated by acid, replace the entire apparatus with a clean one. Soak the contaminated unit in deionized water for 48 hours.

1. Unscrew the filter cup from the filter holder. Make sure it separates properly and that the O-rings are secure and in place.

2. Lift the cup. Using clean, acid-rinsed Teflon forceps place a 0.45-µm membrane filter onto the screen. Moisten the filter with deionized water (from wash bottle) and apply the vacuum to seal the filter to the screen. Be sure the filter is centered and lies smoothly with no tears.

3. Replace the cup onto the holder without disturbing the filter. Tighten the ring securely. If the ring is not properly tightened, it may leak when vacuum is applied. If this happens, obtain a new aliquot bottle of the same type and reprocess the aliquot.

4. Rinse the filter with 5 mL of deionized water, followed by 5 mL of 5 percent HNO_3, followed by two 5-mL rinses with deionized water. A third rinse with deionized water should cover the sides of the cup as well as the filter.

NOTE: For deionized water-washed apparatus, eliminate the 5 percent HNO_3 rinse, rinsing three times with deionized water only.

5. Shut off the vacuum. Break the seal and thoroughly rinse the filter funnel tip with deionized water.

6.5.2 Sample Filtration

NOTE 1: The cap should be kept on the aliquot bottles until the bottle is placed under the funnel to avoid any possible contamination from the chamber.

NOTE 2: If the Cubitainer cap (or its white paper liner) is dropped at any time, rinse it one time with deionized water and one time with sample, then continue processing.

NOTE 3: Keep the hood area clean. Wipe up spills as they occur.

1. Agitate the Cubitainer. Pour no more than 10 mL of sample into the filter cup.

2. Turn on the vacuum and filter the sample into the waste beaker. Turn off the vacuum.

3. Lift the chamber and remove the waste beaker. Empty the beaker and place it behind the apparatus, out of the way. Loosen the lid of the aliquot bottle. Lift the chamber and set the aliquot bottle on the base. Remove the cap and lower the chamber back onto the base. Set the cap upright next to apparatus, in a clean spot.

4. Agitate the Cubitainer. Pour no more than 10 mL of sample into the filter cup.

5. Turn on the vacuum. Filter the sample into the aliquot bottle and turn off the vacuum.

6. Lift the chamber and replace the cap on the aliquot bottle. Remove the bottle and tighten the cap. Rinse the bottle thoroughly by shaking and rotating. Pour the rinse sample into the waste beaker. Loosen the cap, lift the chamber, and replace the bottle under the funnel. Remove the cap and set the chamber on its base.

7. Agitate the Cubitainer. Pour 200 mL of the sample into the filter cup. Apply vacuum pressure and filter the sample into the aliquot bottle. Turn off the vacuum.

8. Use only one aliquot bottle to collect filtered sample. Pour remaining aliquots of the same type from this bottle. Rinse each bottle and cap with 5 to 10 mL of filtered sample.

9. If it is necessary to change the filter before filling all aliquots from one sample because the filter has become clogged, use the following procedure:

   a. Shut off the vacuum. Lift the chamber, cap the aliquot bottle, remove it, and replace with the waste beaker.

   b. Unscrew the filter cup, remove the dirty filter with the "DIRTY" forceps, and replace it with a clean filter using the "CLEAN" forceps.

   c. For aliquots prepared using the acid-rinsed units, rinse the filter as described in Section 6.5.1. For the filtration unit which is deionized water-rinsed, follow the same procedure except <u>eliminate the 5 percent HNO_3 wash</u>; instead rinse three times with deionized water.

10. Aliquots which receive added chemical preservatives should be filled to the bottle shoulder. Aliquots which do not receive added preservatives should be filled to the brim, capped tightly so that no headspace exists, and immediately refrigerated at 4 °C.

6.5.3 <u>Between Sample Rinsing</u>

1. Place a 250-mL plastic beaker (for waste) under each filter funnel.

2. Rinse the filter funnel once with deionized water from a 1-L wash bottle. Be sure water flows evenly over all interior surfaces of the filter cup; turn the cup one complete revolution while rinsing the sides.

3. (Acid-Washed Units only). Rinse the filter funnel once with 5 percent HNO_3 from a 1-L wash bottle. Turn the cup one complete revolution while rinsing the sides.

4. Rinse the filter funnel three times with deionized water from a 1-L wash bottle. Turn the cup one complete revolution for each rinse and allow the water to drain completely.

6.5.4 Unfiltered Aliquots

1. Thoroughly agitate the Cubitainer and rinse the aliquot bottle once with a 10-mL portion of sample. Be sure to rotate the bottle so that the sample contacts all internal surfaces.

2. Agitate the Cubitainer again and fill the bottle to the shoulder with sample, if preservative is to be added. If unpreserved, fill bottle completely with sample, cap tightly so that no headspace exists, and immediately refrigerate at 4 °C.

6.5.5 Preservation

NOTE 1: Dedicate a 40- to 200-µL micropipet for each type of preservative. Colored tape is recommended for identification (e.g., red tape for nitric acid, yellow tape for sulfuric acid). It also is recommended that aliquot labels be color coded to indicate the type of preservative (e.g., pink for nitric acid, yellow for sulfuric acid, white for no preservative).

NOTE 2: A row of similarly preserved aliquots (e.g., those preserved with HNO_3) may be done at one time. Loosen the aliquot bottle caps (for the entire row) but do not remove the caps until it is time for the actual addition of the acid. As long as there is no contamination to the pipet tip, one pipet tip may be used throughout the preservation procedure for each acid type.

1. Add the appropriate quantity of preservative to the aliquot bottle, (generally 100 µL).

2. After the preservative is added, tighten the caps and mix thoroughly.

3. (Acid-preserved aliquots only). Loosen the aliquot bottle caps and using a fresh capillary tube for each bottle, collect and place a drop of preserved sample on pH paper (pH range 1.8 to 3.8). The pH should be less than 2. It may be necessary to add more than 100 µL of acid for the pH to be less than 2. If this situation occurs, continue adding the appropriate acid in 100-µL increments until the pH is less than 2, using a new capillary tube each time the pH is tested.

4. Write the total amount of preservative added to the sample on the aliquot label and in the logbook.

6.5.6 Shipping Instructions

NOTE: The following procedures are recommended if aliquots are to be shipped by a commercial carrier or another method which includes a transfer of physical custody to a third party. Portions of these procedures may not be applicable when physical custody of samples remains within a single laboratory.

1. Refrigerate both preserved and preservative-free aliquots for at least 1 hour at 4 °C before shipping. Check that all labels are correct and tighten the caps firmly. Tape each cap in a clockwise direction with electrical tape. Place each aliquot to be shipped in an individual plastic bag and tie with a twist-tie.

NOTE: Aliquot lids may leak if not tightened after refrigeration.

2. Place each set of aliquot bottles (not including extractable aluminum aliquots) into a 1-gallon Ziploc bag or equivalent. Face the labels in the same direction for easy sample identification. Remove excess air from the bag, seal it, and place the bag in the refrigerator, or directly into the prepared shipping coolers.

3. Line the sides of each shipping container with frozen gel packs.

4. Place numerically sequential sets of aliquots in a container. If there is excess space, fill with gel packs or newspaper.

5. A shipping form similar to Form 3 (Appendix D), is completed by the laboratory coordinator and contains all aliquot information for the batch. At least one copy is placed inside a sealable bag and placed inside the shipping box. The original is retained in the processing laboratory until verification of sample receipt at the analytical laboratory is received.

6. Securely seal the shipping boxes with strapping tape.

7. Clearly label each box. Information should include:

   a. To: Name, address, and telephone number of the analytical laboratory.

   b. From: Name, address, and telephone number of the processing laboratory.

   c. Box __ of __ (box number and total number of boxes)

   d. Weight

   e. Appropriate shipper labels and documentation.

6.6 QUALITY ASSURANCE AND QUALITY CONTROL

There are no specific quality control procedures for aliquot preparation. The use of laboratory blanks is recommended as a check of possible contamination during processing.

6.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelpha, Pennsylvania.

7.0 PREPARATION OF TOTAL EXTRACTABLE ALUMINUM ALIQUOT

7.1 OVERVIEW

7.1.1 Scope and Application

This procedure is for the preparation of a sample extract for subsequent determination of total extractable aluminum by atomic absorption spectroscopy. It is applicable to weakly buffered natural waters of low ionic strength.

7.1.2 Summary of Method

A water sample is filtered in a contamination-free environment. Phenol red is added as a pH indicator. Next, an 8-hydroxyquinoline/sodium acetate reagent is added. This mixture is then buffered to a pH of 8.3 with an ammonium acetate buffer. At this pH, any small dissolved aluminum species existing in the sample will complex with the 8-hydroxyquinoline in the solution.

These organic complexes are removed from the mixture by adding methyl isobutyl ketone (MIBK), an organic solvent in which the aluminum complexes are more soluble. Agitation causes the complex to be almost totally transferred to the organic layer. This organic layer is extracted and stored until it is analyzed for aluminum content by atomic absorption spectroscopy.

7.1.3 Interferences

Numerous interferences have been observed in the MIBK extraction procedure, particularly affecting the phenol red color change and organic layer separation. Suspected interferences include other metals, particularly iron, and, possibly, organic compounds. Observed colors at the phenol red addition stage include pink, red, purple, brown, and black. Extracted aliquots have been observed to range from clear to yellow, green, brown, and black. A bubbly or frothy third layer has been observed following centrifuging; this layer should be considered part of the organic layer.

7.1.4 Safety

MIBK is a hazardous organic liquid and should be handled with care. Keep MIBK in the clean work station at all times. The laminar flow hood must be on, and a slight negative pressure must be maintained throughout the entire MIBK procedure. Analysts are required to wear half-mask respirators, safety glasses, a lab coat, and two pairs of gloves when handling MIBK.

An organic vapor photoionization detector is also employed as a safety precaution (see Appendix F).

7.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Water samples are collected in clean, deionized water-washed containers, filled completely to minimize atmospheric contact. Cubitainers or syringes are recommended for collection of water used in the extractable aluminum aliquot. Collected samples are stored at 4 °C until sample processing; processing should be as close to the time of sampling as possible (generally within 24 hours).

7.3 EQUIPMENT AND SUPPLIES

7.3.1 Apparatus

1. Centrifuge.
2. Volumetric flasks - 1 or 2 each 2-L, 1-L, and 500-mL.
3. Repipet or equivalent dispensers - 1 each 2.00-mL, 5.00-mL, and 10.0-mL.
4. Reagent bottle with dropper - 2 each 60-mL.
5. Polystyrene graduated cylinders - 2 each 25-mL, 100-mL, and 250-mL.

7.3.2 Reagents and Consumable Materials

1. Phenol Red--Fill a 60-mL drop-dispenser bottle, labeled "Phenol Red", with phenol red indicator solution.

2. Methyl Isobutyl Ketone--Attach a labeled ("MIBK") 10.0-mL Repipet dispenser to a 4-L bottle of methyl isobutyl ketone.

3. 1M NH_4OH---

NOTE: Always work with NH_4OH under the hood wearing safety glasses, double gloves, and a lab coat. Exercise caution when working with concentrated NH_4OH.

   a. Fill a clean 100-mL graduated cylinder with approximately 25 mL of deionized water.

   b. Measure 12 mL of 5M NH_4OH (Baker Instra-Analyzed grade or equivalent) in a clean 25-mL graduated cylinder.

   c. Carefully pour NH_4OH into the 100-mL graduated cylinder and dilute to 60 mL. Cover the graduated cylinder with Parafilm and invert 4 to 5 times to mix thoroughly.

   d. Transfer the solution to a 60-mL drop-dispenser bottle labeled "1M NH_4OH".

4. Ammonium Acetate/Ammonia Buffer Solution (NH_4OAc/NH_3)--

   a. Fill a clean 2-L Erlenmeyer flask with 200 mL of deionized water.

   b. Prepare an ice bath by placing a frozen gel pack into a plastic pan and filling the pan 1/3 full of tap water. With the flask sitting in the ice bath, slowly add 112 mL of glacial acetic acid (Baker Instra-Analyzed grade or equivalent) from a 250-mL graduated cylinder. Swirl the flask to mix the solution as the acid is being added. If the solution becomes too hot, allow it to cool prior to adding more acid.

   c. Keep the flask in the ice bath and slowly add 150 mL NH_4OH (5M Baker Instra-Analyzed grade or equivalent) from a 250-mL graduated cylinder. Swirl during the addition to prevent overheating.

   d. Dilute to the 500-mL mark with deionized water and swirl to mix.

   e. Place a small amount of the solution into a small beaker. Using a capillary tube, check the pH of the solution by placing one drop on pH paper (pH range 8.0 to 9.7). The pH should be near 8.3. If the pH is too high, add glacial acetic acid dropwise from a disposable pipet. This may require 20 to 30 drops of acid. Swirl and test frequently, using a new capillary tube and a new rinsing of the beaker. If the pH is too low, add 1M NH_4OH dropwise from the bottle. It may require 15 to 20 drops to adjust the pH. Swirl and test frequently, using a new capillary tube and new rinsing beaker for each test.

   f. When the pH has been adjusted, add an additional 32.0 mL of 5M NH_4OH. If the solution becomes hot, return the flask to the ice bath.

   g. Dilute with deionized water to the 1-L mark in the Erlenmeyer flask. Swirl to mix.

   h. Pour the NH_4OAc/NH_3 buffer into a clean, labeled 2.0-mL Repipet. Rinse the Repipet pump with buffer by depressing it several times.

   i. Store any extra buffer in a labeled, capped, 1-L volumetric flask in the refrigerator.

5. 8-Hydroxyquinoline Solution--

NOTE: Make sure 8-hydroxyquinoline is dissolved totally in the glacial acetic acid before adding water.

a. Measure 12.5 of mL glacial acetic acid (Ultrex or equivalent grade) in a clean 25-mL graduated cylinder.

b. Pour into a clean 500-mL volumetric flask labeled "Hydroxyquinoline Solution".

c. Weigh 5.000 g of 8-hydroxyquinoline in a clean, disposable weighboat. Transfer to the flask containing the glacial acetic acid. Cap and mix until salt is dissolved completely.

d. Dilute with deionized water to the 500-mL mark and mix thoroughly. Store the extra solution in the refrigerator.

6. 1.0M Sodium Acetate (NaOAc)--

a. Fill a clean 500-mL volumetric flask labeled "1.0M NaOAc" with approximately 250 mL of deionized water.

b. Weigh 41.0 g anhydrous sodium acetate (ultrapure grade) and add to flask.

c. When sodium acetate is dissolved, dilute to the 500-mL mark and mix thoroughly. Store extra solution in the refrigerator.

7. 8-Hydroxyquinoline/Sodium Acetate Solution (HO_X)--

NOTE 1: Calibrate the Repipet with deionized water.

NOTE 2: Prepare the HO_X reagent daily.

NOTE 3: 140 mL of reagent is enough for 20 samples. Make enough HO_X reagent to process all samples for that day.

a. Place 20 mL 1.0M NaOAc solution in a clean 100-mL graduated cylinder. Rinse the cylinder with a small amount of reagent prior to measuring the volume.

b. Place 100 mL of deionized water into a clean 250-mL graduated cylinder.

c. Place 20 mL of 8-hydroxyquinoline solution into a clean 100-mL graduated cylinder. Rinse the cylinder with a small amount of reagent prior to measuring the volume.

d. Rinse a 5.0-mL Repipet with deionized water and add the solutions in the following order: 1M NaOAc, deionized water, and 8-hydroxyquinoline; cap and swirl to mix. Flush the plunger with the HO_X reagent.

8. Consumable Materials--

   a. 50-mL graduated centrifuge tubes with sealing caps.
   b. 10-mL or 15-mL centrifuge tubes with sealing caps.
   c. pH indicating paper (pH range 8.00 to 9.7).
   d. Capillary tubes.
   e. Nucleopore membrane filters and filter holder assemblies.

7.4 PREPARATION

7.4.1 Calibration and Standardization

Check the calibration of all of the Repipets daily, as directed in Appendix C, and record the values in the MIBK logbook. Volumes should be delivered exactly, especially for the MIBK and HO_x reagents.

Check the calibration of the analytical balance weekly, as directed in Appendix C.

7.4.2 Maintenance

Prepare syringe filters as directed in Appendix C. Check the operation of the organic vapor photoionization detector weekly, as described in Appendix F.

7.5 PROCEDURE

NOTE 1: Learn how to achieve good reproducibility with a Repipet prior to sample processing.

NOTE 2: See aluminum extraction flowchart, Figure 7-1.

7.5.1 Filtration

NOTE 1: This section is applicable to samples in syringes. If bulk sample is used, filter according to the directions for acid-washed filtration in Section 6.

NOTE 2: A minimal amount of sample is used for rinses to conserve both sample and filters.

1. Obtain the sample syringes. Record the date and time of collection in the MIBK logbook (this information should be recorded on the syringe label).

2. Remove the syringe valve and attach a clean acid-washed Nucleopore filter assembly (see Appendix C). Only remove the valve from the sample syringe long enough to filter. When filtration is complete, replace the valve and set the sample in the refrigerator.

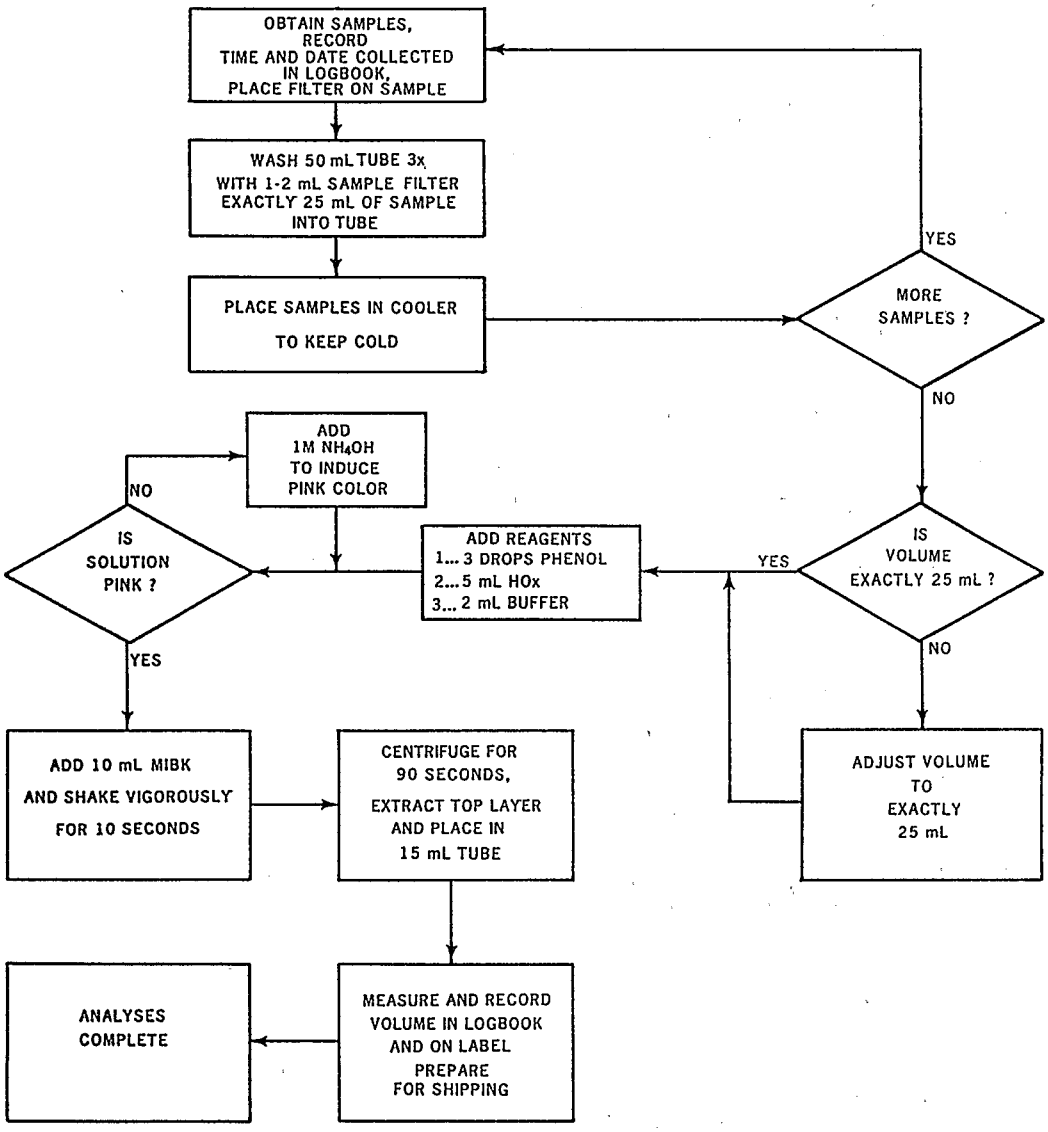


Figure 7-1. Aluminum extraction flowchart.

3. Rinse approximately 1 mL of sample through the filter into a waste beaker. Now filter 1 to 2 mL of sample into an appropriately labeled acid-washed 50-mL centrifuge tube and cap. Rotate the tube so that all interior surfaces are rinsed. Repeat this two more times.

4. Filter exactly 25 mL of sample into the rinsed tube. Be sure to fill all tubes to the same mark. It is crucial that the 25 mL of sample be measured accurately and reproducibly.

5. Cap the tube and place it in the cooler with gel packs. Filter all samples prior to beginning the extraction process.

7.5.2 Extraction

NOTE 1: Successful extraction depends thorough agitation after the addition of MIBK.

NOTE 2: Record in the logbook any abnormalities which appear in the samples (e.g., color, precipitate).

1. Add three drops of phenol red to the sample in the 50-mL centrifuge tube.

2. Add 5.0 mL HO_x reagent, using the Repipet.

3. Using the 2.0-mL Repipet, add 2.0 mL NH_4OAc/NH_3 buffer. Mix for exactly 5 seconds by swirling gently. The tube may be left uncapped. The solution in the tube should turn red to pink throughout the tube, indicating that the pH value is 8.3 or greater.

4. If the solution does not change color, rapidly adjust the pH by adding the 1M NH_4OH solution dropwise until a red-to-pink color is obtained. Record number of drops required in the logbook.

NOTE: See comments on color change in Section 7.1.3.

5. Using the 10.0-mL Repipet, add 10.0 mL MIBK to the centrifuge tube. Cap and shake vigorously for exactly 10 seconds. Time the agitation with a stopwatch and be sure the time is accurate.

6. Place the centrifuge tube with the filtered sample in a small cooler with gel packs. After all samples have been processed, centrifuge the samples, four at a time. Centrifuge the samples for 90 seconds at medium speed.

7. Use a 1-5 mL pipet set at 5.0 mL to transfer the top organic layer (MIBK and HO_x-Al^{+3} complex) to a labeled 15-mL centrifuge tube. Be careful not to withdraw any of the underlying aqueous layer. A total of 8.5 to 10.0 mL of MIBK should be extracted. Cap the tube tightly and check the bottom of the tube to ensure that no water has been extracted.

8. Compare the sample extract volume to a 15-mL centrifuge tube which has exact volume markings from 7.0 to 10.0 mL. Record the volume on the label and in the logbook for each sample. A volume greater than 10.0 mL indicates either the presence of water in the tube or a faulty Repipet. A volume less than 8.5 mL indicates improper extraction or a faulty Repipet. If the Repipet is found to be at fault, the sample should be refiltered and reprocessed completely. Record volumes in the logbook.

9. Store the extracted aliquots in a test tube rack in a cooler with a frozen gel pack until ready to ship. Do NOT store the MIBK sample extracts in the refrigerator.

10. Dispose of any solid or liquid waste materials as described in Section 7.5.3.

7.5.3 Cleanup

NOTE 1: A lab coat, safety glasses, a half-mask respirator, and double gloves must be worn when handling solid or liquid MIBK waste.

NOTE 2: All waste receptacles should be clearly labeled as MIBK waste.

1. Solid Waste Disposal--

   a. During MIBK processing all MIBK solid waste (open centrifuge tubes and caps, gloves, pipet tips, MIBK-soiled Benchkote) should be discarded into a labeled metal container lined with a plastic bag. This container should be located on the floor beside the clean work station.

   b. At the end of daily processing, this bag containing solid MIBK waste is emptied into a large, labeled garbage can stored outside, in a secure area. The gloves being worn also are discarded.

   c. Vent the large garbage can outside for 5 to 6 days; this aeration renders the waste acceptable for regular waste disposal. Upon complete venting of the solid waste, double bag and place in regular waste disposal canisters.

2. Liquid Waste Disposal--

   a. Discard the MIBK liquid waste into a properly labeled solvent waste can stored in the clean work station.

   b. Solvent waste should be emptied daily into a properly labeled container (e.g., 5-gallon metal gasoline can).

3. Glassware Cleanup--

   a. Clean all glassware by rinsing in succession with deionized water (once), 5-percent nitric acid (once), and deionized water (three times).

   b. Disassemble the 5.0-mL Repipet daily. Rinse the bottle with deionized water, then fill it about half full with deionized water. Replace the dispenser on the bottle and rinse it copiously by filling it and dispensing about 10 portions of deionized water. Put the Repipet unit in the clean work station. Be sure to leave the dispenser full of water to prevent build-up of air bubbles.

   c. Disassemble and rinse the 2.0-mL Repipet whenever the NH_4OAc/NH_3 buffer is replaced.

   d. Store all Repipets in the clean work station.

8.0 FRACTIONATION AND DETERMINATION OF ALUMINUM SPECIES

8.1 OVERVIEW

8.1.1 Scope and Application

This method is a semiautomated colorimetric method applicable to the determination of reactive aluminum in natural, surface waters. The method colorimetrically measures in aqueous samples the amount of aluminum which forms a complex with pyrocatechol violet (PCV). The measurement is performed on two sample streams, one directly and one after passage through a cation-exchange column. This method is an adaptation of the method presented by Rogeborg and Henrikson (1985).

For purposes of this analysis, reactive aluminum is defined as the fraction of soluble (dissolved) aluminum that reacts with PCV without preliminary acidification. This fraction is believed to represent the monomeric portion of the total aluminum pool. This includes free inorganic monomeric aluminum, various aluminum hydrous oxides, and aluminum bound to various inorganic and organic ligands. The reactivity of certain aluminum complexes is dependent upon the strength (stability constant) of the complex in relation to the aluminum PCV complex.

Total reactive aluminum is defined as the fraction of the total dissolved aluminum pool that forms a complex with PCV. Dissolved species are species that pass through a 0.45-µm filter. It is known that some particulate forms of aluminum are smaller than 0.45 µm. These forms include soils, colloidal aluminum complex (monomeric and polymeric), and clay minerals. The reactivity of these complexes with PCV is unknown.

Reactive nonexchangeable aluminum is defined as the fraction of total reactive aluminum that is not removed from the sample stream after passage through the cation-exchange column. This fraction consists primarily of organic-aluminum complexes whose stability constants are greater than the affinity of the cation-exchange column for the bound aluminum and yet are less than the stability constant for the aluminum-PCV complex. This fraction is theoretically nontoxic to fish, at least in terms of acute effects.

Toxic aluminum is not measured directly but can be estimated by subtracting reactive nonexchangeable aluminum from total reactive aluminum. This difference estimates the amount of inorganic monomeric aluminum which is believed to manifest acute toxic responses in fish.

The method detection limit (MDL) has been determined to be 7.0 µg L^{-1} for repetitive measurements of a low aluminum standard. The applicable range of this method is 0-3500 µg L^{-1}.

The method presented here does not distinquish between various inorganic monomeric aluminum species, nor does it distinguish between the various neutral organic complexes of aluminum. Furthermore, the definitions of total reactive and nonexchangeable reactive aluminum are based on commonly accepted usage. In actuality, some charged or weakly bound organic-aluminum complexes will be removed by the cation exchange column and are regarded as inorganic monomeric species, and some strongly complexed monomeric aluminum may not be measured in either fraction.

8.1.2 Summary of Method

Samples are collected in syringes to minimize diffusion of carbon dioxide into and out of samples. The aluminum species in each sample are subsequently determined by flow injection analysis (FIA). Samples are loaded into the FIA system directly from the syringe via a syringe pump. The sample fills a fixed-volume (100 µL) sample loop on Channel 1, then passes through a cation exchange column prior to filling the second sample loop (also 100 µL) on Channel 2. The contents of each sample loop, total reactive and nonexchangeable reactive aluminum, respectively, are then injected by operator-prompted computer command. The sample valve switches by computer activation, engaging the deionized water carrier stream. The sample (bolus) is flushed by carrier into the reaction manifold where it reacts with hydroxylamine hyrochloride/1,10-phenanthroline, eliminating iron interference. The bolus is then reacted with PCV. Optimum color development is achieved by adjusting the final pH of the aluminum aluminum-PCV complex to 6.1 by addition of hexamethylene tetraamine buffer. The absorbance of the complex is subsequently determined at 580 nm. Channel 1 measures total reactive ("inorganic" plus "organic" monomeric) aluminum whereas Channel 2 measures nonexchangeable reactive ("organically bound" monomeric) aluminum.

8.1.3 Interferences

Holding time, storage methods, and changes in temperature, dissolved carbon dioxide concentrations, and pH may drastically alter aluminum speciation in water samples. Samples should be analyzed as soon as possible after collection, generally within 24 to 36 hours. Samples are stored at 4 °C in the dark during transit and prior to analysis.

NOTE: The actual holding time is currently unknown. Research on the stability of aluminum species is ongoing. In the interim, holding times exceeding 48 hours should be avoided.

Iron (III) interferes with the determination of aluminum using this method. Therefore, the interference is eliminated by reducing iron (III) to iron (II) with hydroxylamine hydrochloride and subsequent chelation with 1,10-phenanthroline.

8.1.4 Safety

The calibration standards and most chemical reagents encountered in this method pose no serious health hazard due to external contact. Acids and bases may cause burns and they should be handled only under a fume hood. Protective clothing (e.g., safety glasses, gloves, lab coats) should be worn. Hands should be washed thoroughly after handling aluminum standards and reagents.

8.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples are collected in 60-mL linear polyethylene syringes with syringe lock valves affixed to the tips. Use of this type of syringe has been shown to prevent the diffusion of carbon dioxide into and out of samples if they are kept at 4 °C. Sample preservation is limited, therefore, to storage at 4 °C in the dark.

8.3 EQUIPMENT AND SUPPLIES

8.3.1 Equipment Specifications

1. Automated flow injection analyzer--A computer-interfaced FIA capable of automatic injection of samples, mixing of specified reagents for reaction of PCV with aluminum, and detection unit (colorimeter) capable of measuring absorbance at 580 nm.

2. Cation-exchange column--A 100-mm (10 mm I.D.) Teflon column with Teflon fritted inserts.

8.3.2 Consumable Materials

1. Cation-exchange resin--An Amberlite IR 120 (14 to 50 mesh) exchange resin is used to separate the inorganic from the organic monomeric aluminum species.

2. Nucleopore membrane filters and filter holder assemblies or equivalent.

3. Pump tubes.

4. Teflon tubing.

5. Plastic syringes, 60-mL.

8.3.3 Reagents

1. Water--All water used in preparing reagents and cleaning labware should meet the specifications for Type I reagent grade water (ASTM, 1984).

NOTE: All reagents, with the exception of the pyrocatechol violet and degassed DI carrier, may be prepared in large quantities and refrigerated in polyethylene containers between uses.

2. Hexamethylamine Tetraamine Buffer--

   a. Label 250-mL disposable beaker "Hexamethylamine Tetraamine 84.0 g". Place the beaker on the balance and tare. Be sure the balance is set on the high range (0 to 300 g).

   b. Wearing gloves, use a second labeled beaker to scoop out the powdered buffer from the container and measure 84.0 g.

   c. Obtain a clean 1-L volumetric flask. Label it "Hexamethylamine Tetraamine Buffer 84.0 g L^{-1}" and add approximately 200 mL of deionized water.

   d. Using a clean funnel, carefully transfer the powdered buffer to the volumetric flask. It is best to add enough deionized water to the beaker to form a slurry and then pour it through the funnel.

   e. Rinse the beaker well with deionized water and add it to the volumetric flask. Bring the volume up to near the 1-L mark with deionized water and allow the buffer sufficient time to dissolve prior to filling to the 1-L mark. Mix completely. The buffer is now ready for filtering. Follow the procedure in Section 8.3.8.

3. Pyrocatechol Violet--

NOTE 1: PCV is made daily. If depleted before sample analysis is completed, a new calibration must be performed.

NOTE 2: Store the PCV in a cool, dark place when not in use. Be sure the lid is closed tightly.

NOTE 3: Use of an antistatic gun in the empty weighboat will prevent "climbing" of the PCV powder.

   a. Set the balance to the low range (0-30 g) setting. Tare a clean, dry weighboat.

   b. Wearing gloves, use a clean Teflon spatula to place 0.375 g of pyrocatechol violet powder into the center of the weighboat. Be sure to weigh it very accurately.

   c. Obtain a clean 1-L volumetric flask and label "PCV 0.375 g L^{-1}." Add approximately 300 mL deionized water to the flask.

d. Carefully transfer the PCV from the weighboat to the volumetric flask by slowly washing the PCV with deionized water into the flask. Be sure to flush the weighboat completely. Avoid spilling any solution outside of the flask.

e. Dissolve the PCV in the flask by adding additional deionized water. Be sure to rinse the mouth and neck of the flask. Bring the volume up to the 1-L mark and mix thoroughly.

f. Filter this solution following the procedure in Section 8.3.8.

4. Mask--

a. Label a clean 250-mL disposable beaker "Hydroxylamine HCl 7.60 g". Place the beaker on the balance and tare. Be sure the balance is on the low range (0-30 g).

b. Wearing gloves, use a clean Teflon spatula to scoop 7.60 g hydroxylamine HCl into the beaker. This powder scatters easily; be sure that no powder falls outside of the beaker onto the balance stage.

c. Obtain a clean 1-L volumetric flask and label it "Mask-Hydroxylamine HCl 7.60 g; 1,10-Phenanthroline Monohydrochloride 0.560 g". Add approximately 300 mL deionized water to the flask.

d. Dissolve the hydroxylamine HCl in the beaker by adding deionized water. Carefully transfer it to the volumetric flask, being sure to flush all the powder from the beaker. Bring the volume in flask up to about 500 mL with deionized water.

e. Tare a clean weighboat with the balance set on the low range. Use a clean Teflon spatula to place exactly 0.560 g 1,10-phenanthroline monohydrochloride into the weighboat.

f. Transfer the powder into the labeled flask by flushing it from the weighboat with deionized water. Be sure to rinse the weighboat completely.

g. Swirl the flask to dissolve all solids. The solution may have a slight pink hue. Now fill to the 1-L mark with deionized water.

h. Filter this solution following the procedure in Section 8.3.8.

5. Deionized Water Carrier--

NOTE: Be sure to have at least 4 liters of degassed deionized water prepared before beginning analysis. If the sample load is greater than 25 samples, increase the volume prepared.

a. Fill a 4-L Cubitainer or other suitable container with fresh deionized water.

b. Follow the filtration procedure in Section 8.3.8.

6. Cleaning Solution - 0.1N HCl/10 percent Ethyl Alcohol--

NOTE: This solution is used to clean tubing as described in Section 8.5.3. It is not involved in the actual aluminum determination.

a. Place 8.3 mL concentrated (Baker Instra-Analyzed or equivalent) hydrochloric acid into a clean 1-L volumetric flask which contains approximately 500 mL deionized water.

b. Measure 100 mL 95 percent ethyl alcohol in a graduated cylinder. Add this to the 1-L flask.

c. Dilute to the 1-L mark with deionized water. Place in a labeled 1-L plastic bottle and store in the refrigerator.

8.3.4 Amberlite Cation Exchange Resin

NOTE: Prepare as needed.

1. 0.1N HCl--

a. Fill a 1-L volumetric flask with about 500 mL deionized water.

b. Add 8.3 mL concentrated (Ultrex or equivalent) hydrochloric acid.

c. Dilute to the 1-L mark with deionized water.

d. Store the solution in the flask until needed.

2. 0.1N NaCl--

a. Fill a 1-L volumetric flask with about 500 mL deionized water.

b. Add 5.8 g of sodium chloride.

c. Dilute to the 1-L mark with deionized water.

3. Hydrogen form of Amberlite

   a. Tare a clean 250-mL disposable beaker.

   b. Add about 25 g (wet weight) of amberlite cation exchange resin.

   c. Remove from the balance and pour in enough 0.1N HCl to cover the beads.

   d. Stir thoroughly.

   e. Rinse with deionized water for approximately 10 minutes, stirring constantly.

   f. Pour off the rinse water from the last rinse into a separate glass beaker.

   g. Check for the presence of chloride ions by adding a few drops of silver nitrate to the rinse.

   CAUTION: Silver nitrate will turn skin black when exposed to sunlight. Wear gloves during this procedure.

   h. If a white precipitate forms (silver chloride), rinse the resin for another 5 minutes.

   i. Check for chloride as in step g above.

   j. Repeat steps c through i until no precipitate forms. Store the prepared resin in a labeled bottle in the refrigerator.

4. Sodium form of Amberlite resin--Follow the procedure in step 3, above, except substitute 0.1N NaCl for 0.1N HCl.

5. One percent hydrogen form of Amberlite resin--

NOTE: This is the resin used to pack the cation exchange column in Section 8.4.4.

   a. Tare a 250-mL disposable beaker. Add the desired amount of the sodium form of amberlite resin into the beaker. Drain the resin as much as possible.

   b. Record the weight.

   c. Take one percent of that value and add that amount of the drained hydrogen form to the beaker.

d. Add deionized water and mix thoroughly.

e. Place in a labeled polyethylene bottle (deionized water-washed) and store in the refrigerator until needed.

8.3.5 Aluminum Stock Solutions

NOTE 1: Use one source of 1,000 mg L^{-1} aluminum stock solution to make the standard stock solution and another source to make the quality control (QC) stock solution.

NOTE 2: These stock solutions can be stored indefinitely and do not require refrigeration.

1. Standard Stock Solution--

a. Fill a clean 1-L volumetric flask with approximately 500 mL of deionized water.

b. Using a calibrated pipet, add 1.000 mL of concentrated (Ultrex or equivalent) nitric acid to the flask and mix well.

c. Add 10.00 mL 1,000 mg L^{-1} aluminum stock solution to the flask using a calibrated 1-5 mL pipet. Mix thoroughly. Be sure to use the aluminum stock designated for standard preparation.

d. Bring volume up to the 1-L mark with deionized water and mix thoroughly. Transfer to a 1-L clean plastic bottle which has been rinsed one time with a small amount of the stock solution. Label "10 mg L^{-1} Calibration Standard Stock".

2. QC Stock Solution--Using a different source of 1,000 mg L^{-1} aluminum stock solution, follow the procedure above. Use a different 10-L volumetric flask and a new pipet tip. Label the plastic bottle "10 mg L^{-1} QC Stock".

8.3.6 Aluminum Calibration Standards

NOTE 1: Flasks used for the standards should never be acid-washed. Wash only with deionized water. Flasks should be dedicated to a particular standard.

NOTE 2: Standards should be stored in the refrigerator when not in use.

1. Routine Calibration Standards--

NOTE: Check the calibration of all pipets daily prior to use. Use the same pipet tip as used for the calibration or recheck the

calibration if a new tip is required. Be sure to rinse the tip with the standard prior to use.

a. Fill a clean, labeled 100-mL volumetric flask with approximately 30 mL deionized water. Use one flask per standard.

b. Use the appropriate calibrated pipet to deliver the correct volume of stock solution to the flask. See Table 8-1 for values.

c. Mix thoroughly by inversion. Using a disposable pipet, bring up to the 100-mL mark (exactly) with deionized water.

d. Cap flask and store in the refrigerator when not in use.

2. High-Range Calibration Standards--

NOTE: These standards are used for calibration with the "High Al" method. They require a significant volume of stock solution and should only be prepared if a sample is found to be out of range on the routine calibration.

a. These standards are also made in 100-mL volumetric flasks. Follow the procedure for routine calibration standards, but use Table 8-2.

b. Be sure to keep an accurate count of 5-mL portions added to each flask. Standards should be exact.

3. Acidified Blank Preparation--

NOTE: The acidified blank is used in the calibration procedure only.

a. Fill a clean 100-mL volumetric flask with about 50 mL of deionized water. Label "10 percent Nitric Acid".

b. Slowly add 10.0 mL concentrated Ultrex or equivalent nitric acid.

c. Dilute to 100 mL with deionized water. Store in the refrigerator. This may be used as a stock solution to prepare the acidified blank each day.

d. Place 0.1 mL of 10-percent nitric acid in a 500-mL graduated cylinder containing about 250 mL of deionized water. Dilute to 500-mL mark with deionized water. Label "Acidified Blank".

4. 20 μg L^{-1} Detection Limit Standard--Follow the procedure outlined for routine calibration standards using a 200- to 1000-μL pipet to deliver 200 μL of an aluminum calibration stock solution to the flask.

TABLE 8-1. VOLUME OF ALUMINUM STOCK STANDARDS REQUIRED TO PREPARE DAILY STANDARDS

	Standard Concentration ($\mu g\ L^{-1}$)	Micropipet Range	Volume of Standard Stock Required
Calibration Standards	0	40-200 µL	See Section 8.3.6
	25	200-1000 µL	250 µL
	100	200-1000 µL	1000 µL
	200	1-5 mL	2.00 mL
	350	1-5 mL	3.50 mL
	500	1-5 mL	5.00 mL
	750	1-5 mL	7.50 mL
	1000	1-5 mL	10.00 mL

TABLE 8-2. VOLUME OF ALUMINUM STOCK STANDARDS REQUIRED FOR HIGH RANGE CALIBRATION STANDARDS

Standard Concentration ($\mu g\ L^{-1}$)	Micropipet Range	Volume of Standard Stock Required (mL)
1000	1-5 mL	10
2000	1-5 mL	20
3500	1-5 mL	35

8.3.7 Quality Control Standards

1. 75 $\mu g\ L^{-1}$ Routine QC Standard--

   a. Fill a clean, labeled 500-mL volumetric flask with approximately 300 mL of deionized water.

   b. Use a calibrated pipet to deliver 3.75 mL of 10-mg L^{-1} QC stock solution to the flask. Be sure to use a new pipet tip.

   c. Mix thoroughly. Bring up to exact volume with deionized water and mix again.

d. Fill a labeled 60-mL syringe with the standard after rinsing it with the standard three times. Affix syringe valve.

e. Store standards in the refrigerator.

2. 600 µg L^{-1} Routine QC Standard--Follow the above procedure except substitute a 100-mL flask. Use 6.00 mL of 10-mg L^{-1} QC stock solution to prepare this QC solution.

3. 2500 µg L^{-1} High Range QC Standard--

NOTE: This standard is a QC check for the "High Al" method. It requires a significant volume of stock solution and should only be prepared if a sample is found to be out of range on the routine calibration.

a. Fill a clean, labeled 100-mL volumetric flask with approximately 50 mL deionized water.

b. Use a calibrated pipet to deliver 25.0 mL QC stock solution to the flask. Be sure to use a new pipet tip.

c. Mix thoroughly. Bring up to the 100-mL mark with deionized water and mix again.

d. Store flask in the refrigerator.

8.3.8 Reagent Filtering/Degassing

NOTE 1: Glass bell jars are very fragile. Removing the funnel while vacuum pressure is high may result in breakage.

NOTE 2: Thoroughly wash the filtration apparatus between each reagent filtration. Rinse with copious amounts of deionized water. A blue-grey liquid on the filtration apparatus indicates a reaction between the PCV and buffer, a sign of improper rinsing.

1. Rinse the properly labeled 1-L plastic bottle and cap with deionized water. Place the bottle under the bell jar on the vacuum filtration apparatus.

2. For all reagents but the buffer, a 0.45-µm Gelman filter in a small Nalgene filtration funnel should be used. For the buffer, place a Whatman GF/C filter in a large Buchner funnel. (A glass fiber filter may be substituted for the Whatman filter. This filter will also fit into the small Nalgene filtration funnel). Handle the filters with clean forceps.

3. Turn on the vacuum pump and pour a small amount of reagent through the filter and into the 1-L bottle to rinse.

4. Turn off the vacuum and allow the pressure to fall to near zero. Remove the bottle and replace the cap. Shake the bottle to rinse interior surfaces. Discard the reagent rinse.

5. Replace the bottle under the filtration apparatus and filter the remaining reagent into the bottle. Do not overfill the funnel. For the buffer, one filter is good for about 1 L of reagent.

6. Pour the reagent from the bottle into the appropriate reagent reservoir. Store the excess reagents (except PCV) in the refrigerator.

8.4 PREPARATION

NOTE: System assembly is illustrated in Figure 8-1(a) and 8-1(b) for Channels 1 and 2, respectively.

8.4.1 Precalibration Procedure

1. With pump off, remove reagent lines from deionized water flask. Place each line in the appropriate reagent reservoir. Be sure the end is completely submerged in reagent. Cover the opening of each reservoir with Parafilm. Each reservoir should contain two lines, one line for each channel.

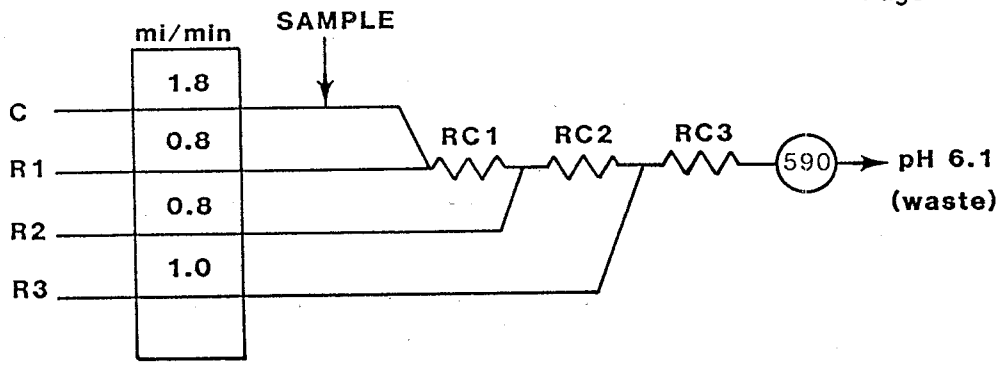
2. Lock down the pressure arms on the pump. Turn on the pump. Adjust tension on each line to be sure optimal pumping is achieved. Worn tubes will not pump well even at highest tension. Bubbles oscillating in a line signal a worn pump tube. Replace it with a pump tube of the same type.

3. Observe the baseline on both channels. Adjust the zero knobs on colorimeter to make both channels as close to 100 as possible. Be sure the baseline is stable for a period of time after adjustment.

4. Recheck the gain knob and be sure it is set to the proper position and locked into place. Do not adjust this knob once the calibration has begun or at any time during sample analysis.

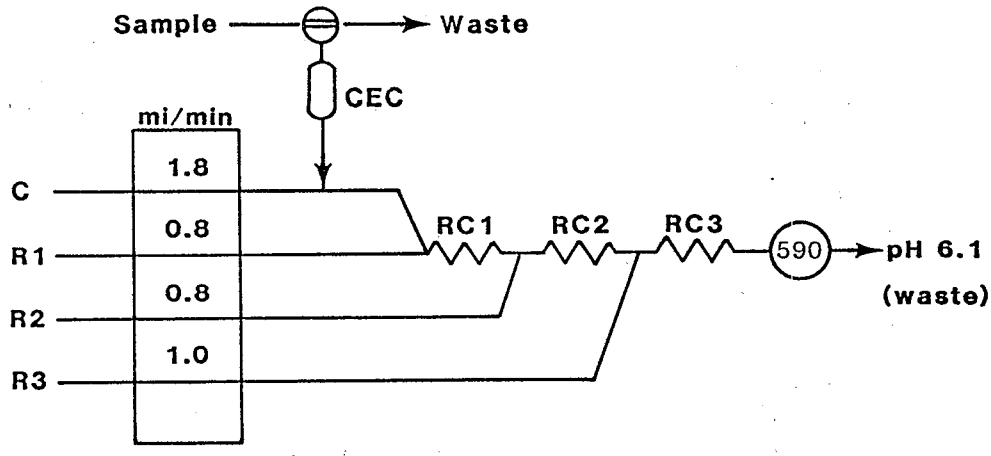
5. Check the sample loops. Be sure the proper size loops are in place. Check all lines for signs of wear.

6. Be sure the waste container is empty and waste is flowing properly. Turn the pH meter knob to "pH" position. Meter should be calibrated using procedure in Section 19.4. Record the calibration data in the logbook. Monitor the waste from one channel by allowing the waste



Key:

Carrier: Deionized water (or 0.1 M HCl)
R1 – Masking solution: Hydroxylammonium chloride and 1,10 Phenanthroline chloride
R2 – Color reagent: Pyrocatacholviolet
R3 – Buffer solution: Haxamethylenetatramine and NaOH
RC1 – Reaction Coil, 10 cm (0.5 mm i.d.)
RC2 – Reaction Coil, 30 cm (0.5 mm i.d.)
RC3 – Reaction Coil, 60 cm (0.5 mm i.d.)



Key:

Carrier: Deionized water (or 0.1 M HCl)
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R3 – Buffer solution: Haxamethylenetatramine and NaOH
RC1 – Reaction Coil, 10 cm (0.5 mm i.d.)
RC2 – Reaction Coil, 30 cm (0.5 mm i.d.)
RC3 – Reaction Coil, 60 cm (0.5 mm i.d.)
CEC – Cation Exchange column

Figure 8-1(a,b). Schematic of flow injection system for aluminum speciation.

from that channel to flow over the pH probe. Check for a stable reading. The pH value should be approximately 6.1 ± 0.1.

7. If using a strip chart recorder, adjust prior to sample analysis. Adjust pen level with zero knob. Check time base and sensitivity settings also.

8.4.2 Calibration and Standardization

Channels 1 and 2--The dilute calibration standards (including the 0.000 µg L^{-1} Al standard) described in Section 8.3.6 are prepared prior to analysis each day. The cation exchange column is disengaged by turning the 6-port switching valve to the "cl/cal/QC" position, allowing the standards to fill the sample loop on Channel 2 without passing through the cation exchange column. A low calibration curve is generated by injecting increasing concentrations of low calibration standards. Each standard is injected twice during calibration. The calibration is obtained by printout from the computer, or manually by plotting absorbance (peak area) versus concentration. The best fit line of response versus concentration is obtained manually or by computer output. Immediately after the low calibration is performed, high calibration standards are injected as routine samples and their respective absorbances are recorded for future use (see "High Calibration" below).

1. Calibration Procedures--Once the system has attained a steady baseline with reagents, place the sample intake line into the 100-mL flask containing the lowest concentration calibration standard (0.00 µg L^{-1} Al). After two injections of standard, remove the sample line from the flask, rinse with deionized water and place the sample line in the next highest standard. Inject this standard twice and continue to the next highest standard until calibration is complete. Be certain that the cation exchange column is disengaged during calibration ("cl/cal/QC").

2. High Calibration--The high calibration standards (500, 750, 1,000 µg L^{-1} Al) and high quality control check sample (QCCS) (600 µg L^{-1} Al) are analyzed daily prior to sample analysis. If a sample exhibits a measured concentration greater than 350 µg L^{-1} Al, but less than 600 µg L^{-1} Al, examine the high QCCS for linearity. If the observed concentration of the 600 µg L^{-1} QCCS is within 10 percent of its nominal concentration (540 to 660 µg L^{-1} Al), the measured concentration of the high sample may be accepted. If a measured concentration of greater than 600, but less than 1,000 µg L^{-1}, is observed for a routine sample, or if the high QC check criteria are not met, a new calibration line should be calculated from the high standard raw data. Also, if more than 20 percent of the samples in a batch contain more than 350 µg L^{-1} Al, a high calibration should be determined, regardless of the acceptable high QCCS. The high calibration is determined from a linear regression of peak area versus concentration of the 350, 500,

750, and 1,000 μg L^{-1} Al standards. Check the linearity of the high calibration by determining the concentration of the high QCCS (600 μg L^{-1} Al) by inserting the peak area into the linear regression equation. The measured concentration should be within 10 percent of the nominal value, or the high standards should be reanalyzed and a new high calibration determined.

If a sample concentration of greater than 1,000 μg L^{-1} Al is observed, an expanded calibration may be performed following completion of the remainder of the batch. Standard concentrations of 1,000, 2,000, and 3,500 μg L^{-1} Al and a QCCS of 2,500 μg L^{-1} Al are used to calibrate in the expanded range. These standards are prepared by adding the specified volumes of 10.0 mg L^{-1} Al standard stock solution to a clean 100-mL volumetric flask and bringing to a final volume of 100 mL. The 2,500 μg L^{-1} Al QCCS should be prepared from the 10.0 mg L^{-1} Al QC stock solution.

Standard Concentration (μg L^{-1} Al)	mL 10.00 mg L^{-1} Al required	
1,000	10.00	
2,000	20.00	
2,500 (QCCS)	25.00	(QC stock)
3,500	35.00	

Calibration is done by reducing the gain to 1.00 (from 4.00) and analyzing the 1,000, 2,000, and 3,500 μg L^{-1} Al standards twice each. This is performed as a separate calibration from the normal calibration. Analyze the QCCS to ensure linearity (within 10 percent). It should be noted that the normal limits of linearity have been reported at 1,000 μg L^{-1} Al. Also, it is important to change the gain rather than sample size in order to retain comparable flow charcteristics. Any samples with aluminum concentrations greater than 3,500 μg L^{-1} Al should be diluted with deionized water which has been adjusted to the pH of the sample with dilute sulfuric acid. This can be done by titrating deionized water with 0.001N Ultrex or equivalent sulfuric acid to the pH of the sample and diluting the sample until its PCV absorbance is on-scale at a gain of 1.00. Return the gain to 4.00 following completion of the high sample analyses. It is very important that samples analyzed by high calibrations be noted as such, along with their corresponding gain and QC values.

8.4.3 Maintenance

After completion of all sample analyses for a given day, flush the system with water for 5 minutes. Next, place the switching valve in the "cl/cal/QC" position to disengage the cation-exchange column, increase the pump speed by 20 percent, and flush with cleaning solution for 5 minutes. Follow with another water rinse. Return pump to normal operating speed. Shut off instrument and computer. If system is not to be used for more than 2 days, pump air through the lines. Release all pump tubes from the peristaltic pump.

Weekly and daily maintenance is critical in keeping an FIA system in proper operation. Deviations in flow rate due to worn or constricted lines alter the flow and mixing characteristics of the system and therefore will affect the chemistry of the method. Monitor the system constantly for any changes in flow, replace pump tubes on a regular basis (determined by extent of use), and release tubes from the pump at the completion of analysis. Spray silicone over the pump rollers weekly to prolong pump tube life. The 0.5-mm I.D. Teflon tubing is also subject to aging. Crimps in the lines can occur due to twisting or pinching and are most often observed at the end of mixing coils. Also, a black precipitate can develop in the lines due to the buffer. Disconnecting the coil and injecting cleaning solution from a syringe will augment the cleaning process. It is also helpful to pass air through the coil with the syringe. PCV also gradually stains the lines. When a line appears fouled or damaged, replace it with a line of equal I.D. and length. If a coiled line is to be replaced, wrap a new coil in a similar fashion. After completing a coil-wrap, reposition the ends to release any pressure or bends that may lead to coil-kinking.

Inspect the flow cell regularly for fingerprints, dirt, or scratches. A dirty flow cell may be cleaned with alcohol, but a scratched or cracked flow cell should be replaced; therefore, exercise caution when handling flow cells. Maintain the colorimeter according to manufacturers' instructions. A poorly functioning colorimeter negates an otherwise properly functioning system; therefore, the colorimeter should be checked regularly. Turn off the light source for the colorimeter prior to activating other system components and turn on after system components to prevent blown fuses.

The rotary valves also require regular maintenance. Weekly (or more frequently if necessary), disassemble the valve by removing the three screws that hold the valve together and cleaning all of the parts with a soft brush. Check the flanged line ends to make sure a good seal is being made and that no constrictions exist. Check the valve housing for wear and replace any worn components. Put the valve back together by installing the three screws as you would when changing a tire. Do not over tighten the screws as the Teflon may become warped. If the valve leaks upon reinstallation, tighten each of the screws a little more. If the valve still leaks, the flanged ends are probably not making a good seal. Reinspect the flanges and reassemble the valve.

8.4.4 Column Packing Procedure

NOTE 1: Soak all reagent lines in deionized water when packing the column. Turn the pump to "OFF" position.

NOTE 2: Wear gloves while packing the column.

NOTE 3: This procedure is used on initial packing of the column or when air is introduced into the column.

1. Unscrew the top of the column. This is the outflow for the deionized water which flushes the column when the valve is in the "CAL" position.

2. Obtain a 1-5-mL pipet tip and cut 1-2 mm from the end. Fit the cut end into the top of the column.

3. If this is the initial packing (or when the Amberlite needs to be replaced) pour activated Amberlite into the column using a funnel and proceed to step 5. (See Section 8.3.4 for Amberlite resin preparation.)

4. If removing an air pocket from the column, use a disposable pipet to remove the air pocket, or agitate the beads with a piece of Teflon tubing to dislodge the air and proceed with step 5.

5. Turn on the pump and allow the deionized water to carry the Amberlite beads to the pipet tip. Turn off the pump. Allow the beads to fall and fully pack the column. Be sure that the level of the beads is flush with the top of the column. If not, add more activated Amberlite and repeat this step. Remove the excess water with a disposable pipet.

6. Check for the presence of air pockets. Repeat steps 4 and 5 if any bubbles persist.

7. Remove the pipet tip and replace the column cap. Be sure that the cap has all components (washer, Teflon frit) and that there are no beads on the column threads. If any beads are present on the threads, dislodge them using a stream of deionized water from a wash bottle.

8. Purge the column deionized water carrier line (preceding the column) of any air which may have been introduced during the packing procedure.

9. Turn on the pump and check for any air pockets in the column.

10. Recharging Amberlite Cation Exchange Resin--

    a. Pump 0.1N HCl through the column for 2 minutes. Do not pump the acid onto the FIA manifold. Collect and discard the effluent from the column.

    b. Pump 0.001N NaCl through the column for 5 minutes. Do not allow the effluent to pump onto the manifold.

    c. Collect 40 mL of effluent in a beaker and measure the pH.

    d. The pH value should fall between 4.0 and 5.5. If it does not, repeat steps 2 and 3 until the pH value is within the desired range.

8.4.5 Troubleshooting

1. If the standards used in calibrating do not give a good calibration after two attempts, remake the standards (recheck pipet calibrations, Appendix C).

2. If the new standards do not improve the calibration, remake the 10-mg L^{-1} aluminum stock solution and prepare new standards.

3. Poorly defined peaks indicate an obstruction in the lines. Flush the lines with ethyl alcohol. Do not flush alcohol through the column.

4. If flushing fails to correct the problem, check all tubing for kinks or other objects which could create dispersion.

5. Check all pump tubing for poor pumping efficiencies.

6. An unstable baseline is caused by intermittent flow interruption. This results in pulsing in the lines and causes baseline fluctuations. Check all connections for leaks or over tightening.

7. Be sure there is no dead (empty) space in the column. Fill with appropriate beads.

8. To check for clogs, place all reagent lines in deionized water. Place the column in-line on channel 2. Mix a few drops of food coloring (red is most obvious) in deionized water. Introduce through the sample inlet line and observe the color progress through the instrument. Manually switch the valves and continue to observe the color moving through the lines. Manually switch the valves again and observe progress through the loops. Switch valves back and observe progress through the manifolds. This procedure may isolate clogs that are present in the lines.

9. Keep the ambient temperature cool and constant. This will help the instrument retain its calibration.

10. If the pH is not within the proper range, check for:

    a. Flow restrictions
    b. Drift in the pH meter calibration
    c. Incorrect solution preparation

8.5 PROCEDURE

8.5.1 Syringe Pump Setup

1. Release the pressure arm on the sample inlet line at the pump. Remove the end from the deionized water to prevent siphoning.

2. Disconnect the line at the prevalve fitting.

3. Replace with tubing which has a syringe-adapter fitting on one end.

4. Set the syringe pump near the sample inlet line. The speed knob should be at "9".

5. Prepare syringes by opening the syringe valve and reducing the volume to just less than 50 mL. Attach an acid-washed Nucleopore membrane filter. Rinse the filter by pushing 2-3 mL of sample through. Close the valve and place the samples in order in the refrigerator; see Appendix C for filter preparation procedure.

8.5.2 Sample Injection

NOTE 1: The Amberlite cation exchange column remains in-line for all sample analyses.

NOTE 2: If any sample exceeds 350 µg L^{-1}, a 600-µg L^{-1} QC sample (without using the column) is analyzed with the next 75-µg L^{-1} QC routine check.

If the value obtained is 600 ± 10 percent, the original sample value is accepted. If not, a recalibration should be performed using standards which bracket the value obtained. If the obtained value is in the 1,000-µg L^{-1} range, the high calibration standards in Table 8-2 should be prepared.

1. Be sure to monitor the effluent pH and record values on the tally sheet. pH values should be between 6.0 and 6.2. If the pH starts to drift, look for possible causes (see Section 8.4.5).

2. A natural audit sample that contains a known amount of nonexchangeable aluminum may be analyzed to test the column function.

3. Check baselines and initiate analysis. Run samples in numerical order.

4. Turn the syringe pump to "OFF" position as soon as the valve lights return to green.

5. Place the next sample on the pump and affix the inlet line. Check baseline values between samples. Record any major changes (i.e., "glitches") on the tally sheet.

6. Monitor the chart recorder and check for samples that require reanalysis. Reanalyze any suspect samples (i.e., those with a noisy baseline, glitches).

7. The 75-µg L^{-1} QC should be analyzed after every 10 samples, or at intervals determined by the QA program, once with the column and once without the column. The acceptable range is 67.5 to 82.5 µg L^{-1}.

8. After the final QC standard has been analyzed, a final natural audit sample is run with the column. Then the column switch is placed in the "CAL" position.

9. A final analysis of the 20-µg L^{-1} detection limit and the deionized water blank is performed.

8.5.3 Cleanup

1. Cap all reservoirs except PCV and store in the refrigerator.

2. Pour PCV into the sink and wash the bottle and cap with deionized water.

3. Clean all dirty glassware by rinsing three times with deionized water only.

4. Place inlet lines into the 2-L flask containing deionized water.

5. Remove the syringe adapter line and reattach the pump line.

6. Bypass the column by turning the valve to "CAL" position. Failure to do this will introduce air into column and repacking the column will be necessary.

7. Turn on the pump. Allow lines to flush with deionized water for 5 minutes.

8. Turn the pump off. Place lines in 0.1N HCl/10 percent ethyl alcohol cleaning solution. Turn the pump on and flush for 5 minutes. Do not flush the deionized water line leading to the column with the cleaning solution; this line may be left in the deionized water and its pressure arm from the pump may be released.

9. Turn the pump off. Replace lines in deionized water and flush for 5 minutes.

10. Turn the pump off. Leave all lines in deionized water. Release pressure arms on pump. Empty the waste reservoir; failure to do this will result in waste siphoning back through the manifold and clogging lines.

11. Remove filters from sample syringes. Remove membranes and discard. Soak filter parts in deionized water and prepare filters using the procedure in Appendix C.

12. Save all samples until all laboratory analyses have been completed and data have been checked by the laboratory supervisor.

13. Turn off power at the monitor, printer, colorimeter, computer, and injection module. Turn pH meter to "STAND-BY" and store the probe in 3M KCl solution.

8.6 QUALITY ASSURANCE AND QUALITY CONTROL

8.6.1 Precision and Accuracy

A single operator in a single laboratory analyzed various concentrations of inorganic monomeric aluminum prepared in distilled/deionized water. The precision and accuracy estimates are shown in Table 8-3. Similarly, precision and accuracy were determined for the high calibration range from 350 to 1,000 $\mu g\ L^{-1}$ Al. These values are displayed in Table 8-4.

Percent recovery was determined for two natural surface water samples, Big Moose Lake (Adirondack Mountains, New York) and Bagley Lake (Cascade Mountains, Washington) spiked with 300 and 100 $\mu g\ L^{-1}$ Al, respectively. The percent recoveries were as shown in Table 8-5.

TABLE 8-3. PRECISION AND ACCURACY FOR SINGLE OPERATOR AND SINGLE LABORATORY ANALYSIS OF INORGANIC MONOMERIC ALUMINUM BY FLOW INJECTION/PYROCATECHOL VIOLET METHOD

Nominal Al Concentration ($\mu g\ L^{-1}$)	Number of Samples	Avg. Observed Concentration ($\mu g\ L^{-1}$)	Precision (Std. Dev.) ($\mu g\ L^{-1}$)	Bias ($\mu g\ L^{-1}$)
0.0	12	4.9	3.3	4.9
10.0	13	9.2	2.5	-0.8
15.0	9	15.0	2.8	0.0
20.0	10	20.5	2.5	0.5
25.0	10	24.0	3.4	-1.0
35.0	10	34.2	2.5	-0.8
50.0	10	49.4	2.8	-0.6
75.0	10	70.0	3.1	-5.0
100.0	10	99.1	2.7	-0.9
150.0	2	150.5	4.8	0.5
350.0	5	350.8	3.8	0.8

TABLE 8-4. PRECISION AND ACCURACY FOR SINGLE OPERATOR AND SINGLE LABORATORY ANALYSIS OF HIGH LEVELS OF INORGANIC MONOMERIC ALUMINUM BY FLOW INJECTION/PYROCATECHOL VIOLET METHOD

Nominal Al Concentration ($\mu g\ L^{-1}$)	Number of Samples	Avg. Observed Concentration ($\mu g\ L^{-1}$)	Precision (Std. Dev.) ($\mu g\ L^{-1}$)	Bias ($\mu g\ L^{-1}$)
350.0	5	356.9	9.2	6.9
500.0	5	494.4	11.3	-5.6
750.0	5	743.9	13.3	-6.1
1000.0	5	1004.8	16.2	4.8

8.6.2 Quality Control Checks

1. Detection Limit Quality Control Check Sample (QCCS)--Analyze the detection limit QCCS (20 $\mu g\ L^{-1}$ Al) (keep the switching valve in "cl/cal/QC" position) immediately after low calibration and high calibration standards. The high calibration standards are not part of the computer-generated low calibration. The measured concentration should be within 20 percent of the actual concentration or the instrument detection limit, whichever is greater. If it is not, the reason

TABLE 8-5. PERCENT RECOVERY OF MONOMERIC ALUMINUM FROM TWO SPIKED NATURAL SURFACE WATER SAMPLES ANALYZED BY THE FLOW INJECTION/ PYROCATECHOL VIOLET METHOD

Sample	Number of Samples	Pre-Spike Concentration ($\mu g\ L^{-1}$)	Spike Concentration ($\mu g\ L^{-1}$)	Sample + Spike Concentration ($\mu g\ L^{-1}$)	Recovery (%)
Big Moose	6	278.2 ± 5.6	300.0	575.8 ± 7.7	99.6
Bagley	10	3.3 ± 1.5	100.0	105.7 ± 2.2	102.3

for the poor sensitivity and accuracy should be isolated and eliminated prior to sample analyses.

2. Routine Quality Control Check Sample--If it is not already in position, turn the switching valve to "cl/cal/QC" to disengage the cation-exchange column. Analyze the routine QCCS (75 $\mu g\ L^{-1}$ Al) after the detection limit QCCS and at intervals determined by the quality assurance program. The observed concentration should be within 10 percent of the calculated concentration. If the routine QCCS 10-percent window is not met, the reason for the poor accuracy should be isolated and eliminated prior to sample analyses. A duplicate injection may be performed, or a QCCS from a freshly prepared batch of routine QCCS (75 $\mu g\ L^{-1}$ Al) can be injected. If the 10-percent window again is not met, the FIA should be recalibrated. Reanalyze the routine QCCS. Continue sample analyses if the QCCS falls within the 10-percent window.

3. Blank--Analyze one blank consisting of water from the same source used to prepare all reagents and standards. Analyze once at the beginning and once at the end of analyses for a given day. The blank should be less than twice the detection limit. If not, the cause for the elevated blank value should be isolated and eliminated.

4. Laboratory Duplicate--Analyze one sample per batch in duplicate (immediately following the first analysis). The duplicate should be less than or equal to 10 percent of its corresponding sample for Channel 1 and Channel 2. If not, the source of the poor precision should be isolated and eliminated prior to continuing sample analyses.

5. Column Breakthrough Sample--The efficiency of the cation exchange column (CEC) in removing inorganic monomeric aluminum is determined by passing a portion of the routine QCCS sample through the CEC every time the routine QCCS is analyzed as described in step 2. The QCCS

is passed through the CEC by keeping the switching valve in the "sample" position and injecting QCCS. Determine column breakthrough BEFORE routine QCCS analyses. The CEC should remove all the aluminum from the QCCS; therefore, Channel 2 should exhibit no detectable aluminum. If the measured value is greater than 20 percent of the blank value, perform the column preparation procedure described in Section 8.4.4.

8.7 References

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

Rogeborg, E. J. S. and A. Henriksen, 1985. An Automated Method for Fractionation and Determination of Aluminum Species in Freshwaters. Vatten v. 41, pp. 48-53.

9.0 DETERMINATION OF AMMONIUM

9.1 OVERVIEW

9.1.1 Scope and Application

This method describes the determination of ammonium in natural surface waters in the range of 0.01 to 2.6 mg L^{-1} NH_4^+. This range is for photometric measurements made at 630 to 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.

9.1.2. Summary of Method

Alkaline phenol and hypochlorite react with ammonia to form an amount of indophenol blue that is proportional to the ammonium concentration. The blue color intensifies with sodium nitroprusside (U.S. EPA, 1983).

9.1.3 Interferences

Calcium and magnesium ions may be present in concentration sufficient to cause precipitation problems during analysis. A 5-percent disodium ethylenediamine tetraacetate (EDTA) solution prevents the precipitation of calcium and magnesium ions.

Sample turbidity may interfere with this method. Turbidity is removed by filtration at the processing laboratory. Sample color that absorbs in the photometric range used also interferes.

9.1.4 Safety

The calibration standards, sample types, and most reagents used in this method pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses should be worn when preparing reagents.

9.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples are collected, filtered, and preserved (addition of H_2SO_4 to pH less than 2). The samples should be stored at 4° C in the dark when not in use.

9.3 EQUIPMENT AND SUPPLIES

9.3.1 Apparatus and Equipment

Technicon AutoAnalyzer Unit (AAI or AAII) or equivalent, consisting of sampler, manifold (AAI) or analytical cartridge (AAII), proportioning pump,

heating bath with double-delay coil (AAI), colorimeter equipped with 15-mm tubular flow cell and 630- to 660-nm filters, recorder, and digital printer for AAII (optional).

9.3.2 Reagents and Consumable Materials

1. Water--Water should meet the specifications for Type I reagent grade water (ASTM, 1984).

2. Sulfuric Acid (5N), Air Scrubber Solution--Carefully add 139 mL concentrated sulfuric acid to approximately 500 mL ammonia-free water. Cool to room temperature and dilute to 1 L with water.

3. Sodium Phenolate Solution--Using a 1-L Erlenmeyer flask, dissolve 83 g phenol in 500 mL water. In small increments, cautiously add with agitation 32 g NaOH. Periodically cool flask under flowing tap water. When cool, dilute to 1 L with water.

4. Sodium Hypochlorite Solution--Dilute 150 mL of a bleach solution containing 5.25 percent NaOCl (such as "Clorox") to 500 mL with water. Available chlorine level should approximate 2 to 3 percent. Clorox is a proprietary product and its formulation is subject to change. The analyst should 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.

5. Disodium Ethylenediamine-Tetraacetate (EDTA) (5 percent w/v)--Dissolve 50 g EDTA (disodium salt) and approximately six pellets NaOH in 1 L of water.

6. Sodium Nitroprusside (0.05 percent w/v)--Dissolve 0.5 g sodium nitroprusside in 1 L deionized water.

7. NH_4^+ Stock Standard Solution (1,000 mg L^{-1})--Dissolve 2.9654 g anhydrous ammonium chloride, NH_4Cl (dried at 105 °C for 2 hours) in water, and dilute to 1,000 mL.

8. Standard Solution A (10.00 mg L^{-1} NH_4^+)--Dilute 10.0 mL NH_4^+ stock standard solution to 1,000 mL with water.

9. Standard Solution B (1.000 mg L^{-1} NH_4^+)--Dilute 10.0 mL standard solution A to 100.0 mL with water.

Using standard solutions A and B, prepare (fresh daily) the following standards in 100-mL volumetric flasks:

NH_4^+ (mg L^{-1})	mL Standard Solution/100 mL
	Solution B
0.01	1.0
0.02	2.0
0.05	5.0
0.10	10.0

NH_4^+ (mg L^{-1})	mL Standard Solution/100 mL
	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

9.4 PREPARATION

9.4.1 Calibration and Standardization

Analyze the series of ammonium standards as described in Section 9.5. Prepare a calibration curve by plotting the peak height versus standard concentration.

9.5 PROCEDURE

9.5.1 Standard Operating Procedure

Since the intensity of the color used to quantify the concentration is pH-dependent, the acid concentration of the wash water and the standard ammonium solutions should approximate that of the samples. For example, if the samples have been preserved with 2 mL concentrated H_2SO_4 L^{-1}, the wash water and standards should also contain 2 mL concentrated H_2SO_4 L^{-1}.

1. For a working range of 0.01 to 2.6 mg L^{-1} NH_4^+ (AAI), set up the manifold as shown in Figure 9-1. For a working range of 0.01 to 1.3 mg L^{-1} NH_4^+ (AAII), set up the manifold as shown in Figure 9-2. Higher concentrations may be accommodated by sample dilution.

2. 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.

3. For the AAI system, sample at a rate of 20 hr^{-1}, 1:1. For the AAII use a 60 hr^{-1} 6:1 cam with a common wash.

4. Load sampler tray with unknown samples.

5. Switch sample line from water to sampler and begin analysis.

6. Dilute and reanalyze samples with an ammonia concentration exceeding the calibrated concentration range.

9.5.2 Calculations

Compute concentration of samples by comparing sample peak heights with the calibration curve. Report results in mg L^{-1} NH_4^+.

9.6 QUALITY ASSURANCE AND QUALITY CONTROL

9.6.1 Precision and Accuracy

In a single laboratory (EMSL-Cincinnati), using surface-water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg L^{-1} NH_3-N, the standard deviation was ±0.005 (U.S. EPA, 1983).

In a single laboratory (EMSL-Cincinnati), using surface-water samples at concentrations of 0.16 and 1.44 mg L^{-1} NH_3-N, recoveries were 107 percent and 99 percent, respectively (U.S. EPA, 1983). These recoveries are statistically significantly different from 100 percent.

9.6.2 Quality Control Checks

Quality control checks include blank, duplicate, and matrix spike analyses and determination of detection limits. Appendix G explains internal quality control procedures.

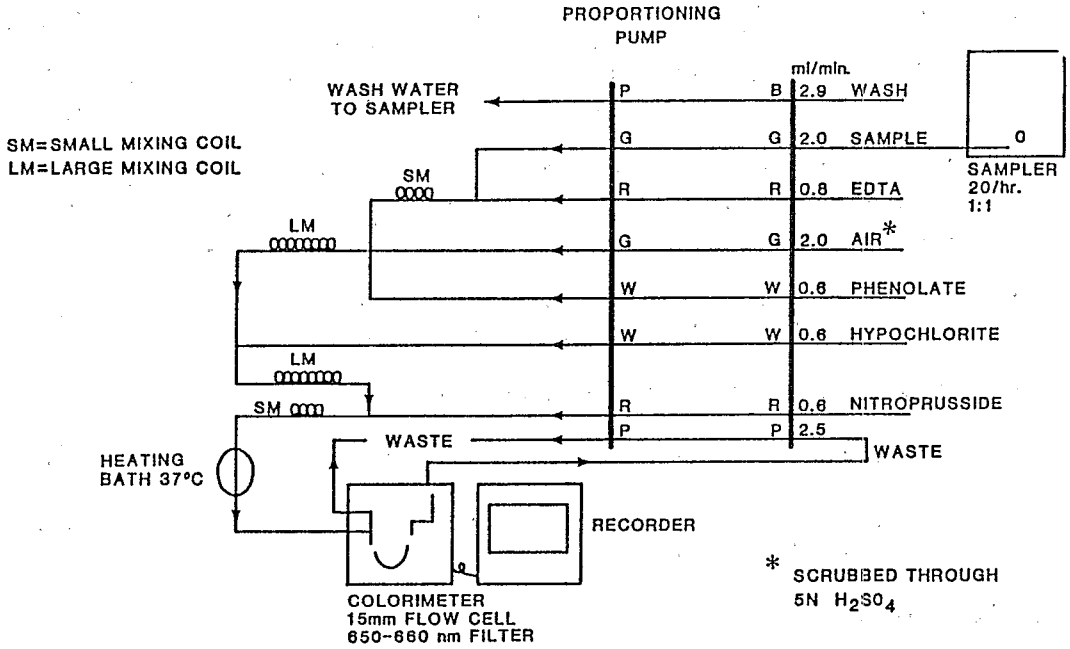

Figure 9-1. Ammonia manifold AAI.

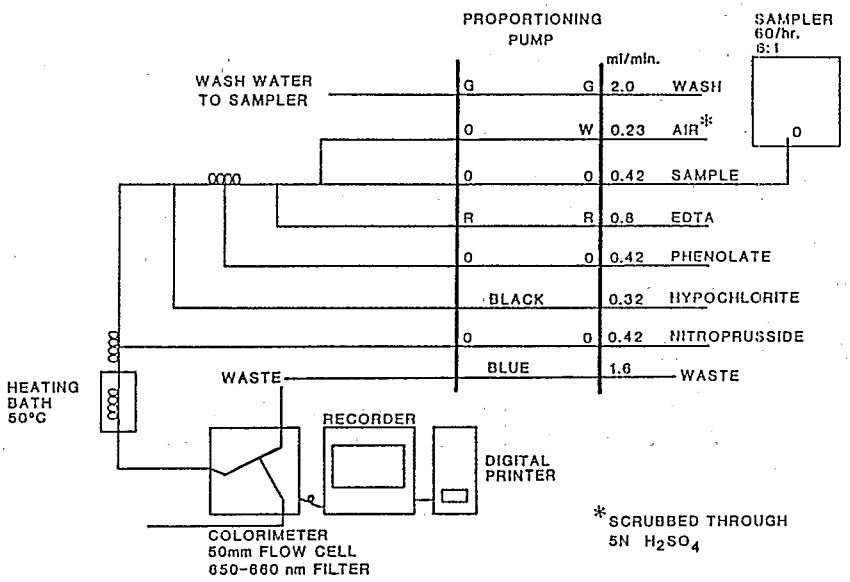

Figure 9-2. Ammonia manifold AAII.

9.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

U.S. Environmental Protection Agency, 1983 (revised). Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency, Cincinnati, Ohio.

10.0 DETERMINATION OF AMMONIUM BY FLOW INJECTION ANALYSIS

10.1 OVERVIEW

10.1.1 Scope and Application

This method covers the determination of ammonium in the range of 0.01 to 0.150 mg L^{-1} NH_4^+. This range is for photometric measurements made at 630 to 660 nm in a 10-mm tubular flow cell. Higher concentrations can be determined by sample dilution. Approximately 60 samples per hour can be analyzed.

10.1.2 Summary of Method

Alkaline phenol and hypochlorite react with ammonia to form an amount of indophenol blue that is proportional to the ammonium concentration. The blue color formed is intensified with sodium nitroprusside.

10.1.3 Interferences

Calcium and magnesium ions may be present in concentration sufficient to precipitate during the analysis. A 5-percent EDTA solution is used to prevent the precipitation of calcium and magnesium ions.

Sample turbidity may interfere with this method. Turbidity is removed by filtration at the processing laboratory. Sample color that absorbs in the photometric range used also interferes.

10.1.4 Safety

The calibration standards, sample types, and most reagents used in this method pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses should be worn when preparing reagents.

10.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples are filtered and preserved (addition of H_2SO_4 to pH less than 2) in the processing laboratory. The samples should be stored at 4 °C in the dark when not in use.

10.3 EQUIPMENT AND SUPPLIES

10.3.1 Equipment and Apparatus

Tecator FIAstar flow injection analyzer or equivalent consisting of:

1. Sampler.
2. Analytical manifold with 200-µL sample loop.
3. In-line heater.
4. Colorimeter equipped with a 10-mm flow cell.
5. Printer.

10.3.2 Reagents and Consumable Materials

1. Water--Water should meet the specifications for Type I reagent grade water (ASTM, 1984).

2. Acidified water--To a 2-L volumetric flask containing 1500 mL water, pipet 0.70 mL of concentrated H_2SO_4 (Ultrex or equivalent). Dilute to 2 L and mix.

3. Sodium Phenate Solution--Using a 400-mL Griffen beaker, dissolve 20.7 g phenol in 200 mL water. Add 8 g NaOH, stirring occasionally. Add water to the 250-mL mark and stir. The final solution should be a light amber color. Pour the solution into a 250-mL amber plastic bottle and store the bottle in a hood until used.

4. Sodium Hypochlorite Solution--Using a 500-mL Erlenmeyer flask, dilute 100 mL of a commercial bleach solution (Chlorox or equivalent, 5 percent NaOCl, minimum) with 100 mL of water.

5. Disodium Ethylenediamine Tetraacetate (EDTA)--Dissolve 50 g EDTA (disodium salt) and approximately 6 pellets of NaOH in 1 L of water and store the solution in a 1-L plastic bottle. To facilitate solution, use of a mechanical shaker is recommended.

6. Sodium Nitroprusside--Dissolve 0.5 g sodium nitroprusside in 1 L of water. Store the solution in a 1-L plastic bottle.

7. Ammonium Stock Solution (1,000 mg L^{-1} NH_4^+)--In a 1-L volumetric flask, dissolve 3.6624 g $(NH_4^+)\cdot 2SO_4$ (dried at 105 °C for 2 hours) in water, add 0.35 mL of 18M H_2SO_4 (Ultrex or equivalent), and dilute the solution to 1 L. Store it in a 1-L plastic bottle.

8. Standard Solutions (10 mg L^{-1} NH_4^+)--In a volumetric flask, dilute 1 mL of ammonium stock solution to 100 mL with acidified water.

9. Working Standards--Using the standard solution and diluting with acidified water, prepare the following standards in 100-mL volumetric flasks:

NH_4^+ (mg L^{-1})	mL standard solution/100 mL
0.010	0.100
0.025	0.250
0.050	0.500
0.100	1.00
0.150	1.50

10.4 PREPARATION

10.4.1 Calibration and Standardization

Analyze the series of standards described above. The calibration curve is calculated by the instrument. Follow the instructions provided by the manufacturer for creating calibration curves.

10.5 PROCEDURE

10.5.1 Standard Operating Procedure

1. Turn the power on to the analyzer and to the data station for at least 30 minutes before use.

2. Set up the ammonium manifold, and pump water through the manifold and lines while making the standards.

3. Prepare the reagents, standards, and QC samples.

4. Check the photometer reference and sample dark current. Consult the owners manual for specific instructions for this adjustment.

5. Load the standards, QC samples and water samples in the sample trays.

6. Enter the required information about the standards into the analyzer.

7. Begin the analysis.

8. Dilute any samples which are outside the calibration range.

10.5.2 Calculations

The concentrations of the samples are computed by the data station.

10.6 QUALITY ASSURANCE AND QUALITY CONTROL

10.6.1 Precision and Accuracy

In a single laboratory with standards at concentrations of 0.125, 0.104 (EPA reference sample WP486 No. 1), 0.100, and 0.050 mg L^{-1} NH_4^+, the average percent relative standard deviation was 5.65 (Chaloud et al., 1987).

Bias for the same samples were 102, 106, 105, 106, respectively.

10.6.2 Quality Control Checks

A batch is defined herein as the number of samples, excluding the standards and QC samples, accommodated by the analyzer at any one time. For the FIAstar, this is approximately 25 samples. The following special sample types are used for quality control.

1. Quality control check solution (QCCS) is a standard having a concentration of approximately the midpoint of the calibration range. Use 0.100 mg L^{-1} HN_4^+ concentration for this procedure. The QCCS is analyzed after the calibration standards (before any samples), then after every tenth sample or at intervals determined by the quality assurance program and as the last sample of any batch of samples. The QCCS should be within the prescribed accuracy limits (within 10 percent of actual concentration). If a QCCS is not within the prescribed limit, all samples analyzed since the last good QCCS are reanalyzed. Prepare the QCCS from an ammonium stock made of ammonium sulfate from a different lot than that used for the ammonium stock used to prepare the standards.

2. Detection limit standard (DL) is a standard 2 to 5 times the required detection limit. Use a 0.050 mg L^{-1} NH_4^+ solution for this standard. The DL is analyzed after the first QCCS and before the first sample and should be within the prescribed accuracy limit (within 20 percent of actual concentration).

3. A blank is run once per batch of samples. The blank is a sample of the acidified water used to make up the standards.

4. External standards from the National Bureau of Standards, EPA or other source should be analyzed twice in any batch of samples.

5. An internal standard (IS) or calibration standard is run three times in a batch; it is run the first time prior to the analysis of the first sample. The additional IS's are spaced at approximately equal intervals in the sample batch. The IS assists in compensating for any drift that may occur during the analysis.

6. One sample in any batch is analyzed in duplicate.

10.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadephia, Pennsylvania.

Chaloud, D. J., L. R. Todechiney, R. C. Metcalf, and B. C. Hess, 1987. Wet Deposition and Snowpack Monitoring Operations and Quality Assurance Manual. EPA 600/8-87/024. U.S. Environmental Protection Agency, Las Vegas, Nevada.

11.0 DETERMINATION OF CHLORIDE, NITRATE, AND SULFATE BY ION CHROMATOGRAPHY

11.1 OVERVIEW

11.1.1 Scope and Application

This method is applicable to the determination of chloride, nitrate, and sulfate in natural surface waters by ion chromatography (IC). It is recommended for use by or under the supervision of analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatogram. The applicable ranges and method detection limits (MDL) of this method are:

	MDL ($mg\ L^{-1}$)	Range ($mg\ L^{-1}$)
Chloride	0.01	0.2-10
Nitrate	0.005	0.01-5
Sulfate	0.05	1-20

11.1.2 Summary of Method

IC is a liquid chromatographic technique that combines ion exchange chromatography, eluent suppression, and conductimetric detection. A filtered sample portion is injected into an ion chromatograph. The sample is pumped through a precolumn, a separator column, a suppressor column, and a conductivity detector. The precolumn and separator columns are packed with a low-capacity anion exchange resin. The sample anions are separated in these two columns based on their affinity for the resin exchange sites.

The suppressor column reduces the conductivity of the eluent to a low level and converts the sample anions to their acid form. Typical reactions in the suppressor column are:

$$\underset{\text{(high-conductivity eluent)}}{Na^+\ HCO_3^-} + R - H \longrightarrow \underset{\text{(low conductivity)}}{H_2CO_3} + R - Na$$

$$Na^+\ A^- + R - H \longrightarrow HA + R - Na$$

Three types of suppressor columns are available: the packed-bed suppressor, the fiber suppressor, and the micromembrane suppressor. The packed-bed suppressor contains a high-capacity cation exchange resin in the hydrogen form. The resin is consumed during analysis and has to be regenerated periodically off-line. The fiber and micromembrane suppressors are based on cation exchange membranes and are preferred for two reasons. These suppressors are regenerated

continuously throughout the analysis. Also, their dead volume is substantially less than that of a packed-bed suppressor.

The separated anions in their acid form are measured using a conductivity cell. Anion identification is based on retention time. Quantification is performed by comparing sample peak heights to a calibration curve generated from known standards (ASTM, 1984a; O'Dell et al., 1984; Topol and Ozdemir, 1981).

11.1.3 Interferences

Interferences can be caused by substances with retention times that are similar to and overlap those of the anion of interest. Natural surface water samples are not expected to contain any interfering species. Large amounts of an anion can interfere with the peak resolution of an adjacent anion. Sample dilution or spiking can be used to solve most interference problems.

The water dip or negative peak that elutes near, and can interfere with, the chloride peak can be eliminated by the addition of the concentrated eluent so that the eluent and sample matrix are similar.

Method interferences may be caused by contaminants in the reagent water, reagents, glassware, or other sample processing apparatus. These interferences lead to discrete artifacts or elevated baselines in ion chromatograms.

Samples that contain particles larger than 0.45 microns and reagent solutions that contain particles larger than 0.20 microns should be filtered to prevent damage to instrument columns and flow systems.

11.1.4 Safety

The calibration standards, samples, and most reagents pose no hazard to the analyst. Protective clothing and safety glasses should be worn when handling concentrated sulfuric acid.

11.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples are collected in deionized water-washed containers and filtered without adding any preservative. Aliquot containers are filled completely (i.e., no headspace) and stored at 4 °C in the dark when not in use.

11.3 EQUIPMENT AND SUPPLIES

11.3.1 Equipment Specifications

1. Ion Chromatograph--Analytical system complete with ion chromatograph and all accessories (conductivity detector, autosampler, data recording system).

2. Anion Preseparator and Separator Columns--Dionex Series AG-4A and AS-4A or equivalents are recommended for use with the ion chromatograph. AG-3 and AS-3 columns are suitable for older model ion chromatographs.

3. Suppressor Column--Dionex AFS fiber suppressor or AMMS membrane suppressor have been found to be appropriate.

11.3.2 Reagents and Consumable Materials

Unless stated otherwise, all chemicals should be at least ACS reagent grade quality. Also, salts used in preparation of standards should be dried at 105 °C for 2 hours and stored in a desiccator.

1. Deionized Water--Water should meet the specifications for Type I reagent grade water (ASTM, 1984b).

2. Eluent Solution (0.0028M $NaHCO_3$/0.0020M Na_2CO_3)--Dissolve 0.94 g sodium bicarbonate ($NaHCO_3$) and 0.85 g sodium carbonate (Na_2CO_3) in water and dilute to 4 L. This eluent strength may be adjusted for different columns according to the manufactuer's recommendations.

3. Fiber Suppressor Regenerant (0.025N H_2SO_4)--Add 2.8 mL concentrated sulfuric acid (H_2SO_4, Baker Ultrex grade or equivalent) to 4 L water.

4. Stock Standard Solutions--Store stock standards in clean polyethylene bottles (cleaned with deionized water only, using the procedure described in Appendix C) at 4 °C. Prepare monthly.

   a. Bromide Stock Standard Solution (1,000 mg L^{-1} Br^-)--Dissolve 1.2877 g sodium bromide (NaBr) in water and dilute to 1.000 L.

   b. Chloride Stock Standard Solution (200 mg L^{-1} Cl^-)--Dissolve 0.3297 g sodium chloride (NaCl) in water and dilute to 1.000 L.

   c. Fluoride Stock Standard Solution (1,000 mg L^{-1} F^-)--Dissolve 2.2100 g sodium fluoride (NaF) in water and dilute to 1.000 L.

   d. Nitrate Stock Standard Solution (200 mg L^{-1} NO_3^-)--Dissolve 0.3261 g potassium nitrate (KNO_3) in water and dilute to 1.000 L.

   e. Phosphate Stock Standard Solution (1,000 mg L^{-1} P)--Dissolve 4.3937 g potassium phosphate (KH_2PO_4) in water and dilute to 1.000 L.

   f. Sulfate Stock Standard Solution (1,000 mg L^{-1} SO_4^{-2})--Dissolve 1.8141 g potassium sulfate (K_2SO_4) in water and dilute to 1.000 L.

5. Mixed Resolution Sample (mg L^{-1} F^-, 2 mg L^{-1} Cl^-, 2 mg L^{-1} NO_3^-, 2 mg L^{-1} P, 2 mg L^{-1} Br^-, 5 mg L^{-1} SO_4^{-2})--Prepare by appropriate dilution and mixing of the stock standard solutions.

11.4 PREPARATION

11.4.1 Calibration and Standardization

Each day (or work shift) for each analyte, analyze a blank and a series of standards which bracket the expected analyte concentration range. Prepare the standards daily by quantitative dilution of the stock standard solutions. Suggested concentrations for the dilute standards are given in Table 11-1. Prepare a calibration curve for each analyte by plotting peak height versus standard concentration.

TABLE 11-1. SUGGESTED CONCENTRATION OF DILUTE CALIBRATION STANDARDS

	Concentration (mg L^{-1})		
Standard	Cl^-	NO_3^-	SO_4^{-2}
1	0	0	0
2	0.020	0.020	0.20
3	0.10	0.10	0.50
4	0.50	0.50	2.00
5	1.00	1.00	5.00
6	3.00	3.00	10.00

11.5 PROCEDURE

11.5.1 Standard Operating Procedure

1. Set up the IC for operation. Typical operating conditions for a Dionex 2010i IC are given in Table 11-2. Other conditions may be used depending upon the columns and system selected.

2. Adjust detector range to cover the concentration range of samples.

3. Load injection loop (manually or via an autosampler) with the sample (or standard) to be analyzed. Load five to ten times the volume required to thoroughly flush the sample loop. Inject the sample. Measure and record (manually or with a data system) the peak heights for each analyte.

TABLE 11-2. TYPICAL ION CHROMATOGRAPH OPERATING CONDITIONS

IC: Dionex 2010i[a]	Eluent Flow Rate: 2.0 mL min^{-1}
Precolumn: AG-4A[a]	Regenerant: 0.025N H_2SO_4
Separator Column: AS-4A[a]	Regenerant Flow Rate: 3 mL min^{-1}
Suppressor Column: AMMS[a]	Sample Loop Size: 250 µL
Eluent: 0.75mM $NaHCO_3$/2.0mM Na_2CO_3	

Typical Ion Retention Time (min)	
Cl^-	1.8
NO_3^-	4.9
SO_4^{-2}	8.1

[a]Or equivalent.

4. Dilute and reanalyze samples with an analyte concentration exceeding the calibrated concentration range.

11.5.2 Calculations

Compute the sample concentration by comparing the sample peak height with the calibration curve. Report results in mg L^{-1}.

11.6 QUALITY ASSURANCE AND QUALITY CONTROL

11.6.1 Precision and Accuracy

Typical single operator results for surface water analyses are listed in Table 11-3 (O'Dell et al., 1984).

11.6.2 Quality Control Checks

General QC procedures and QC checks are described in Appendix G. After calibration, perform a resolution test. Analyze the mixed standard containing fluoride, chloride, nitrate, phosphate, bromide, and sulfate. Resolution between adjacent peaks should equal or exceed 60 percent. If it is not, replace or clean the separator column and repeat calibration.

TABLE 11-3. SINGLE OPERATOR ACCURACY AND PRECISION (O'Dell et al., 1984)[a]

Ion	Spike ($mg\ L^{-1}$)	Number of Replicates	Mean Percent Recovery	Standard Deviation ($mg\ L^{-1}$)
Cl^-	1.0	7	105	0.14
NO_3^-	0.5	7	100	0.0058
SO_4^{-2}	10.0	7	112	0.71

[a]The chromatographic conditions used by O'Dell were slightly different than those listed in Table 11-2. However, the results are typical of what is expected.

11.7 REFERENCES

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12.0 DETERMINATION OF CHLOROPHYLL a

12.1 OVERVIEW

12.1.1 Scope and Application

This procedure is applicable to the determination of chlorophyll a and pheophytin a concentrations in natural waters of low ionic strength. Chlorophyll a is one of several chlorophylls found in planktonic algae and is commonly measured as an indicator of algal biomass (Shelske, 1984).

12.1.2 Summary of Method

Surface-water samples are filtered in the field, and the phytoplankton retained on a polycarbonate filter are frozen at -20 °C until analysis. The filters are extracted at 4 °C with 95 percent methanol. The fluorescence intensity of the extracted pigments at 660 nm is measured and is compared to the measured intensities of chlorophyll a standards (Stainton, et al., 1977). The extract is then analyzed by reverse-phase, high-performance liquid chromatography (HPLC) with fluorescence detection to allow differentiation between fluorescence from chlorophyll a and from other pigments that fluorescence at 660 nm (Reibiz, et al., 1978).

12.1.3 Interferences

With the fluorometer settings recommended, the instrument responds to chlorophyll a in the extract (Stainton, et al., 1977). However, pheophytin a, chlorophyll b, pheophytin b, and other common pigments also fluoresce at 660 nm, resulting in an overestimate of chlorophyll a (Holm-Hansen and Riemann, 1978). HPLC analysis of the extract allows measurement of the exact amounts of chlorophyll a and pheophytin a.

12.1.4 Safety

Diethyl ether can form potentially explosive peroxides when stored; this can be avoided by storage over a sodium alloy (e.g., Dri-Na). Diethyl ether and dimethylamine are very volatile and, along with ethyl acetate, hexane, and methanol, are extremely flammable (NIOSH/OSHA, 1978; Muir, 1980). All work with these compounds should be performed in a fume hood. Dimethylamine is highly toxic; respirators should be worn if ambient concentrations are above 10 ppm. If dimethylamine is used outside a fume hood, laboratory air concentrations and personnel exposure should be monitored (NIOSH, 1977).

Analysts should be careful when handling concentrated acids. Eye protection should be worn, and work should be carried out in a fume hood. Caution should be exercised to assure that centrifuges and centrifuge heads are firmly fastened and are stable.

12.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Surface-water samples are filtered in the field, and the filter pad with plankton is shipped, frozen, to the processing facility. There the sample is logged in and is shipped to the analytical laboratory. Chlorophyll is extremely light-sensitive, and samples must be protected from exposure to light. All sample-handling operations should be carried out under subdued lighting. In addition, samples should never be exposed to acid vapors. After collection, samples should be kept frozen at -20 °C until analysis.

12.3 EQUIPMENT AND SUPPLIES

12.3.1 Equipment Specifications

1. High performance liquid chromatograph, including:

   a. Fluorescence detector: Excitation filter - 430-470 nm,
      Emission filter - 650-675 nm, blue source.

   b. Rheodyne sampling valve, with 10 to 25-µL sampling loop.

   c. HPLC pumping system, dual piston, constant flow, capable of 2.0 to 3.0 mL min^{-1} at 150 bar.

   d. Integrator--Hewlett Packard 3290 or equivalent.

   e. Reverse-phase HPLC column--5 micron Spheracil, 250 mm x 2.6 mm I.D., or equivalent.

   f. Guard column--Waters C-18 Guard-Pak, or equivalent.

   g. Spectrophotometer--Hewlett Packard 8450 photodiode array with flow cell or equivalent, immediately downstream of the fluorescence detector.

2. Turner Model III fluorometer, or equivalent, equipped as follows:

   a. Cuvettes, 1 cm.

   b. Door with standard cuvette holder.

   c. Excitation filter--Kodak Wratten No. 478 (430-450 nm), or equivalent.

   d. Emission filter--Corning S2-64 (650-675 nm), or equivalent.

3. Spectrophotometer--for use at 650, 666, and 700 nm, with a spectral resolution of 2 nm or less and wavelength precision of ±0.05 nm.

12.3.2 Apparatus

1. Centrifuge, slant-head.

2. Centrifuge tubes--15 mL, graduated, with screw cap.

3. Developing chamber for thin-layer chromatography.

4. Filtration equipment--filters, funnels, filtration flask, vacuum source.

5. Syringes--Hamilton 1710, or equivalent.

6. Vials, with Teflon-lined screw-cap, 10 mL (or greater) capacity.

12.3.3 Reagents and Consumable Materials

1. Acetone--HPLC grade.

2. Chlorophyll a--Chlorophyll a extracted from Anacystis nidulans is free of chlorophyll b. Alternatively, chlorophyll from spinach (which contains chlorophyll b) is available from Sigma Chemical Company, St. Louis, Missouri. Chlorophyll can also be extracted from pale green head-lettuce leaves, spinach, or grasses. Chlorophyll a can be isolated from extracts by thin-layer chromatographic techniques (Loftus and Carpenter, 1971). The extract, in a mixture of 95 percent methanol and 10 percent NaCl (aq) (50/50, v/v), is extracted with petroleum ether. The organic phase is freed of water by centrifugation and is evaporated to near dryness under a stream of nitrogen. The remaining solution is spotted on an Eastman 6061 silica-gel chromatogram sheet (previously dried at 50 °C for 30 minutes). The chromatogram is developed with 58:30:12 hexane:ethylacetate:dimethylamine. Chlorophyll a (R_f = 7.4) and chlorophyll b (R_f = 7.1) spots are cut out and are extracted into acetone. Store all chlorophyll standards in the dark at -20 °C.

3. Chlorophyll b--Chlorophyll b can be purchased from Sigma Chemical Company, St. Louis, Missouri, as a crystalline solid. Chlorophyll b can also be isolated by the thin-layer chromatographic techniques described above (Loftus and Carpenter, 1971).

4. Dimethylamine--HPLC Grade.

5. Ethyl Acetate--HPLC Grade.

6. Hexane--HPLC Grade.

7. Hydrochloric acid, 0.12M--Add 1 volume concentrated HCl to 100 volumes deionized water.

8. Methanol--HPLC Grade.

9. Methanol, 95% (v/v)--Add 5 volumes deionized water to 95 volumes methanol. Mix well.

10. Mobile phase for HPLC--Methanol:acetone:water, 68:27:5 by volume. Store over magnesium carbonate, tightly capped, in a cool, well-ventilated place. Do not allow prepared mixtures to evaporate.

11. Nitrogen--High purity.

12. Petroleum Ether--ACS reagent grade.

13. Silica-Gel Chromatography Paper--Eastman 6061, or equivalent.

14. Sodium Chloride--ACS reagent grade.

15. Water--Water used for preparations should conform to the standards for Type I reagent grade water (ASTM, 1984).

12.4 PREPARATION

12.4.1 HPLC Calibration

Liquid chromatograph operating parameters listed below, or ones which give resolution equivalent to that shown in Figure 12-1, should be used:

Column:	Reverse-phase C_{18}, 5 µm
Mobile Phase:	Methanol:acetone:water, 68:27:5 (volume)
Detector:	Fluorescence
Wavelengths:	440 nm (ex), 660 nm (em)
Flow Rate:	2.0 mL^{-1} min

Weigh out approximately 1 mg chlorophyll a. Dissolve the weighed chlorophyll a in 50 mL 95-percent methanol in a stoppered glass bottle which is wrapped in aluminum foil to prevent exposure of the solution to light. Handle the stock standard solution with care at all times, and keep it cold (-10°C) and in the dark when not in use. Exposure to acid vapors should be avoided.

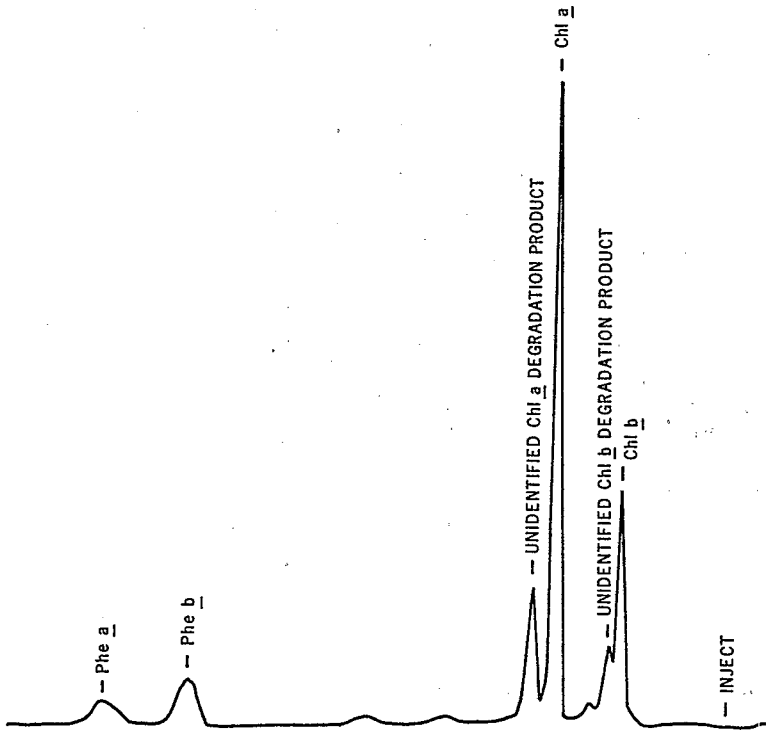

Figure 12-1. Example high performance liquid chromatography chromatogram.

Measure the absorbances of the stock standard in a 1-cm cuvette at 650, 666, and 700 nm, with a 1-cm cuvette of 95-percent methanol in the reference beam. Subtract the absorbance at 700 nm from those at 650 nm and 666 nm to obtain values corrected for nonspecific light losses (e.g., scattering from turbidity). Using these corrected values, calculate the chlorophyll a and chlorophyll b concentrations in the solution:

Chlorophyll a (mg L^{-1}) $16.5\ A_{666} - 8.3\ A_{650}$
Chlorophyll b (mg L^{-1}) $33.8\ A_{650} - 12.5\ A_{666}$

If the concentration of chlorophyll b is greater than 5 percent of that of chlorophyll a, another source of chlorophyll should be used.

Use the procedure described below to prepare mixed calibration standards of chlorophyll a and pheophytin a from the chlorophyll a stock solution at five concentrations spanning the range of 50.0 to 1,000 µg L^{-1}.

NOTE: Prepare all chlorophyll standards under subdued light and store in the dark.

1. Add a known volume of the chlorophyll a stock solution to a volumetric flask. Add 10 percent of that volume of 0.12M HCl to the flask. Swirl the mixture and allow it to stand for 5 minutes.

2. Add 25 mg magnesium carbonate per milliliter of solution and swirl to mix well.

3. After 10 minutes add another measured volume of chlorophyll a stock solution equal to approximately 50 percent of the first volume. Fill the volumetric flask to 75 percent of its volume with HPLC mobile phase.

4. Mix the solution well by inverting the stoppered flask 10 times.

5. Dilute the solution quantitatively and mix well again. Allow the magnesium carbonate to settle or filter the solution in the dark.

6. Analyze each calibration standard by injecting a volume through a nylon sample preparation filter into the HPLC injection loop and injecting it on column (50-µL injections).

7. Tabulate the peak areas of chlorophyll a and pheophytin a.

8. Use these results to prepare calibration functions for chlorophyll a and pheophytin a. If the calibration curve is linear ($r \geq 0.99$ for a linear regression of area on concentration), the mean response factor may be used.

Repeat the calibration each working day using a freshly quantitated stock solution. If the response for chlorophyll a or pheophytin a varies from the expected response by more than 10 percent, prepare fresh stock standards and repeat the calibration.

12.4.2 Fluorometry Calibration

Use the stock standard described above and 95 percent methanol to prepare working standards of chlorophyll a in volumetric flasks wrapped in aluminum foil. Use syringes, not air-displacement micropipets, to measure µL volumes. At least three calibration points should be used for each of the four fluorometer sensitivity ranges (1X, 3X, 10X, 30X). The fluorometer should be zeroed against solvent each time there is a scale change.

Possible dilutions to be used are given in Table 12-1. It is recommended to choose standard concentrations which allow measurement of the instrument response to individual standards on as many scales as possible.

Measure the fluorescence intensity of standard solutions at 660 nm (Stainton, et al., 1977; Baker, et al., 1983). Prepare intermediate and

TABLE 12-1. DILUTIONS OF CHLOROPHYLL a STOCK STANDARD TO MAKE WORKING STANDARDS[a]

Working Standard No.	Volume of Stock Standard	Concentration (µg L^{-1}) (X = stock standard conc. in mg L^{-1})
Blank	0µL	
1	50µL	0.5 X
2	100µL	1.0 X
3	150µL	0.5 X
4	200µL	2.0 X
5	300µL	3.0 X
6	500µL	5.0 X
7	1.00mL	10 X
8	2.00mL	20 X
9	3.00mL	30 X
10	5.00mL	50 X
11	10.00mL	100 X
12	20.00mL	200 X

[a]For each standard, final volume is 100 mL.

additional dilutions as necessary to have three readings for each sensitivity setting (1X, 3X, 10X, 30X). Because of the differences in sensitivity between individual fluorometers, no concentrations that will work with all instruments can be specified here. Plot the scale readings of the chlorophyll a concentration for each sensitivity factor; if the plot is linear ($r \geq 0.99$ for linear regression), the mean scale factor (slope) for each sensitivity setting may be used.

$$F_{(1X,\ 3X,\ 10X,\ 30X)} = \frac{\text{Chlorophyll } a \text{ (µg } L^{-1})}{\text{Scale Reading}}$$

On some fluorometers there will be curvature for high readings on the 1X sensitivity plot. Although the fluorometer calibration is relatively stable, the calibration should be checked daily. A change in instrument response of 10 percent or greater necessitates recalibration as described in Section 12.6.2. The fluorometer should be recalibrated after maintenance, repair, and any changes in configuration.

12.5 PROCEDURE

12.5.1 Sample Extraction

NOTE: Perform sample-handling procedures under subdued light.

1. Place the filter in a 10-mL screw-cap vial. Add a measured volume (3 to 5 mL) of 95 percent (v/v) methanol to the vial to cover the filter and tightly screw on the cap (with Teflon liner).

2. Record the volume to the nearest 0.1 mL. Allow the mixture to stand for 1 hour at 4 °C in the dark, inverting it at 15-minute intervals.

3. After 1 hour, decant the methanolic solution from the vial; filtration or centrifugation of the mixture may be necessary.

4. Store the extract in the dark pending fluorometric and HPLC analysis.

12.5.2 Analysis

From each set of 20 or fewer samples, divide one sample extract into two aliquots and process the two in parallel. Perform the HPLC analysis before the measurement of extract gross fluorescence. Record analytical results for all samples. A form similar to NSWS Form 31, Summary of Analytical Results - Phytopigments (Appendix D) may be used.

12.5.3 Calculations

Calculate the chlorophyll a and pheophytin a concentrations from the HPLC analyses by use of the mean response factor or calibration function (see Section 12.4.1). Calculate the total fluorescence by using the chlorophyll a mean response factor or calibration curve and the total area of the chromatogram. Report results in a format similar to NSWS Form 31, Summary of Analytical Results - Phytopigments (Appendix D) as chlorophyll a ($\mu g\ L^{-1}$), pheophytin a ($\mu g\ L^{-1}$), and total fluorescence ($\mu g\ L^{-1}$ chlorophyll a equivalents).

Calculate the concentration of chlorophyll a (from fluorometry) by using the appropriate scale factor (see Section 12.4.2). Report results as chlorophyll a ($\mu g\ L^{-1}$ uncorrected, fluorometric).

12.6 QUALITY ASSURANCE AND QUALITY CONTROL

12.6.1 Precision and Accuracy

Although these methods have been used in limnological studies, they are still in development, and method performances are not well described. Loftus and Carpenter (1971) report a detection limit of approximately 1 $\mu g\ L^{-1}$ for a fluorometric method. However, the MDL will depend on the size of the sample

filtered (Holm-Hansen and Riemann, 1978). The HPLC method is estimated to have a detection limit of 0.2 μg chlorophyll a on the filter.

12.6.2 HPLC Analysis Quality Control Checks

1. Before processing any samples, demonstrate through analysis of a 95 percent methanol blank that interferences from glassware and reagents are under control.

2. From 10 sequential analyses of the methanol blank, calculate the method detection limit (MDL) using the standard deviation (s) of the detector signal at the retention time of interest:

$$\text{MDL } (\mu g\ L^{-1}) = 3 \times s$$

3. Calibrate the instrument at the start of each working day. In addition, analyze one calibration standard after every 5 samples or at intervals dictated by the quality assurance program; if the mean response changes by more than 10 percent from the initial calibration, evaluate the response with another standard or recalibrate the instrument. Because the distribution of the HPLC response is not known, the interim acceptance criterion of 10 percent has been set, pending availability of better method-performance data.

4. Confirm the identity of peaks identified by HPLC retention time by evaluating the absorption spectrum from the photodiode array detector located immediately downstream from the fluorescence detector. Perform the spectral measurement every 2 seconds.

5. In addition, process a blank daily. A (double-blind) audit sample should be included with each set of 20 or fewer samples. Results from this sample should be evaluated to estimate the relative bias of the measurements. Analyze one extract from each set of 20 or fewer samples in duplicate. A record of the precision of these duplicate measurements should be maintained by the laboratory as a check on analytical precision.

6. Periodically, extracts of phytopigments should be analyzed as a check on the accuracy of HPLC determinations.

The QC results for the HPLC analyses are recorded in a format similar to NSWS Form 33, QC Results - Phytopigments - HPLC, and NSWS Form 34, QC Results - Phytopigments - Time Line (Appendix D).

12.6.3 Fluorometry Quality Control Checks

1. Before analysis of any extracts, make 10 sequential measurements of the fluorescence intensity of a 95 percent methanol reagent blank.

From the estimated standard deviation (SD) of these results, calculate the method detection limit (MDL) by:

$$MDL = 3 \times SD$$

2. In addition, on each working day, or with each batch of 15 or fewer samples, analyze a blank before processing any samples. If the result of the blank analysis is above the MDL, evaluate the system for possible sources of contamination.

3. As a check on instrument response, analyze one or more calibration standards each working day before processing any samples. If the response changes by more than 10 percent from the initial calibration, analyze other standards as a check on the stability of the response. A change of 10 percent or more in instrument response requires recalibration and analysis of two standards each day from that time forward. Because the exact distribution of the instrument response factor is not known, acceptance of variation less than or equal to 10 percent has been set pending better description of method performance. With each batch of samples, one audit sample should be included as a (double-blind) check on combined extraction and analysis relative bias.

4. Analyze one extract from each batch in duplicate. A record of precision of duplicate measurements should be maintained by the laboratory as a check on analytical precision.

The QC results for fluorometry are recorded in a format similar to NSWS Form 32, QC Results - Phytopigments - Fluorometry, and on NSWS Form 34, QC Results Phytopigments - Time Line (Appendix D).

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13.0 DETERMINATION OF DISSOLVED INORGANIC CARBON

13.1 OVERVIEW

This procedure is used to measure the amount of dissolved inorganic carbon (DIC) in natural water and snowpack samples. The amount of carbon in aquatic systems is largely regulated by the bedrock type and, to a lesser extent, photosynthesis and respiration. DIC can exist in several forms which are pH dependent:

pH value	Predominant DIC species
<6	free CO_2
6-10	HCO_3^-
>10	CO_3^{-2}

Aquatic environments containing HCO_3^- and CO_3^{-2} generally have a greater buffering capacity than systems containing predominantly free CO_2. DIC content in combination with pH measurements can, therefore, be a crude indication of the relative buffering capacity of an aquatic system.

13.1.1 Scope and Application

DIC is determined in NSWS processing laboratories using a Dohrmann DC-80 Carbon Analyzer. This method has been written assuming that the DC-80 is being used (Xertex-Dohrmann Corp., 1984). The method, however, can be modified for use with other instruments meeting the same equipment specifications.

The method detection limit (MDL) for DIC determined from replicate analyses of a calibration blank (approximately 0.1 mg L^{-1} DIC) is 0.1 mg L^{-1} DIC. A 1.00-mL sample volume was used to determine the MDL. The applicable analyte concentration range is 0.1 to 50 mg L^{-1} DIC.

13.1.2 Summary of Method

A 1-mL sample is injected into the reaction vessel of the carbon analyzer where the pH is reduced by phosphoric acid in order to convert the existing forms of DIC to CO_2. The CO_2 from the sample is purged from the acid reagent by a continuous flow of nitrogen gas. An infrared (IR) spectrophotometer detects the amount of CO_2 present, and the result in parts per million is displayed and printed.

13.1.3 Interferences

No interferences are known.

13.1.4 Safety

The calibration standards, sample types, and most reagents used in this method pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses should be worn when handling concentrated phosphoric acid.

The nitrogen cylinder should be secured in an upright position. The line pressure should be kept below 40 psi.

13.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples for DIC determination are collected and sealed in syringes; air bubbles are removed. Sealed syringes are kept at 4 °C in the dark until analysis. Analysis should be as close to the time of collection as possible, generally within 24-36 hours. However, a study has shown that DIC does not change significantly over a seven-day period if samples are sealed and stored as described above (Burke, et. al., 1986).

13.3 EQUIPMENT AND SUPPLIES

13.3.1 Equipment Specifications

1. Dohrmann DC-80 Carbon Analyzer or equivalent equipped with high sensitivity sampler (1.00-mL loop).

13.3.2 Apparatus

1. Reagent bottles for DIC standards (equipped with three-valve cap to permit storage under a CO_2-free atmosphere, Rainin No. 45-3200 or equivalent).

2. Luer-Lok syringe valves.

13.3.3 Reagents and Consumable Materials

1. 0.45-µm syringe filters.

2. 60-mL plastic syringes.

3. Nitrogen Gas (99.9 percent)--CO_2-free.

4. Water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).

5. Phosphoric Acid Reagent--

   a. Fill a clean 1-L volumetric flask with approximately 500 mL of deionized water.

b. Add 50 mL concentrated H_3PO_4 and mix thoroughly.

c. Dilute to the 1-L mark with deionized water, mix well, and transfer the solution to a Cubitainer labeled "5% H_3PO_4". Make enough to fill a Cubitainer (4 L).

d. Fill the reaction vessel with the acid reagent (5% H_3PO_4) to just below the arm of the vessel, using a syringe with Tygon tubing.

6. Calibration Standard Stock (1000 mg L^{-1} DIC)--

NOTE: All standards are stored under a CO_2-free atmosphere and refrigerated at 4 °C. Do not allow CO_2 to enter into any of the stock reagents; be sure all valves are closed before removing the syringe from the cap ports.

a. Fill a labeled 1-L volumetric flask with approximately 500 mL of deionized water.

b. Weigh 8.826 g of anhydrous Na_2CO_3 and transfer to the 1-L volumetric flask. Mix well.

c. After complete dissolution, dilute to the 1-L mark and mix thoroughly.

d. Turn on the N_2 gas and adjust the flowrate to approximately 200 mL min^{-1} (50 ml per 15 seconds).

e. Use 10-20 mL of the stock solution to rinse a reagent bottle labeled "Calibration Standard Stock"; then transfer the solution to a reagent bottle. Tighten the cap firmly and record the date on the reagent bottle.

f. Attach the purge line from the injector module to the inlet valve port on the reagent bottle cap. Attach a scrubber line (consisting of first one Mallcosorb, and then one Aquasorb cartridge or equivalents) to the gas outlet valve on the cap. Open the purge line and the scrubber line valves (vertical) and close the third, unused valve (horizontal). Turn the N_2 gas line switch (switch labeled "200") on the injector module to the "UP" position for external flow. Check the flow of the N_2 gas through the system by placing the end of the scrubber line into a beaker of water and confirming the presence of bubbles.

g. Purge the headspace with N_2 gas for at least 20 minutes. Be sure to close all valves before removing the purge line and the scrubber line, thus preventing CO_2 contamination.

7. Daily Calibration Standard (10.00 mg L^{-1} DIC)--

NOTE: Prepare daily, immediately prior to use.

a. Fill a labeled 500-mL volumetric flask with approximately 250 mL of deionized water.

b. Remove the calibration standard stock from the refrigerator. Attach a syringe labeled "Calibration Standard Stock" to the outlet valve of the reagent bottle cap using the female Luer-Lok adapter. Open the valve and withdraw 5 to 10 mL of the stock solution. Close the valve. Rinse the syringe with standard, and empty into a labeled 50-mL beaker. Rinse the beaker. Refill the syringe with approximately 20 mL of stock solution and empty into the beaker.

c. Using a calibrated 1 to 5 mL micropipet, rinse the pipet tip with the standard solution and add 5.000 mL of calibration standard stock to the flask and mix well. Dilute to the 500-mL mark with deionized water and mix thoroughly. Rinse a reagent bottle labeled "10 mg L^{-1} Calibration Standard" with 10 to 20 mL of solution and transfer solution to the reagent bottle. Tighten the cap and purge headspace as described in steps 6f and 6g.

8. QC Standard Stock (1,000 mg L^{-1} DIC)--Repeat step 6, calibration standard stock procedure using a separate source of anhydrous Na_2CO_3. Label a separate set of glassware "QC Standard Stock".

NOTE 1: This solution is prepared the same way as the calibration standard stock. A dilution of the calibration stock is used to calibrate the carbon analyzer whereas dilutions of the QC stock are used to check the function of the instrument.

NOTE 2: Prepare weekly.

9. Daily QC Standards--

NOTE 1: Prepare daily, immediately prior to use.

NOTE 2: If there are more than 30 samples, make a double batch of the 2-mg L^{-1} QC standard in a 1-L volumetric flask.

a. Fill two 500 mL volumetric flasks with approximately 250 mL of deionized water. Label one flask: "2 mg L^{-1} QC Standard" and the other "20 mg L^{-1} QC Standard."

b. Follow the procedure for Daily Calibration Standard, step 7b, substituting the QC standard stock and syringe and beaker labeled "QC Standard Stock". Use this beaker to prepare the 2-mg L^{-1} and 20-mg L^{-1} DIC QC standards.

c. 2-mg L^{-1} QC Standard--Using a calibrated 200-1,000 µL micropipet, rinse the pipet tip with the QC solution and deliver 1.000 mL QC Standard Stock to the flask and mix well. Dilute to the 500 mL-mark and mix again. Rinse a labeled reagent bottle with 10 to 20 mL of solution and transfer the solution to the bottle. Tighten the cap and purge the headspace for 20 minutes (steps 6f and 6g).

d. 20-mg L^{-1} QC Standard--Using a calibrated 1-5 mL micropipet, rinse the pipet tip with the QC solution and deliver 10.00 mL of QC standard stock to the appropriately labeled flask as described in step c. After rinsing, transfer the solution to a labeled reagent bottle and purge for at least 20 minutes (steps 6f and 6g).

13.4 PREPARATION

13.4.1 Instrument Setup

CAUTION: Ultraviolet (UV) lamps are not installed for DIC analysis. Do not turn on UV lamp power switch on the reactor module. The plug for the lamp (inside reactor module) should be covered with electrical tape. DO NOT TOUCH - HIGH VOLTAGE.

NOTE 1: See flowcharts for dissolved inorganic carbon analysis (Figures 13-1 and 13-2).

NOTE 2: See Figures 13-3 to 13-5 for illustrations of the Dohrmann carbon analyzer.

NOTE 3: Allow the IR detector to warm up at least 24 hours prior to initial use. The IR detector should remain on at all times.

1. Check that all electrical and plumbing connections are complete and that the tin scrubber is in place. (See instrument manual and Figures 13-3 to 13-5).

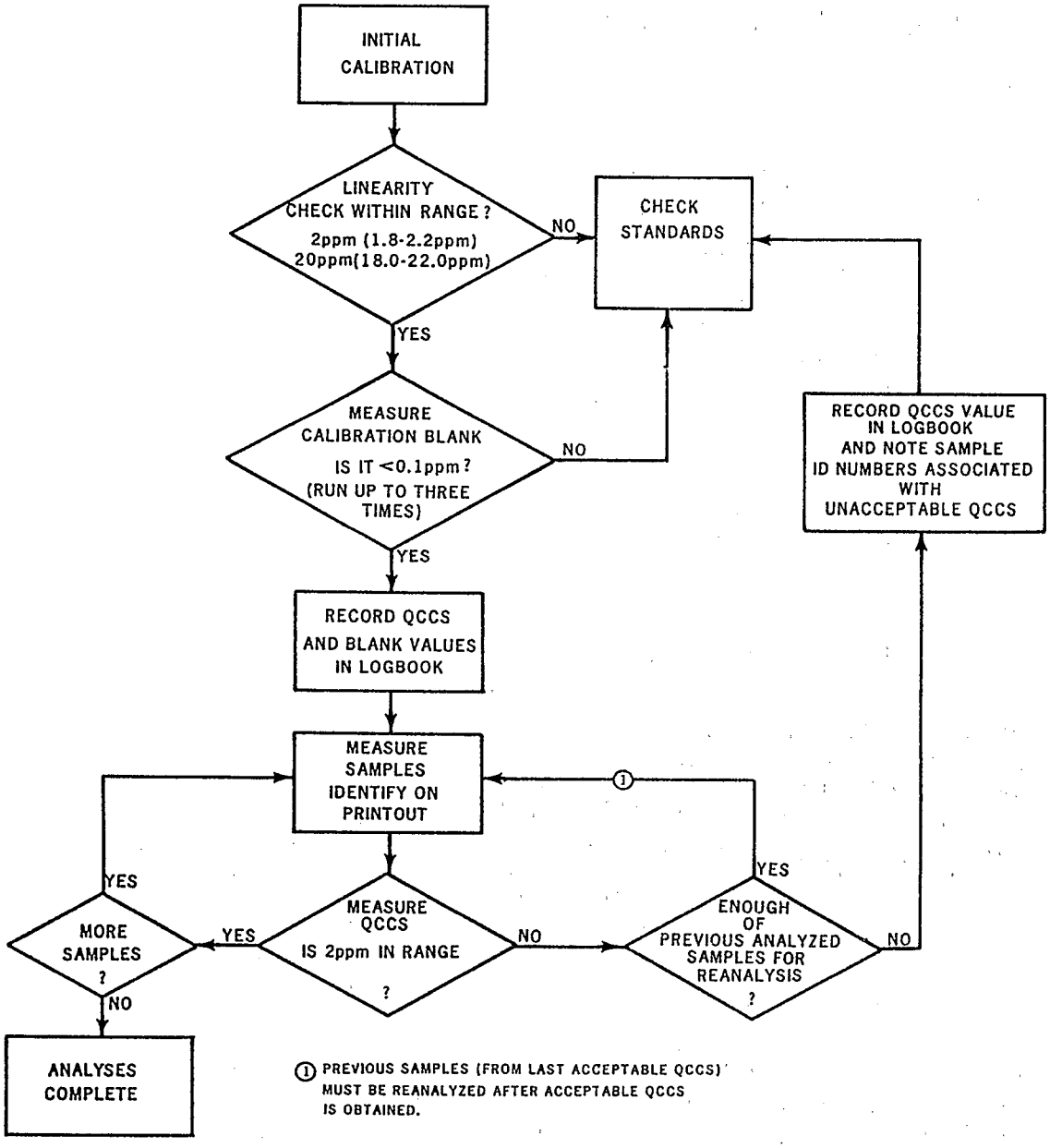

Figure 13-1. Flowchart for dissolved inorganic carbon analysis.

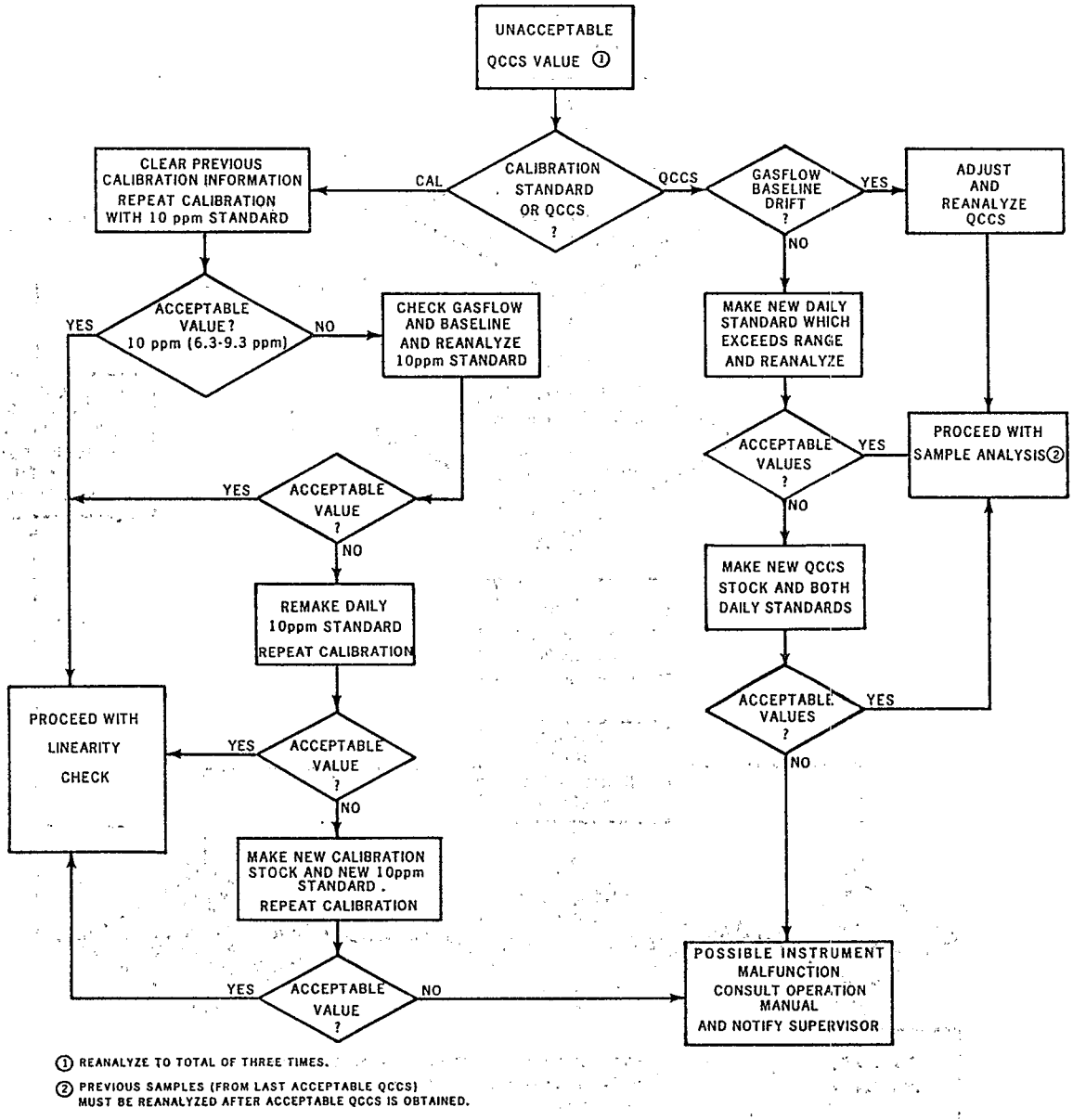

Figure 13-2. Troubleshooting flowchart for dissolved inorganic carbon analysis.

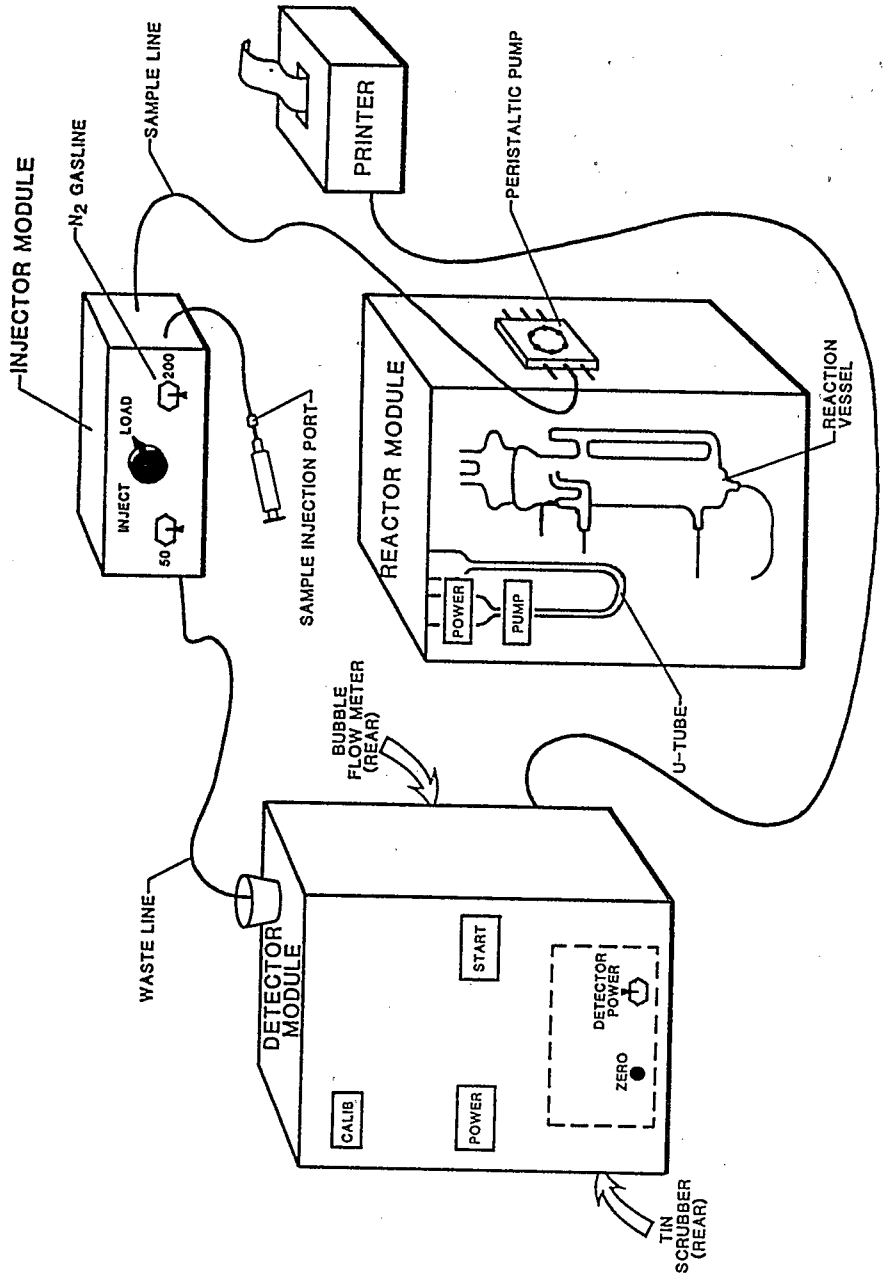

Figure 13-3. Diagram of the Dohrmann carbon analyzer.

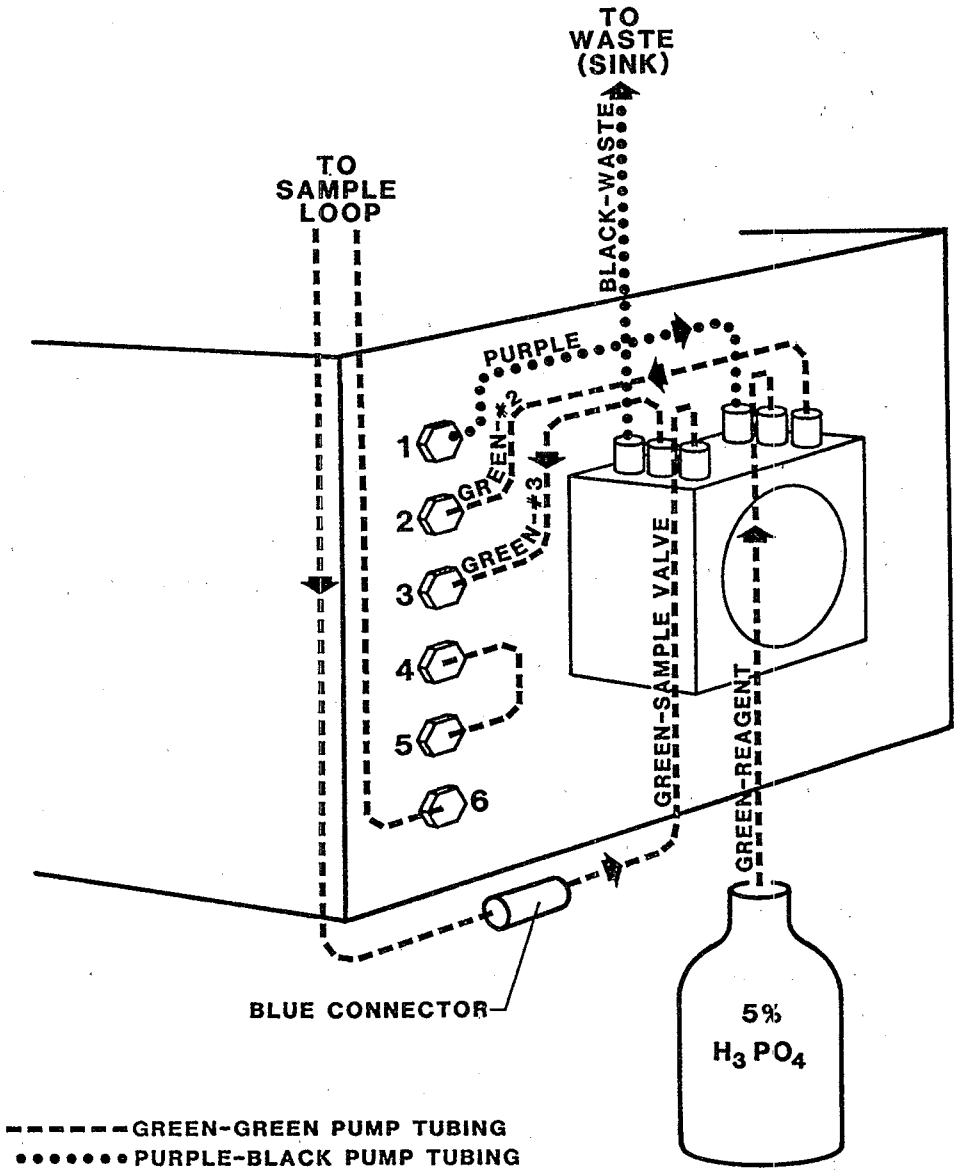

Figure 13-4. External plumbing of the Dohrmann carbon analyzer.

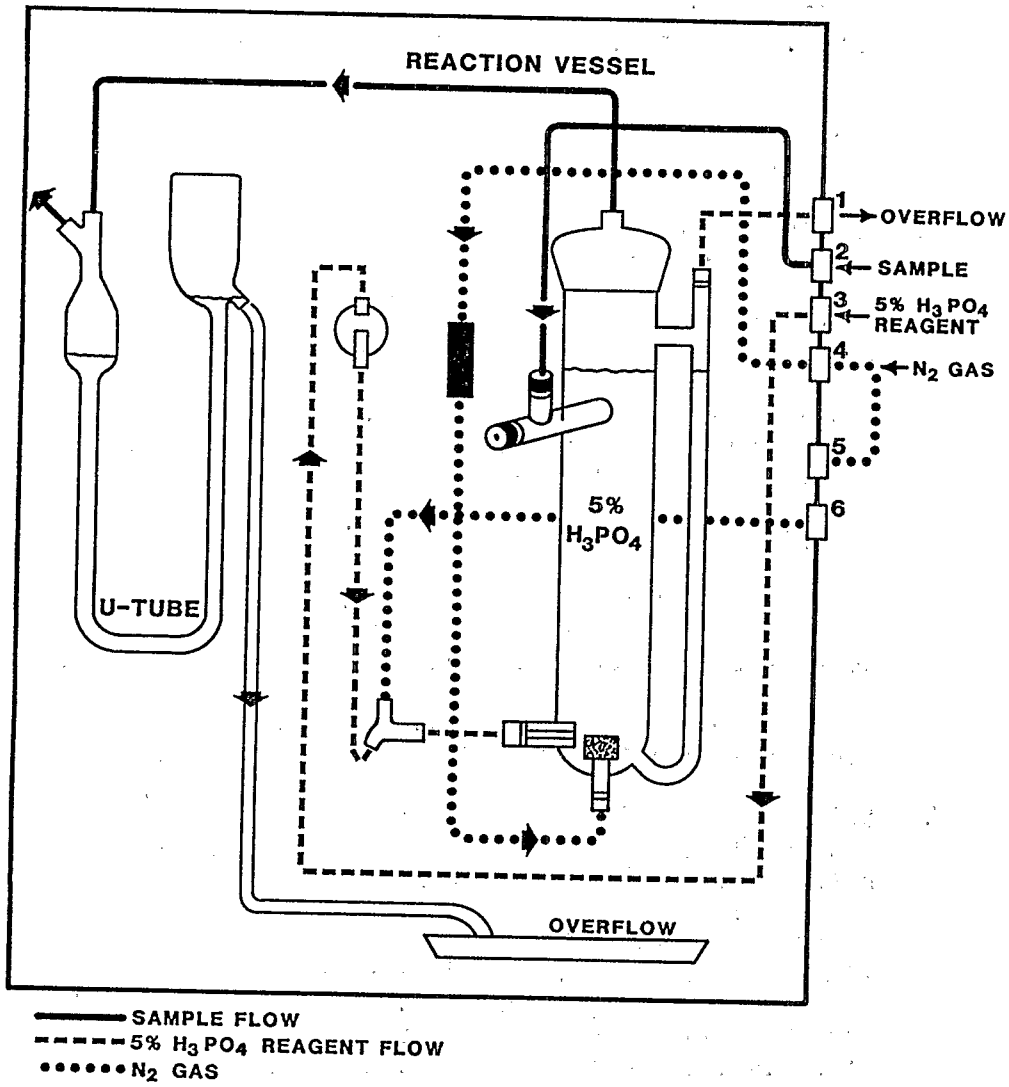

Figure 13-5. Internal connections of the Dohrmann carbon analyzer.

2. Insert the reagent line from the pump into the 5 percent H_3PO_4 container.

3. Place the waste line from the injector module into an empty beaker. Connect the wasteline from the reactor module to a wasteline leading to the sink.

4. Plug the unit in and turn on the IR detector only (switch inside panel of detector).

5. Select the sample volume switch position (1 mL). Select analysis mode switch position (TOC).

6. Fill the U-tube with deionized water to the base of the bulb and place an empty container underneath the U-tube overflow line.

7. Connect the bubble flowmeter with Tygon tubing to the "OUT" port at the back of the detector module using the magnetic metal clamps to secure the bubble flowmeter.

13.4.2 Initial Calibration

1. Check the levels of the acid reagent in the Cubitainer and the reaction vessel. Check the level of deionized water in the U-tube. Be sure all reagent lines and drain lines are connected.

2. Turn on the white power switches on the reactor and detector modules. Start the reagent pump (the white switch on the reactor module).

3. Switch the gas from external flow (purge) to internal flow by switching the N_2 gas line switch to the "Down" position. Adjust the flow rate to 200 mL min^{-1} (50 mL per 15 seconds) using the bubble flowmeter. The values should be 15.0 ± 0.1 seconds. Record the average flow rate of three trials in the logbook. If a rotometer is used, use a bubble flowmeter to set gas flow initially, then attach the rotometer and record the reading. Thereafter adjust the N_2 regulator to the position giving this reading on the rotometer. Recheck the rotometer accuracy every 6 months with the bubble flowmeter. Record the N_2 tank and N_2 regulator readings (psi) in the logbook.

4. Adjust the detector to a baseline of approximately 0.0100 using the "ZERO" knob on the panel inside front of detector module. Record the initial and final baseline readings in the logbook.

5. Fill a syringe labeled "Blank" with deionized water directly from the outlet on the reverse osmosis system using Tygon tubing. Let the

water run about 5 seconds before the collection of water. Avoid any exposure to air. Attach a syringe valve and evacuate any air bubbles. Attach the syringe to the syringe port on the injector module.

6. With the injector module knob on "LOAD", rinse the sample loop with 10-15 mL of deionized water. Inject an additional 5 mL of deionized water. Close the syringe valve and turn knob to "INJECT" and press the "START" button on the detector module. When the instrument signals the completion of sample analysis (it beeps), turn knob to "LOAD". Deionized water should read less than 0.100 ppm. Reanalyze a fresh deionized water sample if this limit is exceeded.

7. Uncalibrate the instrument by pressing the "CALIB" button on the electronics module until the light goes out.

8. Attach the syringe labeled "10 mg L^{-1}" to the valve on the outlet of the reagent bottle containing the daily 10 mg L^{-1} calibration standard. Open the valve and withdraw 5 to 10 mL of solution. Close the valve and rinse syringe. Refill the syringe with 40-50 mL of calibration standard, avoiding exposure to air. Close the valve. Attach the syringe valve to the syringe and evacuate any air bubbles.

9. Attach the syringe to the injection port. The injector module knob should be in the "LOAD" position. Load the sample loop by injecting 5 mL of sample. Close the syringe valve, turn the knob to "INJECT", and press "START". After sample analysis is completed, record the sample identification number on the printout.

10. The acceptable range for the calibration standard is 7.8 ± 1.5 mg L^{-1}. The display prints an error message if the value is outside this range.

11. If the value is out of range, recheck the gas flow and baseline readings. If either of these is the problem, adjust as described in Section 13.4.2, steps 3 and 4 and depress "CALIB" until the light comes on. Press "CALIB" again to uncalibrate (light out) and reanalyze the sample. If the gas flow or the baseline readings are not the problem, remake the daily 10 mg L^{-1} calibration standard and recalibrate the instrument as described above. If the values are still not in range, remake the weekly calibration stock solution and prepare fresh 10 mg L^{-1} standard solution and recalibrate.

12. Repeat step 9 two more times, leaving the syringe in position throughout the analyses. There is no need to rinse between injections.

13. After three sample runs of the 10 mg L^{-1} calibration standard, push "CALIB" until the light comes on. The instrument is now calibrated and the calibration average and adjusted calibration value will appear on the printout. Record these values in the logbook.

13.4.3 Linearity Check

NOTE 1: Rinse the sample loop with 5 to 10 mL of deionized water between injections of the standards.

NOTE 2: Close the syringe valve after each injection of a sample.

1. Using the 2 mg L^{-1} QC standard in a labeled syringe, repeat steps 8 and 9 of Section 13.4.2. The acceptable range is 2.0 ± 0.2 mg L^{-1}.

2. Using the 20-ppm QC standard in a labeled syringe, repeat steps 8 and 9 of Section 13.4.2. The acceptable range is 20.0 ± 2.0 mg L^{-1}.

3. If both values are within acceptable limits, record the sample identification on the printout and record the values in the logbook.

4. If any value is not within range:

   a. Analyze a second sample from the same syringe. If the result is within limits, see step 5; if is not within limits obtain a new syringe and analyze a fresh sample. If the analysis of the sample from the second syringe is within limits, see step 5; if it is not, see step 4b.

   b. Recheck the gas flow and baseline readings. If the baseline or gas flow has drifted, reset as described in steps 3 and 4 of Section 13.4.2, and run the following samples: 20 mg L^{-1}, 10 mg L^{-1}, 2 mg L^{-1}, and a blank. If these values are all within their acceptable limits, continue with step 5; if they are not, see step 4c.

   c. If the gas flow or baseline reading has not drifted, check the accuracy of the QC solutions by remaking any daily QC standard that exceeds the acceptable range and then reanalyzing. If the values are in the acceptable range, proceed with step 5; if they are not, see step 4d.

d. Remake QC standard stock and then remake both of the daily QC solutions. Analyze both the 2-mg L^{-1} and 20-mg L^{-1} QC solutions. If both sample values are within range, continue with step 5 below. If they are not within range, consult the instrument operations manual.

5. Repeat steps 5 and 6 of Section 13.4.2 for the analysis of the "Calibration Blank." The value for the blank should be less than 0.100 ppm. Reanalyze a fresh blank sample if the value is outside of acceptable limits. Record value in logbook and identify on printout.

13.4.4 Maintenance

1. Weekly, replace all of the pump tubes. Remove the reaction vessel and rinse it with deionized water, then refill it with fresh 5 percent H_3PO_4. Remove the U-tube, rinse and refill it with fresh deionized water. Check all tubing connections and replace tubing, connectors, or septa as necessary.

2. Make new Calibration and QC solutions weekly.

3. Weekly, wipe down the inside of the reactor module and the outside of the carbon analyzer with a damp Kimwipe to remove any spilled acid, reagent, or dust.

4. Weekly, replace the glass wool and tin beads of the tin scrubber.

5. Change Aquasorb and Mallcosorb cartridges or scrubber lines when color change is evident.

6. Periodically check the printer for an adequate paper supply.

7. Disassemble the pump roller pressure plate and inspect, clean, and lubricate the bearings after 3 months of daily use.

13.5 PROCEDURE

13.5.1 Sample Analysis

NOTE 1: All sample syringes should be refrigerated at 4 °C.

NOTE 2: A single batch should be analyzed on a single carbon analyzer. Record the instrument identification number in the logbook.

NOTE 3: Do not remove air bubbles from the sample syringes. These bubbles may be the result of CO_2 degassing which may occur between the time of sample collection and analysis. If bubbles are present, record this information in the logbook.

NOTE 4: Record information concerning any damage to the syringe or syringe valve in the logbook.

1. Obtain a sample syringe from the refrigerator and attach a 0.45 µm Acro-disk or equivalent syringe filter to the syringe valve.

2. Rinse the sample loop with 5 to 7 mL of deionized water. Inject a 5-mL portion of the sample. Turn the knob to "INJECT" and push the "START" button to begin analysis. After the sample data is printed, write the sample identification (ID) number next to its printed concentration value.

3. Between injections, rinse the sample loop with 5 to 7 mL of deionized water.

13.5.2 Data Reporting

1. Tape the DIC printout into the logbook and make sure all sample values are identified with the correct sample ID number.

13.5.3 Cleanup

1. Acid wash all glassware by rinsing in succession with deionized water (once), 5 percent nitric acid (once), and deionized water (three times).

2. After the logbook has been checked by the laboratory supervisor and all other analyses are complete, discard the syringe filters empty syringes, and soak all syringe valves in deionized water.

3. Turn off the "PUMP" switch on the reactor module and disengage the pump roller pressure plate. Turn off the power switches on the reactor and the detector modules.

4. Turn off the N_2 gas at its source and remove the reagent line from the acid reagent container (5 percent H_3PO_4).

13.6 QUALITY ASSURANCE AND QUALITY CONTROL

13.6.1 Precision and Accuracy

In a multiple lab study using two lake samples containing 0.42 and 9.9 DIC, respectively, the relative standard deviations were 19 percent (n=41) and 5.2 percent (n=7), respectively.

In a single laboratory (EMSL-Las Vegas), using sodium carbonate in deionized water at concentrations of 0.150, 0.500, 2.00, and 30.00 mg L^{-1} DIC, recoveries were 94, 101, 101, and 98 percent, respectively.

13.6.2 Quality Control Checks

1. 2 mg L^{-1} QC standard--Analyze a 2-mg L^{-1} QCCS initially, after every 10 sample injections or at intervals determined by the quality assurance program. Determine if the value obtained is within range (2.0 ± 0.2 mg L^{-1}). If the value is within range, proceed with sample analysis. If the value is not in range, inject a second 5-mL aliquot. If this value still is not acceptable, fill a new syringe with QCCS and reanalyze. If the reanalyzed value is not within the acceptable range, refer to Section 13.4.3, steps 4b through 4d.

2. Detection Limit--Determine the detection limit by analyzing 20 blank samples. The detection limit is defined as three times the standard deviation and should be less than 0.100 mg L^{-1}.

3. Laboratory Duplicate--Measure one sample in duplicate. It is not necessary to remove the syringe or rinse between injections. The duplicate value should be within 10 percent of the routine value. If it is not, reanalyze a third time or analyze another sample in duplicate.

13.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

Burke, E. M., D. C. Hillman, and E. M. Heithmar, 1986. Stability of pH and DIC in sealed syringe samples. Presented at the Rocky Mountain Conference on Analytical Chemistry, August 3-5, Denver, Colorado.

Xertex-Dohrmann Corporation, 1984. DC-80 Automated Laboratory Total Organic Carbon Analyzer Systems Manual, 6th ed. Xertex-Dohrmann, Santa Clara, California.

14.0 DETERMINATION OF DISSOLVED ORGANIC CARBON AND DISSOLVED INORGANIC CARBON

14.1 OVERVIEW

14.1.1 Scope and Application

This method is applicable to the determination of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) in natural surface waters. This method is written assuming a Dohrmann-Xertex DC-80 Analyzer is being used, but any instrumentation having similar operating characteristics may be used instead.

The method is applicable over the concentration range 0.1 to 30 mg L^{-1} DIC or DOC. The method detection limit is approximately 0.8 mg L^{-1} DOC and 0.1 mg L^{-1} DIC, as determined from replicate analyses of a blank sample.

14.1.2 Summary of Method

DOC is determined (after external sparging to remove DIC) by ultraviolet (UV)-promoted persulfate oxidation, followed by infrared (IR) detection. DIC is determined directly by acidifying to generate CO_2 followed by IR detection (U.S. EPA, 1983; Xertex-Dohrmann, 1984).

14.1.3 Interferences

No interferences are known.

14.1.4 Safety

The sample types, standards, and most reagents pose no hazard to the analyst. Protective clothing (lab coat) and safety glasses should be worn when preparing reagents and operating the instrument.

14.2 SAMPLE COLLECTION, PRESERVATAION, AND STORAGE

The sample for DOC analysis is filtered and preserved (pH adjusted to less than 2 with sulfuric acid). The sample is stored at 4 °C when not in use.

The sample for DIC analysis is not filtered or preserved. It is stored at 4 °C in the dark and is filled completely (i.e., no headspace) to minimize atmospheric exposure.

14.3 EQUIPMENT AND SUPPLIES

14.3.1 Equipment Specifications

1. Carbon analyzer--This method is based on the Dohrmann DC-80 Carbon Analyzer equipped with a high-sensitivity sampler. The essential components of the instrument are a sample injection valve, UV-reaction chamber, IR detector, and integrator. The injection valve should have a 5- to 7-mL sample loop and should permit injection with a standard Luer-Lok syringe. Other instruments having similar performance characteristics also may be used.

14.3.2 Apparatus

1. Reagent bottle for standard storage--Heavy-wall borosilicate glass bottle with three two-way valves in the cap. Suitable suppliers include (but are not limited to) Rainin Instrument Co. (Catalog No. 45-3200) and Anspec Co. (Catalog No. H8332).

2. Disposable plastic Luer-Lok syringes or equivalent (for DIC samples) equipped with Luer-Lok syringe valves.

14.3.3 Reagents and Consumable Materials

1. DOC Calibration Stock Solution (2,000 mg L^{-1} DOC)--Dissolve 0.4250 g potassium hydrogen phthalate (KHP, primary standard grade, dried at 105 °C for 2 hours) in water, add 0.10 mL phosphoric acid (ACS reagent grade), and dilute to 100.00 mL with water. Store in an amber bottle at 4 °C. Prepare monthly.

2. Dilute Daily DOC Calibration Solutions--Using micropipets and volumetric pipets, prepare the following calibration standards daily:

   a. 0.500 mg L^{-1} DOC - dilute 0.125 mL of DOC calibration stock solution plus 0.5 mL of phosphoric acid to 500.00 mL with water.

   b. 1.000 mg L^{-1} DOC - dilute 0.250 mL of DOC calibration stock solution plus 0.5 mL of phosphoric acid to 500.00 mL with water.

   c. 5.000 mg L^{-1} DOC - dilute 1.250 mL of DOC calibration stock solution plus 0.5 mL of phosphoric acid to 500.00 mL with water.

   d. 10.00 mg L^{-1} DOC - dilute 2.500 mL of DOC calibration stock solution plus 0.5 mL of phosphoric acid to 500.00 mL with water.

   e. 30.00 mg L^{-1} DOC - dilute 3.750 mL of DOC calibration stock solution plus 0.25 mL of phosphoric acid to 250.00 mL with water.

   Store calibration standards in amber bottles at 4 °C.

3. DOC QC Stock Solution (1,000 mg L^{-1} DOC)--Dissolve 0.5313 g of KHP in water, add 0.25 mL of phosphoric acid, then dilute to 250.00 mL with water. The QC stock solution should be prepared using an independent source of KHP. Store in an amber bottle at 4 °C. Prepare monthly.

4. Dilute Daily DOC QC Solutions--Prepare the following QC samples daily:

   a. 0.500 mg L^{-1} DOC (Detection Limit QC Sample) - dilute 0.250 mL of QC stock solution plus 0.5 mL phosphoric acid to 500.00 mL with water.

   b. 10.00 mg L^{-1} DOC - dilute 2.500 mL of QC stock solution plus 0.25 mL of phosphoric acid to 250.00 mL with water.

   c. 30.00 mg L^{-1} DOC - dilute 3.000 mL of QC stock solution plus 0.1 mL of phosphoric acid to 100.00 mL with water.

   Store QC samples in amber bottles at 4 °C.

5. DIC Calibration Stock Solution (2,000 mg L^{-1} DIC)--Dissolve 4.4131 g of sodium carbonate (Na_2CO_3, primary standard grade, freshly dried at 105 °C for 2 hours) in water and dilute to 250.00 mL with water. Store in a tightly capped bottle under a CO_2-free atmosphere, as described in Section 13.3.3, step 6. Prepare weekly.

6. Dilute DIC Calibration Solutions--Prepare the following calibration standards daily:

   a. 0.500 mg L^{-1} DIC - dilute 0.250 mL of DIC calibration stock solution to 1.000 L with water.

   b. 1.000 mg L^{-1} DIC - dilute 0.250 mL of DIC calibration stock solution to 500.00 mL with water.

   c. 5.000 mg L^{-1} DIC - dilute 1.250 mL of DIC calibration stock solution to 500.00 mL with water.

   d. 10.00 mg L^{-1} DIC - dilute 2.500 mL of DIC calibration stock solution to 500.00 mL with water.

   e. 30.00 mg L^{-1} DIC - dilute 3.750 mL of DIC calibration stock solution to 250.00 mL with water.

   Store calibration standards in tightly capped bottles under a CO_2-free atmosphere.

7. DIC QC Stock Solution (1,000 mg L^{-1} DIC)--Dissolve 2.2065 g of Na_2CO_3 in water and dilute to 250.00 mL with water. The QC stock solution should be prepared using an independent source of Na_2CO_3. Store in a tightly capped bottle under a CO_2-free atmosphere.

8. Dilute DIC QC Solutions--Prepare the following QC samples daily:

   a. 0.500 mg L^{-1} DIC (Detection Limit QC Sample) - dilute 0.250 mL of QC stock solution to 500.00 mL with water.

   b. 10.00 mg L^{-1} DIC - dilute 2.500 mL of QC stock solution to 250.00 mL with water.

   c. 30.00 mg L^{-1} DIC - dilute 3.000 mL of QC stock solution to 100.00 mL with water.

9. Potassium Persulfate Reagent (2 percent w/v)--Dissolve 20 g of potassium persulfate ($K_2S_2O_8$, ACS reagent grade or better) in water, add 2.0 mL phosphoric acid, then dilute to 1.0 L with water. This reagent is used for DOC analyses.

10. Phosphoric Acid Reagent (5 percent v/v)--Dilute 50.0 mL concentrated phosphoric acid (ACS reagent grade) to 1.0 L with water. This reagent is used for DIC analyses.

11. Water--Water should meet the specifications for Type I reagent grade water (ASTM, 1984).

14.4 PREPARATION

14.4.1 Instrument Setup

1. DOC--Set up the instrument according to the manufacturer's instructions. Adjust all liquid and gas flow rates. Turn on UV lamp and allow the system to stabilize. The IR detector should warm up for at least 2 hours. For best results, leave the IR detector on at all times.

2. DIC--Set up the instrument according to the manufacturer's instructions. Adjust all liquid and gas flow rates, using 5 percent phosphoric acid as the reagent. Do not turn on the UV lamp. Allow the system to stabilize.

14.4.2 DOC Calibration

For the range of interest (0 to 30 mg L^{-1} DOC), the instrument is designed to be calibrated with a single 10.00 mg L^{-1} DOC standard. The linearity of the calibration is checked with the QC samples. If acceptable results are not

obtained for the QC samples, the instrument should be calibrated using the procedure described below for routine calibration.

1. Sparge the 10.00-mg L^{-1} calibration standard for 5 to 6 minutes with CO_2-free gas.

2. Following the instructions in the operating manual, calibrate the instrument using three replicate analyses of the 10.00-mg L^{-1} standard.

3. Analyze a system blank and a reagent blank. Both should contain less than 0.1 mg L^{-1} DOC. If either contains more DOC, then the water is contaminated. In this case, all standards and reagents should be prepared again with DOC-free water, and the instrument should be recalibrated.

4. After sparging for 5 to 6 minutes, analyze the 0.500, 10.00, and 30.00 mg L^{-1} QC samples. Acceptable results are 0.50 ± 0.10, 10.0 ± 0.5, and 30.0 ± 1.5 mg L^{-1}, respectively. If acceptable results are obtained for all QC samples, the instrument calibration is complete. If acceptable results are not obtained for one or more of the QC samples, continue with the steps below.

5. Sparge the 0.500, 1.000, 5.000, 10.00, and 30.00 mg L^{-1} DOC calibration standards for 5 to 6 minutes with CO_2-free gas.

6. Erase the instrument calibration (if present). Analyze each standard and record the uncalibrated response.

7. Plot the response versus standard concentration. Draw or calculate (using linear regression) the best calibration curve.

8. Analyze a system blank and a reagent blank. From their response and the calibration curve, determine their concentrations. Both should contain less than 0.1 mg L^{-1} DOC. If either contains more than 0.1 mg L^{-1} DOC, then the water is contaminated. In this case, the standards and reagents should be prepared again using DOC-free water, and the instrument should be recalibrated.

9. After sparging for 5 to 6 minutes, analyze the 0.500 and 10.00 mg L^{-1} QC samples. From their response and the calibration curve, determine the concentration of each QC sample. Acceptable results are 0.5 ± 0.1 and 10.0 ± 0.5 mg L^{-1} DOC, respectively. If unacceptable results are obtained for one or more of the QC samples, the calibration standards should be prepared again and reanalyzed. Acceptable results should be obtained prior to sample analysis.

14.4.3 DIC Calibration

The calibration procedure is identical to that for DOC with the exception that the DIC standards are not sparged prior to analysis.

14.5 PROCEDURE

14.5.1 DOC Standard Operating Procedure

1. Calibrate the carbon analyzer for DOC.

2. Sparge samples with CO_2-free gas for 5 to 6 minutes (sparge gas should have a flow of 100 to 200 ml min^{-1}). Load and analyze the sample as directed by the instrument operating manual.

14.5.2 DIC Standard Operating Procedure

NOTE: For quality assurance reasons, it is very important that the DIC is measured at the same time pH is measured.

1. Calibrate the carbon analyzer for DIC.

2. Routine Determination--Rinse a clean syringe with sample. Withdraw a fresh sample portion into the syringe. Attach a syringe filter (0.45 µm) and simultaneously filter the sample and inject it into the carbon analyzer. Analyze as directed by the instrument operating manual.

3. Air-Equilibrated Determination--Equilibrate a sample with 300 ppm CO_2 in air (see Section 5.5.3). Rinse a clean syringe with the air-equilibrated sample. Withdraw a fresh portion of the air-equilibrated sample and attach a syringe filter (0.45 µm). Simultaneously filter and inject the sample into the carbon analyzer. Analyze as directed by the instrument operating manual.

14.5.3 Calculations

1. If the routine calibration procedure is satisfactory, the instrument outputs the sample results directly in mg L^{-1}. DOC or DIC calculations are not necessary.

2. If a calibration curve is necessary, determine the sample concentration by comparing the sample response to the calibration curve. Report results as mg L^{-1} DOC or DIC.

14.6 QUALITY ASSURANCE AND QUALITY CONTROL

14.6.1 Precision and Accuracy

1. DOC--In a single laboratory (EMSL-Cincinnati), using raw river water, centrifuged river water, drinking water, and the effluent from a carbon column which had concentrations of 3.11, 3.10, 1.79, and 0.07 mg L^{-1} total organic carbon, respectively, the standard deviations from 10 replicates were 0.13, 0.03, 0.02, and 0.02 mg L^{-1}, respectively (U.S. EPA, 1983).

   In a single laboratory (EMSL-Cincinnati), using potassium hydrogen phthalate in distilled water at concentrations between 5.0 and 1.0 mg L^{-1} total organic carbon, recoveries were 80 percent and 91 percent, respectively (U.S. EPA, 1983).

2. DIC--In a multiple lab study using two lake samples containing 0.42 and 9.9 DIC, respectively, the relative standard deviations were 19 percent (n=41) and 5.2 percent (n=7), respectively.

   In a single laboratory (EMSL-Las Vegas), using sodium carbonate in deionized water at concentrations of 0.150, 0.500, 2.00, and 30.00 mg L^{-1} DIC, recoveries were 94, 101, 100, and 98 percent, respectively.

14.6.2 Quality Control Checks

In addition to the QC inherent in the calibration procedures (Section 14.4), the QC procedures described in Appendix G should be performed.

14.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

U.S. Environmental Protection Agency, 1983 (revised). Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Xertex-Dohrmann Corporation, 1984. DC-80 Automated Laboratory Total Organic Carbon Analyzer Systems Manual, 6th ed. Xertex-Dohrmann, Santa Clara, California.

15.0 DETERMINATION OF TOTAL DISSOLVED FLUORIDE BY ION-SELECTIVE ELECTRODE

15.1 OVERVIEW

15.1.1 Scope and Application

This method is applicable to the determination of total dissolved fluoride in natural surface waters. A fluoride ion-selective electrode (ISE) is used in this method. The applicable concentration range is 0.005 to 2 mg L^{-1} fluoride (F^-). The method detection limit (MDL) is 0.005 mg L^{-1} F^-.

15.1.2 Summary of Method

The total dissolved fluoride in a sample is determined electrometrically using a fluoride ion-selective electrode after addition to the sample of a total ionic strength buffer solution (TISAB). The TISAB adjusts sample ionic strength and pH and breaks up fluoride complexes.

The potential of the fluoride ISE varies logarithmically as a function of the fluoride concentration. A calibration curve is prepared by measuring the potential of known fluoride standards (after TISAB addition) and by plotting the potential versus fluoride concentration (on a semi-log scale). Sample concentrations are determined by comparing the sample potential to the calibration curve.

This method is based on existing methods (U.S. EPA, 1983; Barnard and Nordstrom, 1982; Bauman, 1971; LaZerte, 1984; Kissa, 1983; Warner and Bressan, 1973).

15.1.3 Interferences

The electrode potential is partially a function of temperature. As a result, standards and samples should be equilibrated to the same temperature (±1 °C).

The sample pH should be in the range 5 to 7 to avoid complexation of fluoride by hydronium (pH <5) and hydroxide (pH >7). The addition of TISAB to samples and standards ensures that the pH is maintained in the correct range.

Polyvalent cations may interfere by complexing fluoride, thereby preventing detection by the electrode. The TISAB solution contains a decomplexing agent to avoid potential interferences from polyvalent cations.

Fluoride is ubiquitous. Good laboratory practices and extra care should be used in order to minimize contamination of samples and standards.

15.1.4 Safety

The sample types, calibration standards, and most reagents pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses should be worn when handling concentrated sodium hydroxide.

15.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples are collected in deionized water-washed containers and are filtered without adding any preservative. Aliquot containers should be filled completely (i.e., no headspace). They are stored at 4°C in the dark when not in use.

15.3 EQUIPMENT AND SUPPLIES

15.3.1 Equipment and Apparatus

1. Digital electrometer (pH/mV meter) with expanded mV scale capable of reading 0.1 mV.

2. Combination Reference - Fluoride ion selective electrode.

3. Thermally isolated magnetic stirrer and Teflon-coated stir bar.

15.3.2 Reagents and Consumable Materials

Unless otherwise specified, all chemicals should be ACS reagent grade or better. Use only plasticware (deionized water-cleaned as described in Appendix C) for reagent preparation.

1. TISAB Solution--Add 57 mL glacial acetic acid (Baker Ultrex grade or equivalent), 4 g of CDTA (1,2-cyclohexylene dinitrilo tetraacetic acid) and 58 g of sodium chloride (NaCl, ultrapure) to approximately 500 mL water in a 1-L beaker. Stir to dissolve and cool to room temperature. Adjust the pH of the solution to between 5.0 and 5.5 with 5N NaOH (about 150 mL will be needed). Transfer the solution to a 1-L volumetric flask and dilute to the mark with water. Transfer to a clean polyethylene (LPE) bottle. (Note: Alternatively, commercially available TISAB solution may be used.)

2. Sodium Hydroxide Solution (5N NaOH)--Dissolve 200 g of NaOH in water, cool, then dilute to 1 L. Store in a tightly sealed LPE bottle.

3. Fluoride Calibration Solutions

   a. Concentrated Fluoride Calibration Stock Solution (1,000 mg L^{-1} F^-)--Dissolve 0.2210 g of sodium fluoride (NaF, ultrapure, dried

at 110 °C for 2 hours and stored in a desiccator) in water and dilute to 100.00 mL. Store in a clean LPE bottle.

b. Dilute Fluoride Calibration Stock Solution (10.00 mg L^{-1} F-)--Dilute 1.000 mL of the concentrated fluoride calibration stock solution to 100.00 mL with water.

c. Dilute Fluoride Working Standards--Using micropipets and volumetric pipets, prepare daily a series of dilute working standards in the range 0.0-2 mg L^{-1} F^- by quantitatively diluting appropriate volumes of the 10.00 mg L^{-1} F^- solution and TISAB solution to 50.00 mL. The following series may be used:

mL of TISAB	mL of 10.00 mg L^{-1} F^- Solution	Resulting F^- Concentration When Diluted to 50.00 mL (mg L^{-1})
5.00	0.000	0.0000
5.00	0.050	0.0100
5.00	0.100	0.0200
5.00	0.250	0.0500
5.00	0.500	0.100
5.00	2.50	0.500
5.00	10.00	2.000

4. Water--Water should meet the specifications for Type I reagent grade water (ASTM, 1984).

15.4 PREPARATION

15.4.1. Calibration and Standardization

1. Allow the electrometer to warm up; ensure that the fluoride-ISE contains adequate internal filling solution.

2. With the electrometer set to measure mV, analyze the dilute fluoride working standards (in order of increasing concentration, beginning with the blank), using the procedure described in steps 3 through 5.

3. Prior to use and between determinations, rinse the electrode with water until a potential of at least 200 mV is obtained. Blot dry to avoid carryover.

4. Place 20.00 mL of standard in a clean 30-mL plastic beaker. Add a clean Teflon-coated stir bar, place on a magnetic stirrer, and stir at medium speed.

5. Immerse the electrode in the solution to just above the stir bar and observe the potential. Record the potential when a stable reading is obtained (potential drift less than 0.1 mV min^{-1}). Record the time required to obtain the reading. (It may take 15 to 30 minutes to obtain a stable reading for the low standards.)

6. Prepare a calibration curve on semi-logarithmic graph paper. Plot the concentration of F^- (in mg L^{-1}) on the log axis versus the electrode potential on the linear axis. Determine the slope of the line in the linear portion of the plot. The measured slope should be within ±10 percent of the theoretical slope (obtained from the electrode manual). If it is not, the electrode is not operating properly. Consult the electrode manual for guidance. (Note: The calibration curve may be nonlinear below 0.05 mg L^{-1}.)

15.4.2 Maintenance

The fluoride-ISE should be cleaned and the internal filling solution replaced at regular intervals, as directed by the manufacturer's instructions.

15.5 PROCEDURE

15.5.1 Standard Operating Procedure

NOTE: Use only plasticware when performing fluoride determinations. Clean using the deionized water washing procedure described in Appendix C.

1. Allow samples and standards to equilibrate at room temperature.

2. Analyze fluoride standards and prepare calibration curve as described in Section 15.4.1.

3. Prior to use and between determinations, rinse the electrode with water until a potential of at least 200 mV is obtained. Blot dry to avoid carryover.

4. Place 10.00 mL of sample in a clean 30-mL plastic beaker. Add a clean Teflon-coated stir bar, place on a magnetic stirrer, and stir at a medium speed. Add 1.00 mL of TISAB to beaker. Record the reading when a stable potential is obtained (drift is less than 0.1 mV min^{-1}). Also record the time required to reach the stable reading. (It may take as long as 15 to 30 minutes.) This assists the analyst in detecting electrode problems.

5. At the end of the day, thoroughly rinse the electrode and store it in deionized water.

15.5.2 Calculations

Compute the sample concentration by comparing the sample potential reading to the calibration curve. Report results in mg L^{-1}.

15.6 QUALITY ASSURANCE AND QUALITY CONTROL

15.6.1 Precision and Accuracy

A synthetic sample containing 0.85 mg L^{-1} fluoride and no interferences was analyzed by 111 analysts; the mean result was 0.84 mg L^{-1} and the standard deviation was 0.03 mg L^{-1} (U.S. EPA, 1983).

A synthetic sample containing 0.75 mg L^{-1} fluoride, 2.5 mg L^{-1} polyphosphate, and 300 mg L^{-1} alkalinity was analyzed by 111 analysts: the mean result was 0.75 mg L^{-1} fluoride, and the standard deviation was 0.036 (U.S. EPA, 1983).

15.6.2 Quality Control Checks

The required quality control procedures are described in Appendix G.

15.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

Barnard, W. R., and D. K. Nordstrom, 1982. Fluoride in Precipitation - I. Methodology with the Fluoride-Selective Electrode. Atmos. Environ., v. 16, pp. 99-103.

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Kissa, E. W., 1983. Determination of Fluoride at Low Concentrations with the Ion-Selective Electrode. Anal. Chem., v. 55, pp. 1445-1448.

LaZerte, B. D., 1984. Forms of Aqueous Aluminum in Acidified Catchments of Central Ontario: A Methodological Analysis. Can. J. Fish Aquat. Sci., v. 41, n. 5, pp. 766-776.

U.S. Environmental Protection Agency, 1983 (revised). Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Warner, T. B., and D. J. Bressan, 1973. Direct Measurement of Less Than 1 part-per-billion Fluoride in Rain, Fog, and Aerosols with an Ion-Selective Electrode. Anal. Chim. Acta, v. 63, pp. 165-173.

16.0 DETERMINATION OF METALS (Al, Ca, Fe, K, Mg, Mn, Na) BY ATOMIC ABSORPTION SPECTROSCOPY

16.1 OVERVIEW

16.1.1 Scope and Application

Metals in solution may be readily determined by atomic absorption spectroscopy. The method is simple, rapid, and applicable to the determination of Al, Ca, Fe, K, Mg, Mn, and Na in natural surface waters.

Detection limits, sensitivity, and optimum ranges of the metals vary with the makes and models of atomic absorption spectrophotometers. The data listed in Table 16-1, however, provide some indication of the actual concentration ranges measurable by direct aspiration (flame) and furnace techniques. In the majority of instances the concentration range shown in the table for analysis by direct aspiration may be extended much lower with scale expansion and, conversely, extended upward by using a less sensitive wavelength or by rotating the burner head. Detection limits by direct aspiration may also be extended through concentration of the sample and through solvent extraction techniques. Lower concentrations may also be determined using the furnace techniques. The concentration ranges given in Table 16-1 are somewhat dependent on equipment such as the type of spectrophotometer and furnace accessory, the energy source, and the degree of electrical expansion of the output signal. When using furnace techniques, however, the analyst should be cautioned that chemical reactions may occur at elevated temperatures, which may result in either suppression or enhancement of the signal from the element being analyzed. To ensure valid data, the analyst should examine each matrix for interference effects (matrix spike analysis) and, if detected, should analyze the samples by the method of standard additions.

16.1.2. Summary of Method

In direct aspiration atomic absorption spectroscopy, a sample is aspirated and atomized in a flame. A light beam from a hollow cathode lamp, which has a cathode made of the element to be determined, is directed through the flame into a monochromator and onto a detector that measures the amount of light absorbed. Absorption depends on the presence of free unexcited ground state atoms in the flame. 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.

When using the furnace technique in conjunction with an atomic absorption spectrophotometer, a representative aliquot of a sample is placed in the graphite tube in the furnace, evaporated to dryness, charred, and atomized. As a greater percentage of available analyte atoms are vaporized and dissociated for

TABLE 16-1. ATOMIC ABSORPTION CONCENTRATION RANGES[a]

Metal	Flame			Furnace[b,c]	
	Detection Limit (mg L^{-1})	Sensitivity (mg L^{-1})	Optimum Concentration Range (mg L^{-1})	Detection Limit (μg L^{-1})	Optimum Concentration Range (μg L^{-1})
Aluminum	0.1	1	5 to 50	3	20 to 200
Calcium	0.01	0.08	0.2 to 7	-	-
Iron	0.03	0.12	0.3 to 5	1	5 to 100
Magnesium	0.001	0.007	0.02 to 0.5	-	-
Manganese	0.01	0.05	0.1 to 3	0.2	1 to 30
Potassium	0.01	0.04	0.1 to 2	-	-
Sodium	0.002	0.015	0.03 to 1	-	-

[a]The concentrations shown are obtainable with any satisfactory atomic absorption spectrophotometer.
[b]For furnace sensitivity values, consult instrument operating manual.
[c]The listed furnace values are those expected when using a 20-μL injection and normal gas flow.

absorption in the tube than in the flame, the use of small sample volumes or detection of low concentrations of elements is possible. The principle is essentially the same as with direct aspiration atomic absorption except a furnace, rather than a flame, is used to atomize the sample. Radiation from a given excited element is passed through the vapor containing ground state atoms of that element. The intensity of the transmitted radiation decreases in proportion to the amount of the ground state element in the vapor.

The metal atoms to be measured are placed in the beam of radiation by increasing the temperature of the furnace, thereby causing the injected specimen to be volatilized. A monochromator isolates the characteristic radiation from the hollow cathode lamp and a photosensitive device measures the attenuated transmitted radiation.

Dissolved metals (Ca, Fe, K, Mg, Mn, and Na) are determined in a filtered sample by flame atomic absorption spectroscopy (U.S. EPA, 1983).

Total Al is determined in an unfiltered, nitric acid preserved sample after digestion by graphite furnace atomic absorption spectroscopy (U.S. EPA, 1983).

Total extractable Al is determined in a sample that has been treated with 8-hydroxyquinoline and has been extracted into MIBK (see Section 7) by graphite furnace atomic absorption spectroscopy (Barnes, 1975; May et al., 1979; Driscoll, 1984).

16.1.3 Definitions

1. Optimum Concentration Range--This is a range, defined by limits expressed in concentration, below which scale expansion should be used and above which curve correction should be considered. This range varies with the sensitivity of the instrument and the operating conditions employed.

2. Sensitivity--Sensitivity is the concentration in milligrams of metal per liter that produces an absorption of 1 percent.

3. Dissolved Metals--Dissolved metals are those constituents (metals) which can pass through a 0.45-μm membrane filter.

4. Total Metals--The concentration of metals is determined on an unfiltered sample following vigorous digestion.

16.1.4 Interferences

1. Direct Aspiration--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 and calcium determinations. Similarly, silica interference in the determination of manganese can be eliminated by the addition of calcium. 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.

   Ionization interferences occur when 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.

   Although quite rare, 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. Also, interference can occur when resonant energy from another element in a multi-element lamp or a metal impurity in the lamp cathode falls within the bandpass of the slit setting, and that metal is present in the sample. This type of interference may sometimes be reduced by narrowing the slit width.

2. Flameless Atomization--Although the problem of oxide formation is greatly reduced with furnace procedures because atomization occurs in an inert atmosphere, the technique is still subject to chemical and matrix interferences. The composition of the sample matrix can have a major effect on the analysis. It is this effect which should be determined and taken into consideration in the analysis of each different matrix encountered. To verify the absence of matrix or chemical interference, a matrix spike sample is analyzed using the following procedure:

   a. Withdraw two equal aliquots from the sample.

   b. Add a known amount of analyte and dilute both aliquots to the same predetermined volume. The dilution volume should be based on the analysis of the undiluted sample. Preferably, the dilution should be 1:4 while keeping in mind the optimum concentration range of the analysis. Under no circumstances should the dilution be less than 1:1.

   c. Analyze the diluted aliquots.

   d. Multiply the unspiked results by the dilution factor and compare to the original determination.

   Agreement of the results (within ±10 percent) indicates the absence of interference. Comparison of the actual signal from the spike to the expected response from the analyte in an aqueous standard helps confirm the finding from the dilution analysis. Those samples which indicate the presence of interference should be analyzed by the method of standard additions.

   Gases generated in the furnace during atomization may have molecular absorption bands encompassing the analytical wavelength. When this occurs, either the use of background correction or selection of an alternate wavelength outside the absorption band should eliminate this interference. Background correction can also compensate for non-specific broadband absorption interference.

Interference from a smoke-producing sample matrix can sometimes be reduced by extending the charring time at a higher temperature or utilizing an ashing cycle in the presence of air. Care should be taken, however, to prevent loss of the element being analyzed.

The chemical environment of the furnace may cause certain elements to form carbides at high temperatures. This problem is greatly reduced and the sensitivity is increased with the use of pyrolytically-coated graphite.

16.1.5 Safety

The calibration standards, sample types, and most reagents pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses shoud be worn when preparing reagents, especially when concentrated acids and bases are used. The use of concentrated hydrochloric acid, ammonium hydroxide solutions, and MIBK should be restricted to a hood.

Follow the manufacturer's safety precautions when operating the atomic absorption spectrophotometers. Store compressed gases in an upright position.

16.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

The sample for dissolved metals is filtered through a 0.45-µm membrane filter, then preserved by acidifying to a pH less than 2 with nitric acid. (NOTE: Preservation is not absolutely necessary if all analyses are completed within 30 days). The sample for total Al analysis is preserved by acidifying unfiltered sample to a pH less than 2 with nitric acid. The sample for total extractable Al is prepared by mixing a portion of sample with 8-hydroxyquinoline followed by extraction with MIBK (see Section 7).

After processing, the samples are transferred to an analytical laboratory. For total extractable Al samples, only the MIBK layer from the extraction is shipped.

16.3 EQUIPMENT AND SUPPLIES

16.3.1 Equipment and Apparatus

1. Atomic Absorption Spectrophotometer--The required spectrophotometer is a single- or dual-channel, single-or double-beam instrument having a grating monochromator, photomultiplier detector, adjustable slits, a wavelength range of 190 to 800 nm, and provisions for interfacing with a strip chart recorder.

2. Burner--The burner recommended by the particular instrument manufacturer should be used. For certain elements, a nitrous oxide burner is required.

3. Hollow Cathode Lamps--Single element lamps are preferred, but multi-element lamps may be used. Electrodeless discharge lamps may also be used when available.

4. Graphite Furnace--Any furnace device capable of reaching the specified temperatures is satisfactory.

5. Strip Chart Recorder--A recorder is recommended for furnace work so that there will be a permanent record and so that any problems with the analysis (i.e., drift, incomplete atomization, losses during charring, changes in sensitivity) can be recognized easily.

16.3.2 Reagents and Consumable Materials

General reagents used in each metal determination are listed in this section. Reagents specific to particular metal determinations are listed in the particular procedure description for that metal.

1. Concentrated Hydrochloric Acid (12M HCl)--Ultrapure grade (Baker Instra-Analyzed or equivalent).

2. HCl (1 percent v/v)--Add 5 mL of concentrated HCl to 495 mL water.

3. Nitric Acid (0.5% v/v HNO_3--Carefully dilute Ultrapure grade HNO_3 (Baker Instra-Analyzed or equivalent) in water in the ratio of 0.5 to 100.

4. Stock Standard Metal Solutions--Prepare as directed in the individual metal procedures. Commercially available stock standard solutions may also be used.

5. Dilute Calibration Standards--Prepare a series of standards of the metal by dilution of the appropriate stock metal solution to cover the concentration range desired.

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 required for certain determinations. Standard, commercially available argon and nitrogen are required for furnace work.

7. Water--Water should meet the specfications for Type I reagent grade water (ASTM, 1984).

16.4 PREPARATION

16.4.1 Calibration and Standardization

The calibration procedure varies slightly with the various atomic absorption instruments. For each analyte, calibrate the atomic absorption instrument by analyzing a calibration blank and a series of standards, following the instructions in the instrument operating manual. The concentration of standards should bracket the expected sample concentration; however, the linear range of the instrument should not be exceeded.

When indicated by the matrix spike analysis, the analytes should be quantified by the method of standard additions. In this method, equal volumes of sample are added to a deionized water blank and to three standards containing different known amounts of the test element. The volume of the blank and of each standard should 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 to zero absorbance, the point of intersection 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 Figure 16-1. The method of standard additions can be very useful; however, for the results to be valid the following limitations should be taken into consideration:

1. The absorbance plot of sample and standards should be linear over the concentration range of concern. For best results, the slope of the plot should be nearly the same as the slope of the aqueous standard curve. If the slope is significantly different (more than 20 percent), caution should be exercised.

2. The effect of the interference should not vary as the ratio of analyte concentration to sample matrix changes, and the standard addition should respond in a similar manner as the analyte.

3. The determination should be free of spectral interference and corrected for nonspecific background interference.

16.5 PROCEDURE

16.5.1 Flame Atomic Absorption Spectroscopy

Differences among 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 a 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 unless operated in a double-beam mode.

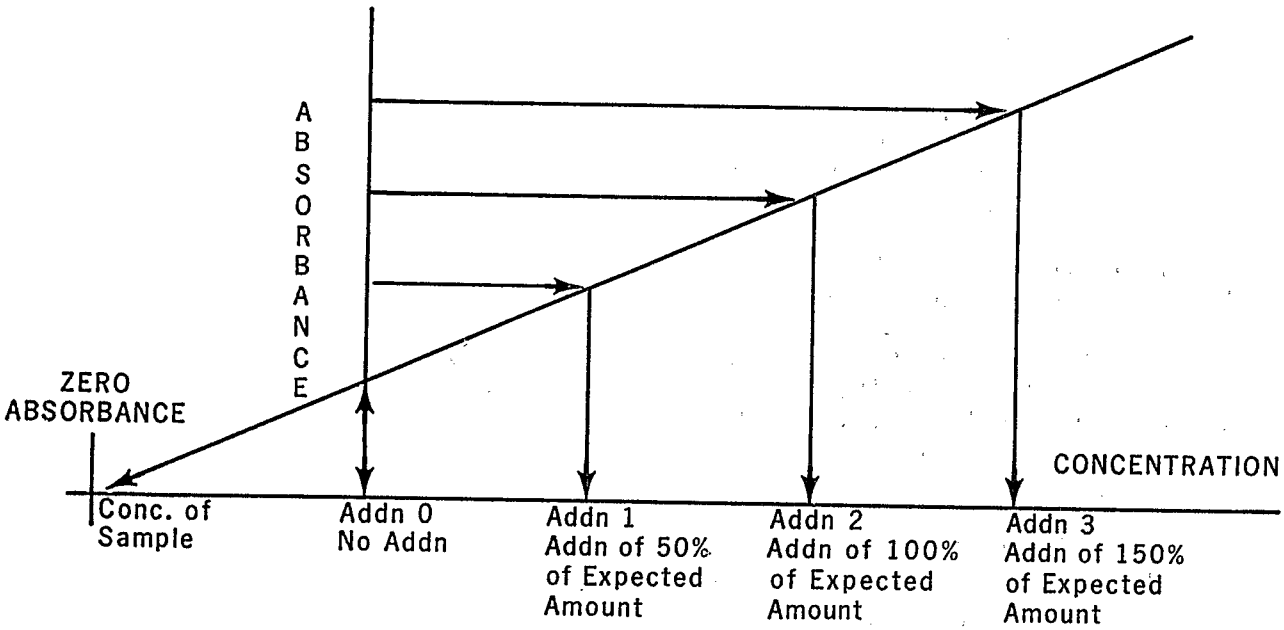

Figure 16-1. Standard addition plot.

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 calibrate the instrument. Aspirate the samples and determine the concentrations either directly (if the instrument reads directly in concentration units) or from the calibration curve.

16.5.2 Furnace Atomic Absorption Spectroscopy

Furnace devices (flameless atomization) are a most useful means of extending detection limits. Because of differences among various makes and models of satisfactory instruments, no detailed operating instuctions can be given for each instrument. Instead, the analyst should follow the instructions provided by the manufacturer of a particular instrument and use as a guide the temperature settings and other instrument conditions listed in Sections 16.5.3 through 16.5.10 (which are those recommended for the Perkin-Elmer HGA-2100). In addition, the following points may be helpful:

1. With flameless atomization, background correction is important, especially below 350 nm. This is because certain samples, when atomized, may absorb or scatter light from the hollow cathode lamp. These effects 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.

2. If all of the analyte is not volatilized during atomization and removed from the furnace, memory effects will occur. This condition depends on several factors (i.e., the volatility of the element and its chemical form, whether pyrolytic graphite is used, the rate of atomization, and furnace design). If this situation is detected through blank burns, the tube should be cleaned by operating the furnace at full power for the required time period at regular intervals in the analytical scheme.

3. Some of the smaller size furnace devices, or newer furnaces equipped with feedback temperature control (Instrumentation Laboratories Model 555, Perkin-Elmer Models HGA 2200 and HGA 76B, and Varian Model CRA-90) employing faster rates of atomization, can be operated using lower atomization temperatures for shorter time periods than those listed in this method.

4. In many cases, prior digestion of the sample is not required if a representative aliquot of sample can be pipeted into the furnace. However, prior digestion provides a more uniform matrix and possibly lessens matrix effects.

5. Inject a measured microliter aliquot of the sample into the furnace and atomize. If the concentration found is greater than the highest standard, the sample should be diluted in the same acid matrix and should be reanalyzed. The use of multiple injections can improve accuracy and can help detect furnace pipetting errors.

16.5.3 Procedure for Determination of Total Aluminum

To determine total aluminum, a portion of sample is digested and the digestate is analyzed for aluminum by furnace atomic absorption spectroscopy (U.S. EPA, 1983).

1. Preparation of Aluminum Standard Solutions--Aluminum stock solution (1,000 mg L^{-1} Al)--Carefully weigh 1.000 g of aluminum metal (analytical reagent grade). Add 15 mL concentrated HCl and 5 mL concentrated HNO_3 to the metal, cover the beaker, and warm gently. When the metal is completely dissolved, transfer solution quantitatively to a 1-L volumetric flask and bring to volume with water. Alternatively, a commercially available, certified Al standard may be used.

Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. These solutions are also to be used for "standard additions." The calibration standards should be prepared in 0.5 percent (v/v) HNO_3.

2. Sample Preparation--The sample should be digested prior to analysis. Due to the low concentrations of analyte expected, contamination from atmospheric sources can be a major problem. To avoid contamination, all preparations should be performed in a laminar flow hood. Perform digestion as follows:

   a. Quantitatively transfer a 50.00-mL aliquot of the well-mixed sample to a Griffin beaker.

   b. Add 3.0 mL of concentrated nitric acid.

   c. Place the beaker on a hot plate and cautiously evaporate to near dryness, making certain that the sample does not boil. (DO NOT BAKE.)

   d. Allow the beaker to cool, then again add 3.0 mL of concentrated nitric acid. Cover the beaker with a watch glass and return to the hot plate.

   e. Increase the temperature of the hot plate until a gentle reflux action occurs. Continue refluxing, adding acid as necessary, until the digestion is complete (indicated by a light-colored residue or no change in appearance with continued refluxing).

   f. When complete, evaporate to near dryness. Allow to cool.

   g. Add 0.5 mL of 50 percent nitric acid and warm slightly to dissolve any precipate or residue resulting from evaporation.

   h. Wash down the beaker walls and watch glass with water.

   i. Quantitatively filter the sample (to remove silicates and other insoluble materials) and adjust to 50.00 mL. The sample is now ready for analysis.

3. Suggested Instrument Conditions (General)--

   a. Drying time and temperature--30 seconds at 125 °C
   b. Ashing time and temperature--30 seconds at 1,300 °C
   c. Atomizing time and temperature--10 seconds at 2,700 °C
   d. Purge gas atmosphere--Argon
   e. Wavelength--309.3 nm

Other operating conditions should be set as specified by the particular instrument manufacturer.

NOTE 1: The above instrument conditions are for a Perkin-Elmer HGA-2100, based on the use of a 20-µL injection, continuous-flow purge gas, and nonpyrolytic graphite.

NOTE 2: Background correction may be required if the sample contains a high level of dissolved solids.

NOTE 3: It has been reported that chloride ion and that nitrogen, used as a purge gas, suppress the aluminum signal. Therefore, the use of halide acids and nitrogen as a purge gas should be avoided.

NOTE 4: The ashing temperature can be increased to 1,500 to 1,700 °C by adding 30 µg magnesium nitrate ($MG(NO_3)_2$) (Manning, et al., 1982).

NOTE 5: If blanks indicate that sample contamination is occurring, the use of Teflon labware is recommended.

4. Analysis Procedure--

a. Calibrate the instrument as directed by the instrument manufacturer.

b. Analyze the samples (including required QC samples).

c. If a sample concentration exceeds the linear range, dilute (with acidic media) and reanalyze.

d. Report results as mg L^{-1} Al.

16.5.4 Procedure for Determination of Total Extractable Aluminum

Samples for extractable aluminum are prepared in the processing laboratory and are obtained as the 8-hydroxyquinoline complex in MIBK. The MIBK solution is analyzed for aluminum by graphite furnace atomic absorption (GFAA) (Barnes, 1975; May et al., 1979; Driscoll, 1984).

1. Preparation of Reagents--

a. Glacial acetic acid (HOAc, 18M)--Baker Ultrex grade or equivalent.

b. Ammonium hydroxide (NH_4OH, 5M)--Baker Ultrex grade or equivalent.

c. Sodium acetate solution (NaOAc, 1.0M)--Dissolve 8.2 g NaOAc (Alfa Ultrapure grade or eqivalent) in 100 mL of water.

d. Methyl isobutyl ketone (MIBK)--HPLC grade or equivalent.

e. Phenol red indicator solution (0.04 percent w/v)--ACS reagent grade.

f. Hydrochloric acid (HCl, 12M)--Baker Ultrex grade or equivalent.

g. 2.5M HCl--Dilute 208 mL of 12M HCl to 1.0 L.

h. NH_4^+/NH_3 buffer--Add 2.5M HCl to 21 mL of 5M NH_4OH until the pH = 8.3, then dilute to 100 mL.

NOTE: Do this cautiously in a fume hood.

i. 8-hydroxyquinoline solution (10 g L^{-1})--Dissolve 5 grams of 8-hydroxyquinoline (99 plus percent purity) in 12.5 mL HOAc, then dilute to 500 mL.

j. 8-hydroxyquinoline sodium acetate reagent--Mix, in order, 10 mL of 1.0M NaOAc, 50 mL of water, and 10 mL of hydroxyquinoline solution.

NOTE: This reagent should be prepared daily.

2. Preparation of Aluminum Standard Solutions--

a. Aluminum stock solution--See Section 16.5.3, Preparation of Aluminum Standard Solutions.

b. Dilute calibration standards--Each day, quantitatively dilute the Al stock solution to prepare a series of calibration standards over the range 0 to 0.1 mg L^{-1} Al. A blank should be prepared. Prior to analysis, the blank, standards, and any QC samples should be extracted using the following procedure:

1) Pipet 25.00 mL of a calibration standard (or calibration blank or QC sample) into a clean 50-mL separatory funnel (or a clean 50-mL disposable centrifuge tube with cap).

2) Add 2 to 3 drops phenol red indicator and 5.00 mL 8-hydroxyquinoline NaOAc reagent. Swirl to mix.

3) Rapidly adjust the pH to 7 by dropwise additions of 5M NH_4OH until the solution turns red. Immediately add 2.0 mL of NH_4^+/NH_3 buffer and 10 mL of MIBK. Cap and shake vigorously for 10 seconds using a rapid, end-to-end motion. Be careful of pressure buildup.

4) Allow the phases to separate (10 to 15 seconds) and isolate the MIBK layer. If an emulsion forms, separation can be hastened by centrifugation. Keep the MIBK layer tightly capped to prevent evaporation.

3. Suggested Instrument Conditions (General)--

a. Drying cycle--Ramp 10 seconds, hold 10 seconds

b. Drying temperature --100 °C

c. Ashing cycle--Ramp 5 seconds, hold 20 seconds

d. Ashing temperature--1,500 °C

e. Atomization cycle--Hold 5 seconds (no ramp, power heating)

f. Atomization temperature--2,500 °C

g. Purge gas--Argon at 20 cc/minute

h. Lamp--Al HCl at 25 mA

i. Wavelength--309.3 nm

j. Graphite tube--Nonpyrolytic

k. Sample size--25 µL

These operating conditions are for a Perkin-Elmer 5000 with a HGA-500 graphite furnace and AS-40 autosampler.

4. Analysis Procedure--

a. Calibrate the instrument as directed by the instrument manufacturer.

b. Analyze the samples (including required QC samples).

c. If a sample concentration exceeds the linear range, dilute with MIBK, and reanalyze.

d. Report results as mg L^{-1} Al.

NOTE: By using the same volumes for standards as for samples, concentration factors are taken into account.

16.5.5 Procedure for Determination of Dissolved Calcium

Samples for determination of dissolve calcium (filtered and preserved with nitric acid; see comment in Section 16.2) are analyzed by flame atomic absorption spectroscopy for calcium (U.S. EPA, 1983).

1. Preparation of Lanthanum chloride matrix modifier solution ($LaCl_3$)--Dissolve 29 g of La_2O_3, slowly and in small portions, in 250 mL of concentrated HCl (Caution: Reaction is violent) and dilute to 500 mL with water.

2. Preparation of Calcium Standard Solutions--

   a. Calcium stock solution (500 mg L^{-1} Ca)--Suspend 1.250 g of $CaCO_3$ (analytical reagent grade, dried at 180 °C for 1 hour before weighing) in water and dissolve cautiously with a minimum of dilute HCl. Dilute to 1,000 mL with water.

   b. Dilute calibration standards--Each day, quantitatively prepare a series of dilute Ca standards from the calcium stock solution to span the desired concentration range.

3. Suggested Instrumental Conditions (General)--

   a. Lamp--Ca, hollow cathode

   b. Wavelength--422.7 nm

NOTE: The 239.9 nm line may also be used. This line has a relative sensitivy of 120.

   c. Fuel--acetylene

   d. Oxidant--air

   e. Flame--reducing

4. Analysis Procedure--

   a. To each 10.0-mL volume of dilute calibration standard, blank, and sample add 1.00 mL of $LaCl_3$ solution (e.g., add 2.0 mL of $LaCl_3$ solution to 20.0 mL of sample).

   b. Calibrate the instrument as directed by the manufacturer.

   c. Analyze the samples, including QC samples.

d. Dilute and reanalyze any samples with a concentration exceeding the calibrated range.

e. Report results as mg L^{-1} Ca.

NOTE 1: Phosphate, sulfate, and aluminum interfere but are masked by the addition of lanthanum. Because low calcium values result if the pH of the sample is above 7, both standards and samples are prepared in dilute acid solution. Concentrations of magnesium greater than 1,000 mg L^{-1} also cause low calcium values. Concentrations of up to 500 mg L^{-1} each of sodium, potassium, and nitrate cause no interference.

NOTE 2: Anionic chemical interferences can be expected if lanthanum is not used in samples and standards.

NOTE 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.

16.5.6 Procedure for Determination of Dissolved Iron

The samples for determination of dissolved iron (filtered and preserved with nitric acid; see note in Section 16.2) are analyzed by flame atomic absorption spectroscopy (U.S. EPA, 1983).

1. Preparation of Iron Standard Solutions--

a. Fe stock solution (1,000 mg L^{-1} Fe)--Carefully weigh 1.000 g of pure iron wire (analytical reagent grade) and dissolve in 5 mL of concentrated HNO_3, warming if necessary. When iron is completely dissolved, bring the volume of the solution to 1 L with water.

b. Dilute calibration standards--Each day, quantitatively prepare a series of calibration standards spanning the desired concentration range. Match the acid content of the standards to that of the samples (ca. 0.1 percent (v/v) HNO_3).

2. Suggested Instrumental Conditions (General)--

a. Lamp--Fe, hollow cathode

b. Wavelength--248.3 nm

NOTE: 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.

c. Fuel--acetylene

d. Oxidant--air

e. Flame--oxidizing

3. Analysis Procedure--

a. Calibrate the instrument as directed by the instrument manufacturer.

b. Analyze the samples.

c. Dilute and reanalyze any samples with concentrations exceeding the calibrated range.

d. Report results in mg L^{-1} Fe.

16.5.7 Procedure for Determination of Dissolved Magnesium

The samples for determination of dissolved magnesium (filtered and preserved with nitric acid, see note in Section 16.2) are analyzed by flame atomic absorption spectroscopy for magnesium.

1. Preparation of Lanthanum chloride solution ($LaCl_3$)--Dissolve 29 g of La_2O_3, slowly and in small portions, in 250 mL concentrated HCl (Caution: Reaction is violent), and dilute to 500 mL with water.

2. Preparation of Magnesium Standard Solutions--

a. Stock solution (500 mg L^{-1} Mg)--Dissolve 0.829 g of magnesium oxide (MgO, analytical reagent grade), in 10 mL of HNO_3 and dilute to 1 L with water.

b. Dilute calibration standards--Each day, quantitatively prepare from the Mg stock solution a series of Mg standards that span the desired concentration range.

3. Suggested Instrumental Conditions (General)--

a. Lamp--Mg, hollow cathode

b. Wavelength--285.2 nm

NOTE: The line at 202.5 nm may also be used. This line has a relative sensitivity of 25.

c. Fuel--acetylene

d. Oxidant--air

e. Flame--oxidizing

4. Analysis Procedure--

a. To each 10.0 mL of dilute calibration standard, blank, and sample, add 1.00-mL of $LaCl_3$ solution (e.g., add 2.0 mL $LaCl_3$ solution to 20.0 mL of sample).

b. Calibrate the instrument as directed by the manufacturer.

c. Analyze the samples.

d. Dilute and reanalyze any samples with a concentration exceeding the linear range.

e. Report results as mg L^{-1} Mg.

NOTE 1: The interference caused by aluminum at concentrations greater than 2 mg L^{-1} is masked by additional lanthanum. Sodium, potassium, and calcium cause no interference at concentrations less than 400 mg L^{-1}.

NOTE 2: To cover the range of magnesium values normally observed in surface waters (0.1 to 20 mg L^{-1}), it is suggested that either the 202.5-nm line be used or that the burner head be rotated. A 90 ° rotation of the burner head will produce approximately one-eighth the normal sensitivity.

16.5.8 Procedure for Determination of Dissolved Manganese

The samples for determination of dissolved manganese (filtered and preserved with nitric acid, see note in Section 16.2) are analyzed by flame atomic absorption spectroscopy for manganese (U.S. EPA, 1983).

1. Preparation of Manganese Standard Solutions--

a. Mn stock solution (1,000 mg L^{-1} Mn)--Carefully weigh 1.000 g of manganese metal (analytical reagent grade) and dissolve in 10 mL of HNO_3. When the metal is completely dissolved, dilute the solution to 1 L with 1 percent (v/v) HCl.

b. Dilute calibration standards--Each day, quantitatively prepare a series of calibration standards spanning the desired concentration range. Match the acid content of the standards to that of the samples (ca. 0.1 percent (v/v) HNO_3).

2. Instrumental Conditions (General)--

a. Lamp--hollow cathode

b. Wavelength--279.5

NOTE: The line at 403.1 nm may also be used. This line has a relative sensitivity of 10.

c. Fuel--acetylene

d. Oxidant--air

e. Flame--oxidizing

3. Analysis Procedure--

a. Calibrate the instrument as directed by the manufacturer.

b. Analyze the samples.

c. Dilute and reanalyze any samples with a concentration exceeding the calibrated range.

d. Report results as mg L^{-1} Mn.

16.5.9 Procedure for Determination of Dissolved Potassium

The samples for determination of dissolved potassium (filtered and preserved with nitric acid, see note in Section 16.2) are analyzed by flame atomic absorption spectroscopy for potassium (U.S. EPA, 1983).

1. Preparation of Potassium Standard Solutions--

a. Potassium stock solution (100 mg L^{-1} K)--Dissolve 0.1907 g of KCl (analytical reagent grade, dried at 110 °C) in water and bring the volume of the solution to 1 L.

b. Dilute calibration standards--Each day, quantitatively prepare a series of calibration standards spanning the desired concentration range. Match the acid content of the standards to that of the samples (ca. 0.1 percent (v/v) HNO_3).

2. Suggested Instrumental Conditions (General)--

   a. Lamp--K, hollow cathode

   b. Wavelength--766.5

NOTE: The 404.4 nm may also be used. This line has a relative sensitivity of 500.

   c. Fuel--acetylene

   d. Oxidant--air

   e. Flame--slightly oxidizing

3. Analysis Procedure--

   a. Calibrate the instrument as directed by the manufacturer.

   b. Analyze the samples.

   c. Dilute and reanalyze any samples with a concentration exceeding the calibrated range.

   d. Report results as mg L^{-1} K.

NOTE 1: In air-acetylene or other high-temperature flames (greater than 2,800 °C), potassium can experience partial ionization which indirectly affects absorption sensitivity. The presence of other alkali salts in the sample can reduce this ionization suppressive effect and thereby enhance analytical results. The ionization suppressive effect of sodium is small if the ratio of Na to K is under 10. Any enhancement due to sodium can be stabilized by adding excess sodium (1,000 µg mL^{-1}) to both sample and standard solutions. If more stringent control of ionization is required, the addition of cesium should be considered. Reagent blanks should be analyzed to correct for potassium impurities in the buffer stock.

NOTE 2: To cover the range of potassium values normally observed in surface waters (0.1 to 20 mg L^{-1}), it is suggested that the burner head be rotated. A 90 ° rotation of the burner head provides approximately one-eigth the normal sensitivity.

16.5.10 Procedure for Determination of Dissolved Sodium

The samples for determination of dissolved sodium (filtered and preserved with nitric acid, see note in Section 16.2) are analyzed by flame atomic absorption spectroscopy for sodium (U.S. EPA, 1983).

1. Preparation of Sodium Standard Solutions--

   a. Sodium stock solution (1,000 mg L^{-1} Na)--Dissolve 2.542 g of NaCl (analytical reagent grade, dried at 140 °C) in water and bring the volume of the solution to 1 L.

   b. Dilute calibration standards--Each day, quantitatively prepare a series of calibration standards spanning the desired concentration range. Match the acid content of the standards to that of the samples (ca. 0.1 percent (v/v) HNO_3).

2. Suggested Instrumental Conditions (General)--

   a. Lamp--Na, hollow cathode

   b. Wavelength--589.6 nm

NOTE: The 330.2 nm resonance line of sodium, which has a relative sensitivity of 185, provides a convenient way to avoid the need to dilute more concentrated solutions of sodium.

   c. Fuel--acetylene

   d. Oxidant--air

   e. Flame--oxidizing

3. Analysis Procedure--

   a. Calibrate the instrument as directed by the manufacturer.

   b. Analyze the samples.

   c. Dilute and reanalyze any samples with a concentration exceeding the calibrated range.

   d. Report results as mg L^{-1} Na.

NOTE: Low-temperature flames increase sensitivity by reducing the extent of ionization of this easily ionized metal. Ionization may also be controlled by adding potassium (1,000 mg L^{-1}) to both standards and samples.

16.5.11 Calculations

Generally, instruments are calibrated to give sample results directly in concentration units. If they do not, then a manual calibration curve must be prepared and sample concentrations are determined by comparing the sample signal to the calibrated curve. If dilutions were performed, the appropriate factor should be applied to sample values. Report results as mg L^{-1} for each analyte.

16.6 QUALITY ASSURANCE AND QUALITY CONTROL

16.6.1 Precision and Accuracy

1. Determination of Total Aluminum--

   In a multiple laboratory study using 84 lake samples containing 0.03 to 5 mg L^{-1} Al, the overall duplicate relative standard deviation was 10.5 percent (note that this represents the overall within-laboratory precision).

   In a multiple laboratory study using synthetic, simulated lake samples containing 0.02 and 0.19 mg L^{-1} Al, respectively, recoveries of 115 (n=21) and 103 (n=21) percent were obtained.

2. Determination of Total Extractable Aluminum--

   In a multiple laboratory study using 74 lake samples containing 0.005 to 3 mg L^{-1} extractable Al, the overall duplicate relative standard deviation was 7.4 percent (note this is the overall within-laboratory precision).

   Accuracy data are not available.

3. Determination of Dissolved Calcium--

   In a single laboratory (EMSL-Cincinnati), using distilled water spiked at concentrations of 9.0 and 36 mg Ca L^{-1}, the standard deviations were ±0.3 and ±0.6, respectively. Recoveries at both these levels were 99 percent.

4. Determination of Dissolved Iron--

   An interlaboratory study on trace metal analyses by atomic absorption was conducted by the Quality Assurance and Laboratory Evaluation Branch of the EPA Environmental Monitoring Systems Laboratory in Cincinnati, Ohio (EMSL-Cincinnati). Six synthetic concentrates containing varying levels of aluminum, cadmium, chromium, copper, iron,

manganese, lead, and zinc were added to natural water samples. The statistical results for iron were as follows:

Number of Labs	True Value (µg L^{-1})	Mean Value (µg L^{-1})	Standard Deviation (µg L^{-1})	Accuracy as Percent 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

5. Determination of Dissolved Magnesium--

In a single laboratory (EMSL-Cincinnati), using distilled water spiked at concentrations of 2.1 and 8.2 mg L^{-1} Mg, the standard deviations were ±0.1 and ±0.2, respectively. Recoveries at both of these levels were 100 percent.

6. Determination of Dissolved Manganese--

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

Number of Labs	True Value (µg L^{-1})	Mean Value (µg L^{-1})	Standard Deviation (µg L^{-1})	Accuracy as Percent 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

7. Determination of Dissolved Potassium--

In a single laboratory (EMSL-Cincinnati), using distilled water samples spiked at concentrations of 1.6 and 6.3 mg L^{-1} K, the standard deviations were ±0.2 and ±0.5, respectively. Recoveries at these levels were 103 percent and 102 percent, respectively.

8. Determination of Dissolved Sodium--

In a single laboratory (EMSL-Cincinnati), using distilled water samples spiked at levels of 8.2 and 52 mg L^{-1} Na, the standard deviations were ±0.1 and ±0.8, respectively. Recoveries at these levels were 102 percent and 100 percent.

16.6.2 Quality Control Checks

The required QC procedures are described in Appendix G.

16.7 REFERENCES

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17.0 DETERMINATION OF DISSOLVED METALS (Ca, Fe, Mg, and Mn) BY INDUCTIVELY COUPLED PLASMA EMISSION SPECTROSCOPY

17.1 OVERVIEW

17.1.1 Scope and Application

This method is applicable to the determination of dissolved Ca, Fe, Mg, and Mn in natural surface waters.

Table 17-1 lists the recommended wavelengths and typical estimated instrumental detection limits using conventional pneumatic nebulization for the specified elements. Actual working detection limits are sample-dependent, and as the sample matrix varies, these concentrations may also vary.

17.1.2 Summary of Method

The method describes a technique for the simultaneous or sequential determination of Ca, Fe, Mg, and Mn in natural water samples. The method is based on the measurement of atomic emission by optical spectroscopy. Samples are nebulized to produce an aerosol. The aerosol is transported by an argon carrier stream to an inductively coupled argon plasma (ICP), which is produced by a radio frequency (RF) generator. In the plasma (which is at a temperature of 6,000 to 10,000 °K), the analytes in the aerosol are atomized, ionized, and excited. The excited ions and atoms emit light at their characteristic wavelengths. The spectra from all analytes are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes. The photocurrents from the photomultiplier tubes are processed by a computer system. The signal is proportional to the analyte concentration and is calibrated by analyzing a series of standards (U.S. EPA, 1983; Fassel, 1982).

A background correction technique is required to compensate for variable background contribution to the determination of trace elements. Background should be measured adjacent to analyte lines during sample analysis. The position selected for the background intensity measurement, on either or both sides of the analytic line, is determined by the complexity of the spectrum adjacent to the analyte line. The position used should be free of spectral interference and should reflect the same change in background intensity as occurs at the analyte wavelength measured. Generally, each instrument has different background handling capabilities. The instrument operating manual should be consulted for guidance.

The possibility of additional interferences listed in Section 17.1.3 should also be recognized and appropriate corrections should be made.

TABLE 17-1. RECOMMENDED WAVELENGTHS AND ESTIMATED INSTRUMENTAL DETECTION LIMITS

Element	Wavelength (nm)[a]	Estimated detection limit ($\mu g\ L^{-1}$)[b]
Calcium	317.933	10
Iron	259.940	7
Magnesium	279.079	30
Manganese	257.610	2

[a]The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference.

[b]The estimated instrumental detection limits as shown are taken from Fassel (1982). They are given as a guide for an instrumental limit. The actual method detection limits are sample-dependent and may vary as the sample matrix varies.

17.1.3 Interferences

The following types of interference effects may contribute to inaccuracies in the determination of trace elements:

1. Spectral Interferences--Spectral interferences can be categorized as (a) overlap of a spectral line from another element; (b) unresolved overlap of molecular band spectra; (c) background contribution from continuous or recombination phenomena; and (d) background contribution from stray light from the line emission of high-concentration elements. The first of these effects can be compensated by utilizing a computer correction of the raw data which requires the monitoring and measurement of the interfering element. The second effect may require selection of an alternate wavelength. The third and fourth effects usually can be compensated by a background correction adjacent to the analyte line. In addition, users of simultaneous multi-element instrumentation should assume the responsibility of verifying the absence of spectral interference from an element that could occur in a sample but for which there is no channel in the instrument array. Table 17-2 lists some interference effects for the recommended wavelengths given in Table 17-1. The interference information is expressed as analyte concentration eqivalents (i.e., false analyte concentrations) arising

TABLE 17-2. ANALYTE CONCENTRATION EQUIVALENTS (mg L^{-1}) ARISING FROM INTERFERENCES AT THE 100-mg L^{-1} LEVEL

Analyte	Wavelength, (nm)	Interference									
		Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Ti	V
Calcium	317.933	--	--	0.08	--	0.01	0.01	0.04	--	0.03	0.03
Iron	259.940	--	--	--	--	--	--	0.12	--	--	--
Magnesium	279.079	--	0.02	0.11	--	0.13	--	0.25	--	0.07	0.12
Manganese	257.610	0.005	--	0.01	--	0.002	0.002	--	--	--	--

from 100 mg L^{-1} of the interfering element. The values in the table are only approximate and should be used as a guide for determining potential interferences. Actual values should be determined for each analytical system when necessary.

Only those interferences listed were investigated. The blank spaces in Table 17-2 indicate that measurable interferences were not observed for the interferent concentrations listed in Table 17-3. Generally, interferences were discernible if they produced peaks or background shifts corresponding to 2 to 5 percent of the peaks generated by the analyte concentrations (also listed in Table 17-3).

2. Physical Interferences--Physical interferences generally are considered to be effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples that contain high dissolved solids or acid concentrations. The use of a peristaltic pump may lessen these interferences. If these types of interferences are operative, they can be reduced by dilution of the sample or utilization of standard addition techniques.

   High dissolved solids may also cause salt buildup at the tip of the nebulizer. This affects aerosol flow rate, causing instrumental drift. Wetting the argon prior to nebulization, using a tip washer, or diluting the sample can be used to control this problem. Better control of the argon flow rate improves instrument performance. This is accomplished with the use of mass flow controllers.

3. Chemical Interferences--Chemical interferences are characterized by molecular compound formation, ionization effects, and solute vaporization effects. Normally these effects are negligible with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (i.e., incident power, observation position), by buffering of the sample, matrix matching, and standard addition procedures. These types of interferences can be dependent on matrix type and the specific analyte element.

17.1.4 Interference Tests

Whenever a new or unusual sample matrix is encountered, a series of tests should be performed prior to reporting concentration data for analyte elements. These tests, as outlined below, ensure that neither positive nor negative interference effects are operative on any of the analyte elements, thereby distorting the accuracy of the reported values.

TABLE 17-3. INTERFERENCE AND ANALYTE ELEMENTAL CONCENTRATIONS USED FOR INTERFERENCE MEASUREMENTS IN TABLE 17-2

Analytes	(mg L^{-1})	Interferences	(mg L^{-1})
Ca	1	Al	1,000
Fe	1	Ca	1,000
Mg	1	Cr	200
Mn	1	Cu	200
		Fe	1,000
		Mg	1,000
		Nn	200
		Ni	200
		Ti	200
		V	200

1. Serial Dilution--If the analyte concentration is sufficiently high (minimally a factor of 10 above the instrumental detection limit after dilution), an analysis of a dilution should agree within 5 percent of the original determination (or within some acceptable control limit that has been established for that matrix). If it does not, a chemical or physical interference effect should be suspected.

2. Spiked Addition--The recovery of a spiked addition added at a minimum level of 10X the instrumental detection limit (maximum 100X) to the original determination should be recovered to within 90 to 110 percent or within the established control limit for that matrix. If not, a matrix effect should be suspected. The use of a standard addition analysis procedure can usually compensate for this effect.

NOTE: The standard addition technique does not detect coincident spectral overlap. If overlap is suspected, use of computerized compensation, an alternate wavelength, or comparison with an alternate method is recommended.

3. Comparison with Alternate Method of Analysis--When investigating a new sample matrix, a comparison test may be performed with other analytical techniques, such as atomic absorption spectrometry or other approved methodology.

4. Wavelength Scanning of Analyte Line Region--If the appropriate equipment is available, wavelength scanning can be performed to detect potential spectral interferences.

17.1.5 Safety

Generally, the calibration standards, sample types, and most reagents pose no hazard to the analyst. Protective clothing (lab coats and gloves) and safety glasses should be worn when handling concentrated acids. Follow the instrument manufacturer's safety recommendations for the operation of the ICP.

The toxicity or carcinogenicity of each reagent used in this method has not been defined precisely. Each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals should be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets also should be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (DHEW, 1977; OSHA, 1976; ACS, 1979) for the information of the analyst.

17.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

For the determination of trace elements, contamination and loss are a prime concern. Dust in the laboratory environment, impurities in reagents, and impurities on laboratory apparatus which the sample contacts are sources of potential contamination. Sample containers can introduce either positive or negative errors in the measurement of trace elements by (a) contributing contaminants through leaching or surface desorption and (b) depleting concentrations through adsorption. Thus the collection and treatment of the sample prior to analysis requires particular attention. Labware should be washed thoroughly as described in Appendix C.

Samples are filtered and acidified (0.1-mL increments) with nitric acid until the pH is less than 2. The processed samples are analyzed for dissolved metal (Ca, Fe, Mg, Mn) content.

17.3 EQUIPMENT AND SUPPLIES

17.3.1 Equipment Specifications

1. Inductively Coupled Plasma-Atomic Emission Spectrometer.

2. Computer-controlled ICP emission spectrometer with background correction capability.

17.3.2 Reagents and Consumable Materials

1. Acids used in the preparation of standards and for sample processing should be ultra-high purity grade or equivalent (e.g., Baker Ultrex grade or SeaStar Ultrapure grade).

   a. Hydrochloric Acid, concentrated (sp. gr. 1.19).

   b. Hydrochloric Acid (50 percent v/v)--Add 500 mL of concentrated HCl to 400 mL of water and dilute to 1 L.

   c. Nitric Acid, concentrated (sp. gr. 1.41).

   d. Nitric Acid (50 percent v/v)--Add 500 mL concentrated HNO_3 to 400 mL of water and dilute to 1 L.

2. Water--Water should meet the specifications for Type I reagent grade water (ASTM, 1984).

3. Standard Stock Solutions--Solutions may be purchased or prepared from ultra-high purity grade chemicals or metals. All salts should be dried for 1 hour at 105 °C unless otherwise specified.

CAUTION: Many metal salts are extremely toxic and may be fatal if swallowed. Wash hands thoroughly after handling.

   a. Calcium Stock Standard Solution (100 mg L^{-1})--Suspend 0.2498 g of $CaCO_3$ (dried at 180 °C for 1 hour before weighing) in water and dissolve cautiously with a minimum amount of 50 percent HNO_3. Add 10.0 mL of concentrated HNO_3 and dilute to 1 L with water.

   b. Iron Stock Standard Solution (100 mg L^{-1})--Dissolve 0.1430 g of Fe_2O_3 in a warm mixture of 20 mL of 50 percent HCl and 2 mL of concentrated HNO_3. Cool, add an additional 5 mL of concentrated HNO_3, and dilute to 1 L with water.

   c. Magnesium Stock Standard Solution (100 mg L^{-1})--Dissolve 0.1658 g of MgO in a minimum amount of 50 percent HNO_3. Add 10.0 mL of concentrated HNO_3 and dilute to 1 L with water.

   d. Manganese Stock Standard Solution (100 mg L^{-1})--Dissolve 0.1000 g of manganese metal in an acid mixture consisting of 10 mL of concentrated HCl and 1 mL of concentrated HNO_3 and dilute to 1 L with water.

17.4 PREPARATION

17.4.1 Calibration and Standardization

Prepare a calibration blank and a series of dilute calibration standards from the stock solutions spanning the expected sample concentration range. Match the acid content of the standards to that of the samples. A multi-element standard may be prepared.

The calibration procedure varies with the various ICP instruments. Calibrate the ICP for each analyte following the instrument operating conditions.

17.5 PROCEDURE

Because of the differences among makes and models of satisfactory instruments, no detailed instrumental operating instructions can be provided. Instead, the analyst should refer to the instructions provided by the manufacturer of the particular instrument.

17.5.1 Standard Operating Procedure

1. Set up instrument as recommended by the manufacturer or as experience dictates. The instrument should be allowed to become thermally stable before beginning (10 to 30 minutes).

2. Profile and calibrate instrument according to instrument manufacturer's recommended procedures. Flush the system with the calibration blank between each standard. (The use of the average intensity of multiple exposures for both standardization and sample analysis has been found to reduce random error.)

3. Begin sample analysis, flushing the system with the calibration blank solution between each sample. Analyze required QC samples at intervals determined by the quality assurance program.

4. Dilute and reanalyze any samples with a concentration exceeding the calibration range.

17.5.2 Calculations

Generally, instruments are calibrated to output sample results directly in concentration units. If not, then a manual calibration curve should be prepared and sample concentrations determined by comparing the sample signal to the calibrated curve. If dilutions were performed, the appropriate factor should be applied to sample values. Report results as mg L^{-1} for each analyte.

17.6 QUALITY ASSURANCE AND QUALITY CONTROL

17.6.1 Precision and Accuracy

In an EPA round-robin Phase I study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been dosed with various metal concentrates. Table 17-4 lists the true value, the mean reported value, and the mean percent relative standard deviation (%RSD) (U.S. EPA, 1983).

17.6.2 Quality Control Checks

The required QC procedures are described in Appendix G.

17.7 REFERENCES

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TABLE 17-4. INDUCTIVELY COUPLED PLASMA EMISSION SPECTROSCOPY PRECISION AND ACCURACY DATA[a]

	Sample 1			Sample 2			Sample 3		
Element	True Value ($\mu g\ L^{-1}$)	Mean Reported Value ($\mu g\ L^{-1}$)	Mean %RSD[b]	True Value ($\mu g\ L^{-1}$)	Mean Reported Value ($\mu g\ L^{-1}$)	Mean %RSD[b]	True Value ($\mu g\ L^{-1}$)	Mean Reported Value ($\mu g\ L^{-1}$)	Mean %RSD[b]
Mn	350	345	2.7	15	15	6.7	100	99	3.3
Fe	600	594	3.0	20	19	15	180	178	6.0

[a]Not all elements were analyzed by all laboratories. Ca and Mg were not determined.
[b]%RSD = percent relative standard deviation.

18.0 DETERMINATION OF TOTAL NITROGEN

18.1 OVERVIEW

18.1.1 Scope and Application

This method is applicable to the determination of total nitrogen in natural surface waters. Total nitrogen includes inorganic nitrogen compounds (nitrate, nitrite, and ammonia) as well as organically fixed nitrogen (proteins, etc.).

This method is applicable to the determination of total nitrogen in the range of 0.01 to 20 mg L^{-1} N. The minimum detection limit is approximately 0.007 mg L^{-1} (three times the standard deviation of replicate blank analyses).

This method may give poor recoveries for organic compounds which contain nitrogen-to-nitrogen double bonds as well as terminal nitrogen groups (e.g., HN = C).

18.1.2 Summary of Method

Samples are oxidized in an autoclave at 120 °C with an alkaline persulfate mixture. The oxidation process converts all nitrogen-containing compounds to nitrate. The nitrate is subsequently determined colorimetrically by flow injection analysis (FIA). During FIA, nitrate is reduced to nitrite by cadmium reduction; the nitrite 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 at 540 nm. The procedure is based on the published methods of Ebina et al. (1983), Smart et al. (1981), D'Elia et al. (1977), Nydahl (1978), and Tecator (1983).

18.1.3 Definitions

Total Persulfate Nitrogen--In a water sample, this is the total nitrogen present that is digested by the persulfate method, including organic N, NH_4-N, NO_3-N, and NO_2-N.

18.1.4 Interferences

Turbidity may interfere with this method. If the digestate is turbid, it should be filtered through a 0.45-µm membrane prior to analysis. Ethylenediamine tetraacetate (EDTA) is used to reduce interference from iron, copper, and other metals.

18.1.5 Safety

The calibration standards, sample types, and most reagents used in this method do not pose a hazard to the analyst. Protective clothing (lab coat, gloves, and safety glasses) should be worn when preparing reagents.

WARNING

- -

Cadmium present in the reduction column is poisonous. Extreme caution should be taken when handling grains and solutions.

- -

18.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

An unfiltered 100-mL sample contained in a bottle washed with HCl (wash as described in Appendix C, substituting HCl for HNO_3) is preserved with 0.05 mL Ultrex or equivalent grade concentrated H_2SO_4 to pH less than 2. Store sample at 4 °C in the dark when not in use.

18.3 EQUIPMENT AND SUPPLIES

18.3.1 Equipment and Apparatus

1. Flow injection analyzer--Analyzer consists of injection valve, spectrophotometer, printer/integrator, cadmium reduction column, and recorder/computer data handler.

2. Autoclave.

3. Teflon-lined screw-top digestion vessels.

NOTE: Clean all labware with hot 5 percent HCl and rinse copiously with nitrogen-free water. Keep labware tightly sealed from the atmosphere to reduce contamination.

18.3.2 Reagents and Consumable Materials

Reagents should be ACS reagent grade unless otherwise stated.

1. Ammonium chloride-EDTA solution--Dissolve 85 g reagent grade ammonium chloride and 0.1 g disodium ethylenediamine tetraacetate (CASRN 60-00-4) in 900 mL water. Adjust the pH to 8.5 with concentrated ammonium hydroxide and dilute to 1 L.

2. Copper sulfate solution (2% w/v)--Dissolve 20 g $CuSO_4 \cdot 5H_2O$ in 500 mL water, then dilute to 1 L.

3. Hydrochloric acid (HCl)--Concentrated (d = 1.19, 37 percent, Baker Ultrex grade or equivalent).

4. Dilute HCl (1 + 1)--Add 50 mL concentrated HCl (Baker Instra-Analyzed grade or equivalent) to 50 mL water.

5. NED solution--Dissolve 0.5 g N-(1-naphthyl)-ethylenediamine dihydrochloride (CASRN 551-09-7) in 500 mL water. Filter and degas. Store in an amber bottle at 4 °C. Prepare fresh weekly.

6. Oxidizing reagent--Dissolve 3.0 g sodium hydroxide (NaOH) and 20.0 g potassium persulfate ($K_2S_2O_8$, N<0.001%) in 1 L of water. If the total nitrogen in a reagent blank is too high (>0.010 ppm), then the potassium persulfate may be purified by recrystallization. Recrystallize potassium persulfate as follows:

   a. Dissolve 75 g potassium persulfate (reagent grade containing less than 0.001% N) in 500 mL water heated to 60 °C.

   b. Filter rapidly through loosely stoppered Pyrex wool and cool in ice water to about 4 °C while stirring continuously.

   c. Isolate the crystals by vacuum filtration on a sintered-glass filter. Wash with small amounts of ice water (4 °C).

   d. Dry in vacuum over anhydrous calcium chloride. Rapid drying in an efficient vacuum is essential in minimizing sulfuric acid formation on the crystals.

   e. Store the crystals in a vacuum desiccator over calcium chloride.

7. Sodium hydroxide(NaOH)--Crystals (98.00%, Baker Instra-Analyzed grade or equivalent, N<0.0003%).

8. Sodium hydroxide (50% w/w)--Dissolve 50 g sodium hydroxide in 50 mL water. Cool to room temperature. Separate supernatant from any precipitate by transferring supernatant to a clean plastic bottle. Store bottle tightly capped.

9. Sodium hydroxide (0.36N)--Dilute 7.2 mL 50% NaOH to 250 mL. Store in a borosilicate glass reagent bottle equipped with an Ascarite or equivalent CO_2 trap.

10. Sulfanilamide solution--Dissolve 5 g of sulfanilamide ($C_6H_8N_2O_2S$, CASRN 63-74-1) in a mixture of 26 mL of concentrated HCl and 300 mL of water, then dilute to 500 mL. Filter and degas. Store at 4 °C. This solution is stable for several months.

11. Water--All water used in preparing reagents and in cleaning labware should meet the specifications for Type I reagent grade water (ASTM, 1984).

18.3.3 Reduction Column and Reagents

WARNING

- -

Cadmium is poisonous. Handle with extreme caution. Dispose of solution from the following treatments as hazardous wastes.

- -

1. Granulated cadmium--40 to 60 mesh.

2. Copperized cadmium--Prepare copperized cadmium as described below:

   a. Wash the cadmium with dilute HCl and rinse with water. The color of the cadmium so treated should be silver.

   b. Swirl 10 g of cadmium in 100 mL copper sulfate solution for 5 minutes, or until the blue color partially fades. Decant and repeat with fresh copper sulfate solution. Continue washings until a brown colloidal precipitate forms.

   c. Wash the cadmium-copper with water (at least 10 times) to remove all the precipitated copper. The color of the cadmium so treated should be black.

18.3.4 Standard Solutions

1. Concentrated stock standard solution (1,000 mg L^{-1} NO_3-N total nitrogen)--Dissolve 0.60681 g sodium nitrate ($NaNO_3$, ultrapure grade, dried at 110 °C for 2 hours and stored in a desiccator) in water and dilute to 100.00 mL with water. Store at 4 °C. Prepare weekly.

2. Dilute stock standard solution (10.00 mg L^{-1} NO_3-N total nitrogen)--Dilute 1.000 mL of the 1,000-mg L^{-1} total-nitrogen solution to 100.00 mL with water. Store at 4 °C.

3. Daily calibration standards--Daily, prepare the calibration standards listed in the table below by adding the appropriate volume of 10.00-mg L^{-1} total-nitrogen standard and diluting to 100 mL.

Total-nitrogen standard (mg L^{-1})	mL of 10.00-mg L^{-1} total-nitrogen standard required	Total-nitrogen standard (mg L^{-1})	mL of 10.00-mg L^{-1} total-nitrogen standard required
0.000	0.000	0.100	1.000
0.010	0.100	0.500	5.000
0.030	0.300	1.000	10.000
0.050	0.500		

4. Concentrated column efficiency (CE) stock standard solution (100 mg L^{-1} NO_2-TN)--Dissolve 0.4502 g sodium nitrite ($NaNO_2$, ACS reagent grade, dried at 100 °C for 2 hours and stored in a desiccator) in water and dilute to 100.00 mL. Prepare daily.

5. CE standard (5.000 mg L^{-1} NO_2-TN)--Daily, dilute 0.500 mL of the 1,000-mg L^{-1} NO_2-TN solution to 100.00 mL with water.

18.3.5 Quality Control (QC) Standards

1. QC stock solution (1,000 mg L^{-1} total nitrogen)--Dissolve 0.60681 g of $NaNO_3$ (ultrapure grade, dried at 110 °C for 2 hours and stored in a desiccator) in water and dilute to 100.00 mL. Store at 4 °C. $NaNO_3$ should be from a source independent of that used to prepare the concentrated stock standard solution.

2. Detection limit QC sample (0.030 mg L^{-1} total nitrogen)--Daily, dilute 0.0300 of mL QC stock solution to 1.000 L with water.

3. Routine QCCS (0.500 mg L^{-1} total nitrogen)--Daily, dilute 0.0500 mL of QC stock solution to 100.00 mL with water.

4. CE QC stock solution (100 mg L^{-1} NO_2-TN)--Dissolve 0.4502 g of $NaNO_2$ (ACS reagent grade, dried at 110 °C for 2 hours and stored in desiccator; should be from a source independent of that used to prepare the concentrated CE stock standard solution) in water and dilute to 100.00 mL. Store at 4 °C.

5. CE QCCS (0.500 mg L^{-1} NO_2-TN)--Daily, dilute 0.0500 mL of CE QC stock solution to 100.00 mL with water.

18.4 PREPARATION

18.4.1 Calibration and Standardization

The colorimeter is calibrated before each batch of samples is analyzed. The seven daily total-nitrogen calibration standards (including 0.000 mg L^{-1}) are analyzed, and a calibration curve is generated from their responses.

18.4.2 Preparation of Reduction Column

The reduction column is an 8-mm by 50-mm low-pressure glass chromatography column. Pack the reduction column with copperized cadmium as follows:

1. Insert a fritted Teflon bed support into one end of the column. Place a column plug in the same end. Fill the column with water.

2. Add copperized cadmium granules to the column while gently vibrating the column with an electric engraving pencil or similar device. This procedure will ensure even column packing. When the column is completely packed, insert another fritted Teflon bed support on the top of the column.

3. Insert the packed column into the flow system using standard 1/4-28 chromatography fittings. The column is now ready for use.

Keep the column filled with water at all times. If air bubbles become trapped in the column, they can be dislodged by vibrating the column while pumping carrier through the system. Repack the column if void volumes are apparent.

18.5 PROCEDURE

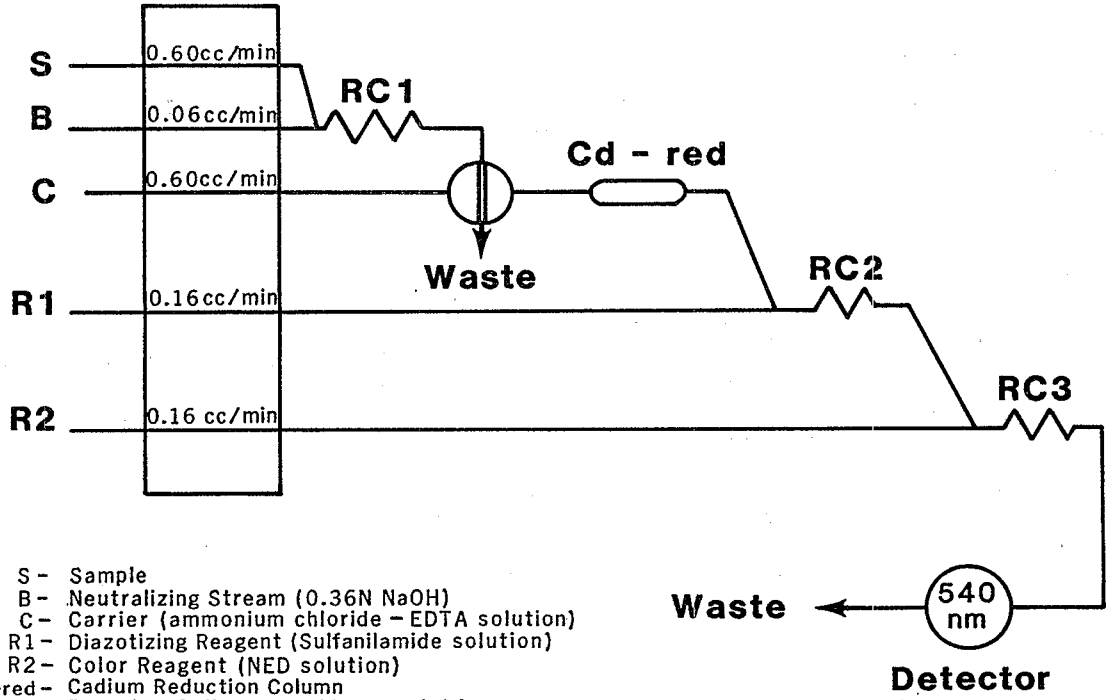
18.5.1 Standard Operating Procedure

1. Set up the FIA system as indicated in Figure 18-1.

2. Allow all reagents to run through the system for 10 minutes.

3. Analyze a 0.500-mg L^{-1} NO_2-N standard on an ion chromatograph to determine if any nitrate is present.

4. Add 5.00 mL oxidizing reagent to 5.00 mL of sample (routine samples, calibration standards, reagent blank, and QC samples included) in a Teflon digestion vessel, and cap the vessel.



Key:
S - Sample
B - Neutralizing Stream (0.36N NaOH)
C - Carrier (ammonium chloride - EDTA solution)
R1 - Diazotizing Reagent (Sulfanilamide solution)
R2 - Color Reagent (NED solution)
Cd-red - Cadium Reduction Column
RC1 - Reaction Coil, 12 cm (0.5 mm i.d.)
RC2 - Reaction Coil, 30 cm (0.5 mm i.d.)
RC3 - Reaction Coil, 60 cm (0.5 mm i.d.)

Figure 18-1. Schematic of flow injection system for determination of total nitrogen.

5. Autoclave sample at 120 °C for 30 minutes, then cool to room temperature.

6. Analyze a 0.500-mg L^{-1} NO_2-N sample and a 0.500-mg L^{-1} NO_3-N sample. Calculate the column efficiency as described in Section 18.6.3. If the column is less than 95 percent efficient, reactivate or replace the column until 95 percent or greater efficiency is achieved.

7. Load the autosampler of the FIA system, and start the analysis. Analyze the samples in the following order:

a. Calibration Standards

b. CE QCCS and 0.500-mg L^{-1} NO_3-TN standard

c. Reagent Blank

d. Detection Limit QCCS

e. Routine QCCS

f. CE QCCS

g. Ten Samples, or interval determined by the quality assurance program

h. Routine QCCS

i. 0.500-mg L^{-1} Calibration Standard

j. Calibration Blank

8. Repeat steps 7e through 7i until all samples are analyzed.

9. Dilute and reanalyze all samples that exceed the calibrated range.

18.5.2 Calculations

Construct a calibration curve for total nitrogen by plotting the measured response for the calibration standards versus concentration. From the calibration curve and response for the samples, calculate the sample concentration. Report results as mg L^{-1} total nitrogen.

18.6 QUALITY ASSURANCE AND QUALITY CONTROL

18.6.1 Precision and Accuracy

In previous studies (Ebina et al., 1983; Tecator, 1983) for total nitrogen concentrations within the range 0.14 to 2.0 mg L^{-1}, the relative precision of the method ranged from 0.4 to 2.5 percent. In a single laboratory (Ebina et al., 1983), using river water spiked with total nitrogen in the range 2.5 to 10.0 µg L^{-1}, the recovery (accuracy) varied from 99 to 103 percent.

18.6.2 Quality Control Checks

1. Laboratory Duplicate--Analyze one sample per batch in duplicate (including digestion). Duplicate precision (expressed by percent relative standard deviation) should not exceed 10 percent.

2. Reagent Blank--Prepare and analyze one reagent blank per batch. A reagent blank contains only the reagents used in processing. It should contain less than 0.010 mg L^{-1} total nitrogen.

3. Detection Limit QC Check--Analyze the detection limit QCCS once per batch prior to sample analysis. The measured result should be within 20 percent of the actual concentration.

4. Routine QC check--Analyze the routine QCCS prior to sample analysis, after every 10 samples are analyzed or at intervals determined by the quality assurance program, and after the final sample is analyzed. The measured concentration should be within 10 percent of the actual concentration.

18.6.3 Reduction Column Quality Control Checks

To ensure that the reduction column completely reduces nitrate to nitrite, nitrite samples (CE standards and CE QCCS) should be analyzed.

1. CE Standard--Analyze a 5.000-mg L^{-1} NO_2-N standard after the calibration standards have been run. Determine the efficiency of the column using the equation below:

$$\text{Column Efficiency (\%)} = \frac{NO_3 \text{ peak height}}{NO_2 \text{ peak height}} \times 100$$

If the column is less than 95 percent efficient, reactivate or replace the column so that 95 percent or greater efficiency is achieved.

2. CE QC Check--Analyze the CE QCCS after every routine CE standard analysis. The measured concentration should be within 10 percent of the actual concentration. If it is not, check the instrument operation and sample preparation.

18.7 REFERENCES

ASTM, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

D'Elia, C. F., P. A. Stendler, and N. Corwin, 1977. Determination of Total Nitrogen in Aqueous Samples Using Persulfate Digestion. Limnol. Oceanogr., v. 22, pp. 760-764.

Ebina, J., T. Tsutsui, and T. Shirai, 1983. Simultaneous Determination of Total Nitrogen and Total Phosphorus in Water Using Peroxodisulfate Oxidation. Water Res., v. 17, pp. 1721-1726.

Nydahl, F., 1978. On the Peroxodisulfate Oxidation of Total Nitrogen in Waters to Nitrate. Water Res., v. 12, pp. 1123-1130.

Smart, M. M., F. A. Reid, and J. R. Jones, 1981. A Comparison of Persulfate Digestion and the Kjeldahl Procedure for Determination of Total Nitrogen in Freshwater Samples. Water Res., v. 15, pp. 19-21.

Tecator, 1983. Determination of the Sum of Nitrate and Nitrite in Water by Flow Injection Analysis. Technical Sub Note ASN62-01/83g Tecator, Höganäs, Sweden.

19.0 DETERMINATION OF pH (CLOSED SYSTEM)

19.1 OVERVIEW

The pH of an aquatic environment is regulated by both abiotic (inorganic CO_2 equilibria, surficial geology, and anthropogenic pollutants) and biotic (photosynthesis, respiration, and decomposition) factors. A pH balance is usually maintained by the presence of buffering reactions within the aquatic system. If this balance is shifted, both chemical and biotic repercussions may result.

The pH is defined as the negative logarithm of the activity of hydrogen ions (H^+). The H^+ activity is a measure of the "effective" concentration of hydrogen ions in solution; it is always equal to or less than the true concentration of hydrogen ions in solution. Values usually range from pH 1 to pH 14, with pH 1 being most acidic, pH 7 neutral (at 25 °C), and pH 14 most alkaline. Each pH unit represents a tenfold change in H^+ activity, i.e., a pH 4 solution is 10 times as acidic as a pH 5 solution.

When the pH of a sample solution is measured, the hydrogen ions come into equilibrium with the ion exchange surface (glass) of a calibrated pH electrode which creates an electrical potential. This voltage difference is measured by the pH meter in millivolts, then is converted and displayed as pH units.

19.1.1 Scope and Application

This method is applicable to the determination of pH in surface waters of low ionic strength. For the AERP studies, pH is determined in the processing laboratory using an Orion Model 611 pH meter and an Orion Ross combination pH electrode. The method has been written assuming that the Orion meter and electrode are used (Orion, 1983). The method, however, can be modified for use with other instrumentation meeting equivalent specifications.

The applicable pH range is 3.0 to 11.0.

19.1.2 Summary of Method

pH is measured in a closed system on a sample collected without exposure to the atmosphere to prevent gaseous exchange between the samples and the atmosphere. The measurement is performed by attaching the sample syringe to the pH sample chamber (Figures 19-1 and 19-2), injecting the sample, and determining the pH using a pH meter and electrode.

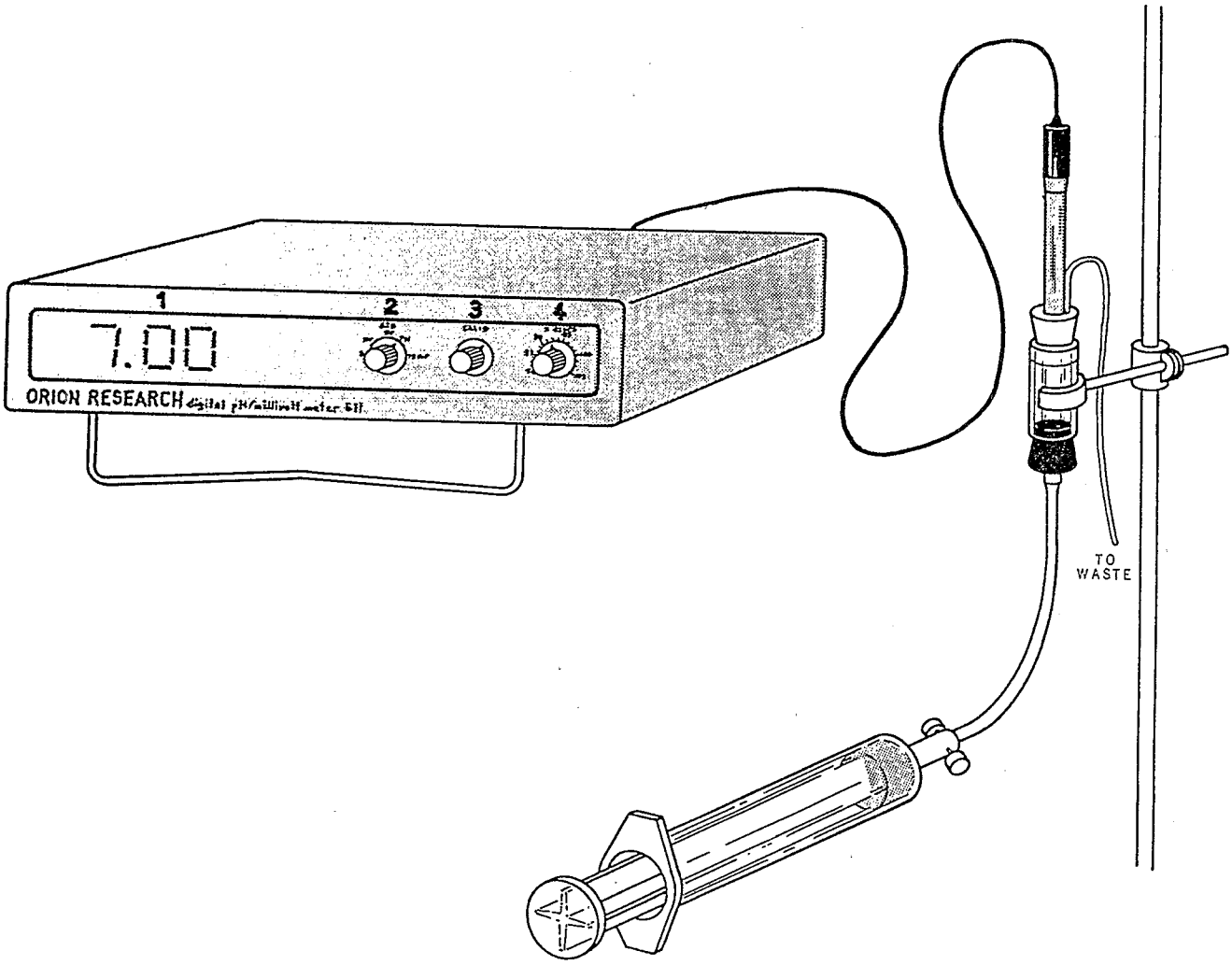


Figure 19-1. Schematic of pH measurement system.

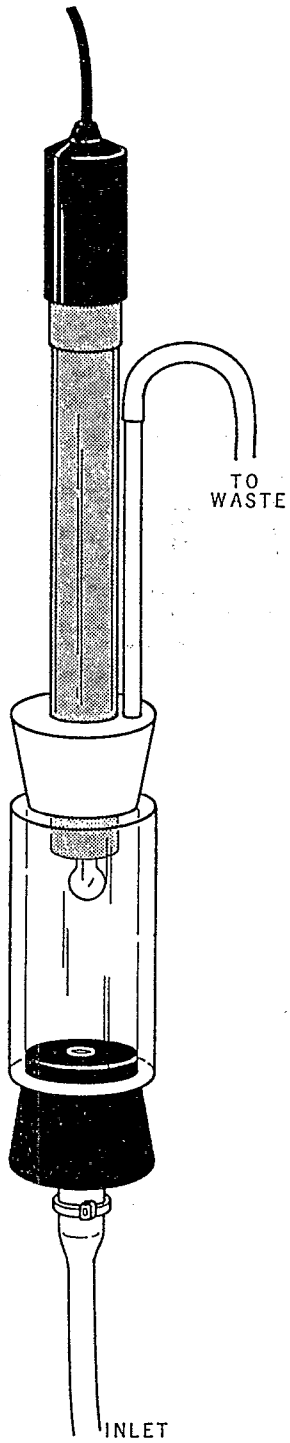


Figure 19-2. pH sample chamber.

19.1.3 Interferences

No interferences are known.

19.1.4 Safety

The calibration standards, sample types, and reagents used in this method pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses should be worn when handling sulfuric acid.

19.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples are collected and sealed without air bubbles in 60-mL plastic syringes. They are stored at 4 °C in the dark until used. Analysis should be as close to the time of collection as possible, generally within 24-36 hours, although a study has shown that pH does not change significantly over a seven-day period if samples are sealed and stored as described above (Burke, et al., 1986).

19.3 EQUIPMENT AND SUPPLIES

19.3.1 Apparatus and Equipment

1. Orion Model 611 pH meter or equivalent.

2. Orion Ross combination pH electrode or equivalent.

NOTE: Only combustion electrodes are recommended.

3. pH sample chamber.

4. 60-mL plastic syringes.

5. Luer-Lok or equivalent syringe valves.

19.3.2 Reagents and Consumable Materials

1. pH Calibration Buffers (pH 4 and 7)--Commercially available pH calibration buffers (NBS-traceable) at pH values of 4 and 7.

2. Potassium Chloride (3M)--Dissolve 75 g KCl in 1 L of water.

3. pH 4.00 Quality Control Check Solution (QCCS) (0.0001N H_2SO_4)--Add 1.000 mL of 0.1N H_2SO_4 to a volumetric flask, dilute to 1 L. Prepare daily.

4. Dilute pH 7.00 Buffer Intermeter Comparability Solution--Dilute 5.000 ± 0.001 g of NBS-traceable pH 7.00 buffer to 1 L in a volumetric flask. Prepare daily.

NOTE: The dilute buffer is used only when more than 1 pH meter is being used; see Section 19.5.4.

5. Water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).

19.4 PREPARATION

19.4.1 Instrument Preparation

1. Plug in the instrument and verify that the control knob is on "STD BY." Allow at least 30 minutes for instrument warm-up prior to use.

NOTE: If instrument is used frequently, leave on and in "STD BY" mode between uses.

2. Connect the combination electrode to the meter. Consult the pH electrode manual for the proper procedure.

3. Verify that the level of reference filling solution (3M KCl) in the electrode is just below the fill hole and that the fill hole is uncovered during measurement (slide the plastic sleeve down).

4. Calibrate the meter for temperature weekly using a two-point standardization (one point at approximately 5 °C to 10 °C and the other point at room temperature).

   a. Room Temperature--Place the electrode and an NBS-traceable thermometer into deionized water which is at room temperature. Swirl the electrode for 5 to 10 seconds.

   b. Turn the knob on the meter to "TEMP." Using a small screwdriver, adjust the display, using the "TEMP ADJ" screw on back of the pH meter, to the temperature reading of the thermometer.

   c. Cold Temperature--Place the probe and the NBS-traceable thermometer into a 250-mL beaker containing cold deionized water (5 to 10 °C). Repeat Step b by adjusting the display with the "TEMP SLOPE" screw on the back of the meter.

   d. Continue steps a through c until no further adjustments are necessary and record all values in the logbook.

19.4.2 Calibration and Standardization

1. Check the meter temperature calibration daily with a beaker of room temperature deionized water and an NBS-traceable thermometer. If the display differs from the NBS-traceable thermometer by more than 1.0 °C, complete adjustments as described in Section 19.4.1, step 4.

2. Pour fresh pH 7.00 and pH 4.00 buffer solutions into labeled 50-mL beakers (one "RINSE," one "CALIBRATION," and one "CHECK" beaker for each buffer). Rinse all beakers three times and fill with the appropriate buffer solutions.

3. Rinse the electrode with deionized water. Place the electrode into the pH 7.00 "RINSE" beaker and swirl for 30 seconds. Place the electrode into the "CALIBRATION" beaker, turn the knob to "pH", swirl for 30 to 60 seconds (or until the pH reading is stable), and read the value on the display. Consult the pH-temperature chart, Table 19-1. Use the "CALIBRATE" knob to adjust the pH reading on the meter to the theoretical pH of the buffer solution at the appropriate temperature.

4. Repeat step 3 for pH 4.00 buffer using the "% SLOPE" knob to adjust the pH reading.

5. Repeat steps 3 and 4 until both the pH 7.00 and the pH 4.00 buffers agree with the theoretical pH of the buffer solution at the appropriate temperature.

6. Check the standardization using the buffer solutions in the "CHECK" beakers. If the values differ by more than ±0.03 units from the theoretical value, repeat the standardization process. When the meter standardization is acceptable, record the pH and temperature readings for each buffer solution in the pH logbook.

19.4.3 Maintenance

1. Weekly, drain the 3M KCl filling solution from the electrode using a disposable pipet with Teflon tubing attached.

2. Refill the electrode chamber with the 3M KCl filling solution and rinse by inverting the electrode. Drain the solution as in step 1.

TABLE 19-1. pH VALUES OF BUFFERS AT VARIOUS TEMPERATURES
(from Orion Research Instruction Manual, 1983)

NBS buffer, nominal value at 25 °C	Temperature										
	0 °C	5 °C	10 °C	20 °C	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
1.68	1.67	1.67	1.67	1.67	1.68	1.69	1.71	1.72	1.74	1.77	1.79
3.78	3.86	3.84	3.82	3.79	3.77	3.75	3.75	--	--	--	--
4.01	4.00	4.00	4.00	4.00	4.02	4.03	4.06	4.08	4.13	4.16	4.21
6.86	6.98	6.95	6.92	6.87	6.85	6.84	6.83	6.84	6.85	6.86	6.88
7.00	7.11	7.08	7.06	7.01	6.98	6.97	6.97	--	--	--	--
7.41	7.53	7.50	7.47	7.43	7.40	7.38	7.37	--	--	--	--
9.18	9.46	9.40	9.33	9.23	9.14	9.07	9.01	8.96	8.92	8.89	8.85
10.01	10.32	10.25	10.18	10.06	9.97	9.89	9.83	--	--	--	--

3. Refill the electrode with the filling solution to just below the fill hole.

4. Gently spin the electrode overhead for approximately 1 minute by the leader to remove any air bubbles. Be careful to stand clear of any obstacles when swinging the electrode.

19.4.4 pH Meter Electronic Checkout

NOTE: This procedure should be performed whenever a new pH meter is set up or when calibration problems occur.

1. Connect the shorting strap as outlined in the Orion pH meter manual.

2. Turn the "TEMP ADJ" and "TEMP SLOPE" screws fully counterclockwise and record the display pH value (turn knob to "pH" position).

3. Turn the "TEMP SLOPE" screw 7.5 turns clockwise and record the display pH value. The difference between the "TEMP SLOPE" value in step 2 and step 3 should be between 7.0 to 15.0.

4. Turn the "TEMP ADJ" screw until a value between 50.0 ± 0.1 appears on display.

5. Press the test button. A value of 42.2 ± 2.0 should appear on the display when the knob is in the "TEMP" position. If this value is not displayed, keep depressing the test button and use the "TEMP SLOPE" screw to adjust the reading to 4.0 ± 0.1. Release the test button and use the "TEMP ADJ" screw to obtain a reading of 50.0 ± 0.1. Press the test button again. The reading should be 42.2 ± 2.0. Repeat this procedure several times if the value is not in range.

19.4.5 Electrode Etching Procedure

NOTE 1: Use Extreme Caution when using the NaOH pellets. Be sure to wear gloves, eye protection, and a rubber apron.

NOTE 2: If the electrode response is sluggish or if the instrument cannot be standardized, the following procedure is recommended for cleaning the ceramic junction of the electrode and improving the electrode response time.

NOTE 3: Etch electrodes in groups of three when possible. Prepare a fresh NaOH solution for each group of electrodes.

1. Drain the filling solution from the electrode.

2. Rinse the filling chamber with deionized water and drain it.

3. Refill the chamber with deionized water.

4. Prepare a 50 percent (w/v) NaOH solution by slowly adding 30 g of NaOH to 30 mL of deionized water.

5. Gently stir the solution with up to three electrodes to dissolve the NaOH. The solution will be very hot and may boil and splatter, caution must be used.

6. Stir the solution an additional 2 minutes with the electrodes.

7. Rinse the electrodes with deionized water.

8. Rinse the electrodes in pH 7.00 buffer for 2 minutes.

9. Drain the deionized water from the filling chambers.

10. Refill each electrode with 3M KCl, agitate the electrodes, and drain the chambers.

11. Refill the chambers once more with 3M KCl and spin each electrode from the leader to remove air bubbles.

19.5 PROCEDURE

19.5.1 Sample Chamber Assembly

1. Using a 3-pronged clamp, attach the pH sample chamber to the ringstand so that the overflow from the chamber will drain into the sink.

2. Insert the electrode through the rubber silicone stopper. Rinse the electrode and the chamber copiously with deionized water.

19.5.2 Initial Quality Control Check

NOTE: Refer to Figures 19-3 and 19-4.

1. Rinse and fill a beaker with pH 4.00 QCCS. Rinse the syringe with QCCS. Fill the syringe with 50 mL of QCCS and attach a syringe valve.

2. Attach the syringe to the inflow tubing of the pH sample chamber. Inject enough standard to fill the chamber. Rinse the electrode by swirling it in the chamber for 15 to 30 seconds. Drain the chamber. Repeat.

3. Fill the chamber a third time. Loosely insert the electrode and inject it with an additional 5 to 10 mL of QCCS to expel air bubbles. Seal the electrode into chamber. Check for air bubbles.

4. Turn the knob to "pH" and start the stopwatch. Record the initial pH, temperature, and time (0:00) in the pH logbook (see Figure 19-5, Column 1).

5. Wait until the readings seem fairly consistent and then note the time and pH values on a loose sheet of paper. If the pH reading does not

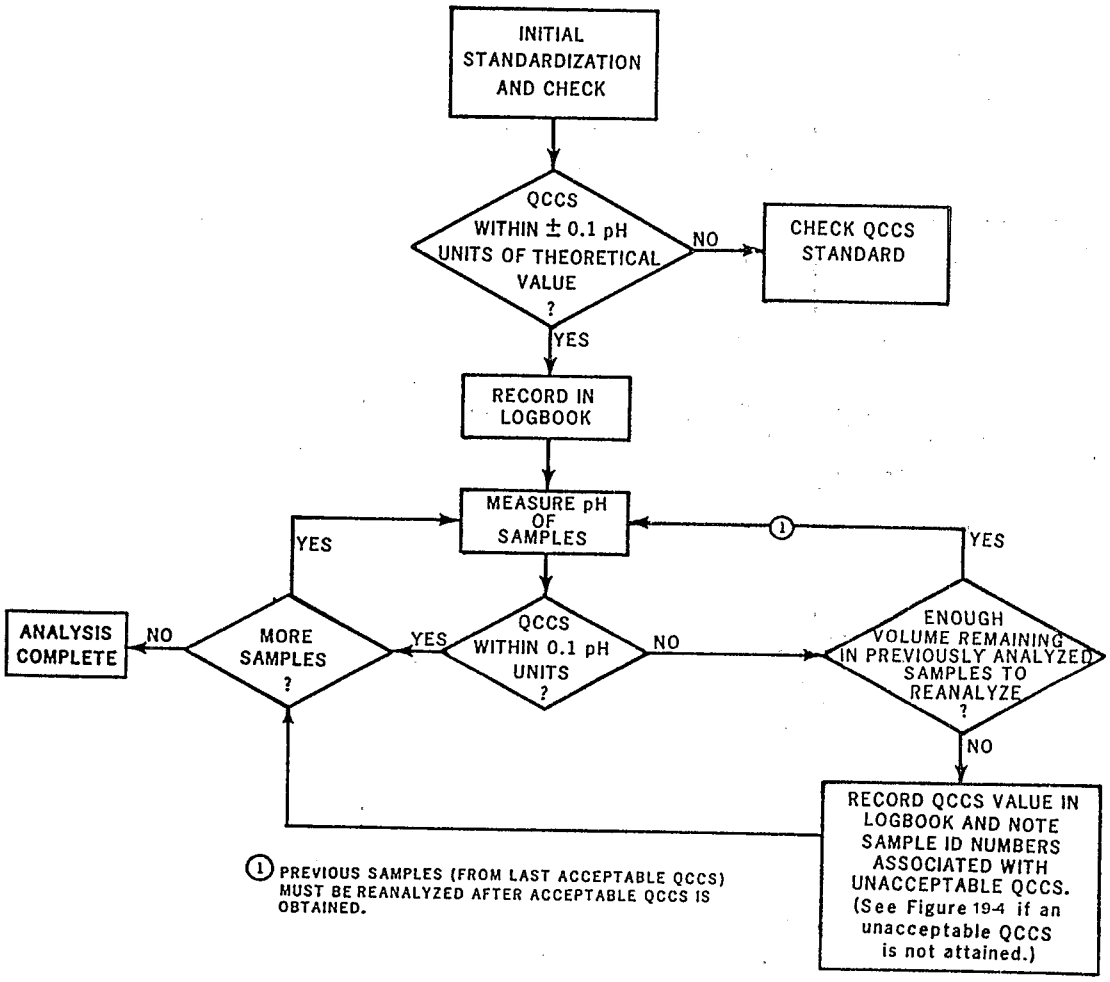
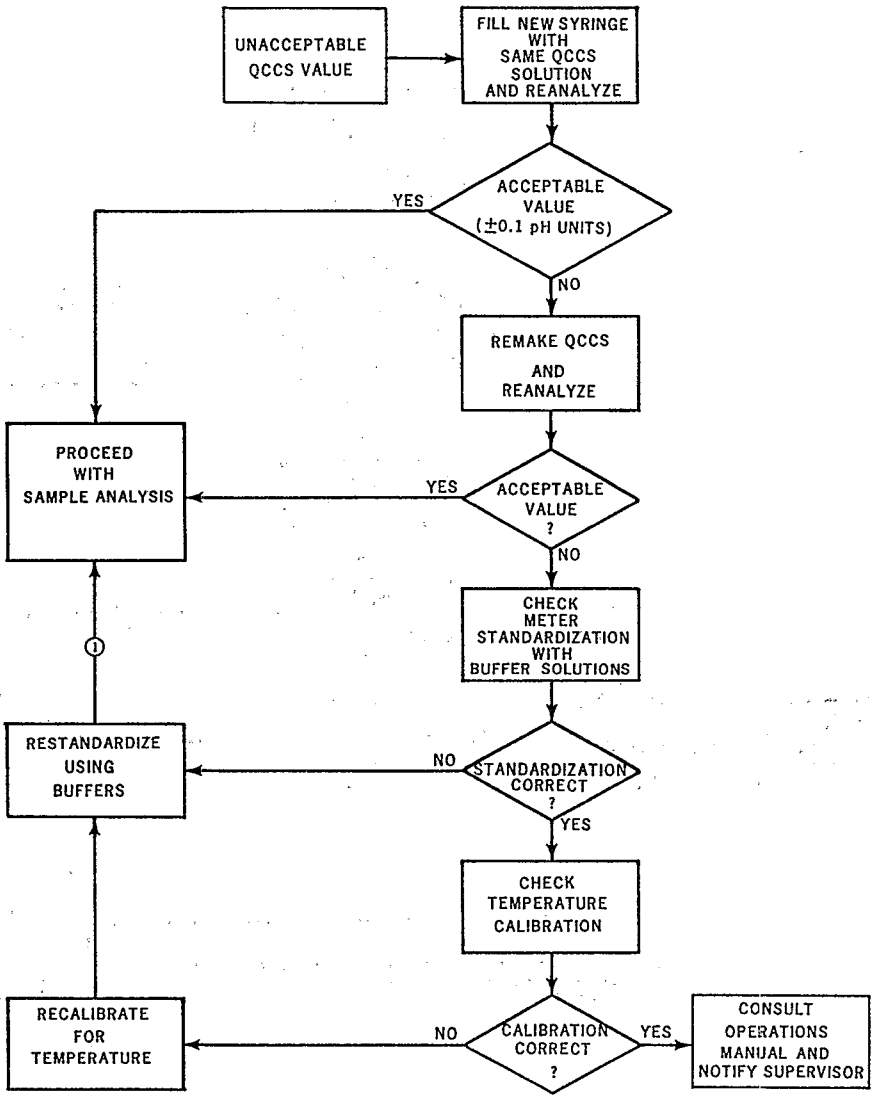


Figure 19-3. Flowchart for pH determination.



1 PREVIOUS SAMPLES (FROM LAST ACCEPTABLE QCCS) MUST BE REANALYZED AFTER ACCEPTABLE QCCS IS OBTAINED.

Figure 19-4. Troubleshooting flowchart for pH detemination.

							INJECT			INJECT			INJECT		
SAMPLE ID	INTIAL pH	INITIAL TEMP	INITIAL TIME	pH	TEMP	TIME	pH	TEMP	TIME	pH	TEMP	TIME	pH	TEMP	TIME
	COLUMN	1		COLUMN	2		COLUMN	3		COLUMN	4		COLUMN	5	

NOTE 1: DO NOT COMPARE SUCCESSIVE pH VALUE UNTIL DATA COLUMN 4. ALL EARLIER DATA ARE INDEPENDENT.

NOTE 2: SOME pH DETERMINATIONS MAY REQUIRE CONTINUATION ON TO THE NEXT LINE.

Figure 19-5. pH logbook and example page; organization of raw data.

vary by more than 0.02 pH units in one direction throughout a 1-minute interval, the reading is considered stable. Record the stable pH and temperature readings, and the total elapsed time in the logbook (see Figure 19-5, Column 2).

6. Inject a 5-mL portion of QC solution into the chamber. Repeat step 5. Record the first stable pH reading, temperature, and time in the logbook (see Figure 19-5, Column 3).

7. Inject a second 5-mL portion of QC solution into the chamber. Repeat step 5 and record the data in the logbook (see Figure 19-5, Column 4).

8. Check the pH values from Column 3 and Column 4 in the logbook. If they agree within ±0.03 pH units, the sample measurement is completed. If they do not, continue to inject 5-mL portions of solution into the chamber and record the first stable pH, temperature, and elapsed time values in additional columns until two successive stable pH readings agree within ±0.03 units.

19.5.3 Sample Measurement

NOTE: Allow syringe samples to warm to room temperature before measuring pH.

1. Rinse the sample chamber and electrode copiously with deionized water.

2. Attach a pH syringe to the chamber and determine the sample pH as described in Section 19.5.2, steps 2 through 8.

3. Measure and record the value of the QCCS at predetermined intervals.

   a. If the measured QCCS pH is acceptable (pH 4.00 ± 0.10), proceed with routine sample pH determinations.

   b. If the QCCS pH is not acceptable, follow the steps below until an acceptable value is obtained.

      1) Repour the pH 4.00 QCCS into a beaker, refill a new rinsed syringe, and reanalyze.

      2) Remake the pH 4.00 QCCS (see Section 19.3.2) and reanalyze.

      3) Repeat the standardization steps (see Section 19.4.2) and reanalyze the QCCS. If the pH meter requires recalibration

to obtain an acceptable QCCS reading, make a notation in the pH logbook. Determine which of the samples have sufficient volume to reanalyze. Reanalyze all samples which have sufficient sample volume back to the last acceptable QCCS.

c. Obtain an acceptable QCCS pH measurement prior to continuing analysis.

19.5.4 Additional Procedures Using Two pH Meters

1. Analyze a dilute buffer intermeter comparability check sample (see Section 19.3.2) after each QCCS is analyzed. The acceptable range for the dilute buffer is pH 7.31 ± 0.07. If the values are not acceptable, follow the steps below (for initial comparability only):

   a. If both meter values are out of range, remake the dilute buffer and reanalyze on both meters.

   b. If only one meter is out of range, check the meter calibration and recalibrate if necessary. If the calibration is correct, reanalyze a new syringe of dilute buffer. If the calbration still does not come within range, perform the electronic checkout described in Section 19.4.4 and repeat calibrations.

2. The dilute buffer values of both meters should agree within 0.05 pH unit. If they do not, follow the steps below (for initial comparability only):

   a. Check the calibration of both meters. Recalibrate one or both meters if necessary.

   b. If the calibrations of both meters are accurate, obtain new syringes and reanalyze the dilute buffer.

   c. If intermeter comparability cannot be obtained, all samples should be analyzed on the meter with the pH values closest to 7.31.

3. If the dilute buffer comparability checks that are analyzed mid-batch after the QCCS are within limits, but do not agree within 0.05 pH unit, follow the steps below:

   a. Obtain a new syringe and reanalyze the dilute buffer.

   b. If the values are within 0.05 pH unit, continue with sample analysis.

c. If the values are still not within 0.05 pH unit, recalibrate the meter which deviates the most from 7.31 pH units and reanalyze the dilute buffer. If the values agree within 0.05 pH unit, two meters can be used but reanalyze all samples which were analyzed on the recalibrated meter back to the last acceptable QCCS and the last dilute buffer check.

d. If the meters still are not within the 0.05 pH unit limit, samples should be analyzed on one meter; use the meter with the pH value closest to 7.31. All samples on the deviant meter should be reanalyzed, back to the last acceptable QCCS and the last dilute buffer values on the acceptable meter.

19.5.5 Cleanup

1. Copiously rinse the sample chamber and glassware with deionized water.

2. Remove the electrode from the silicone stopper of the sample chamber. Cover the fillhole of the electrode with the plastic sleeve and store the electrode in 3M KCl.

3. Make sure the meter is on "STD BY."

19.6 QUALITY ASSURANCE AND QUALITY CONTROL

19.6.1 Precision and Accuracy

A single laboratory, using a 5.00×10^{-5} H_2SO_4 solution for 485 chronological pH measurements by seven different operators and nine different electrodes of the same model (Orion Ross 81-04b combination electrode), achieved a precision of ±0.05 pH units and an accuracy of +0.03 pH units. (Metcalf, 1987).

19.6.2 Quality Control Checks

1. Laboratory Duplicate--One sample is analyzed in duplicate (without rinsing the chamber). The pH value of the duplicate sample should be within 0.1 pH unit of the routine sample value. If the value is outside the acceptable range, reanalyze a third time, if sample volume permits.

2. Field-Laboratory pH comparison--If, for a given sample the laboratory value differs from the *in situ* pH value by 0.5 pH unit or more, reanalyze the sample.

19.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

Burke, E. M., D. C. Hillman, and E. M. Heithmar, 1986. Stability of pH and DIC in sealed syringe samples. Presented at the Rocky Mountain Conference on Analytical Chemistry, August 3-5, Denver, Colorado.

Metcalf, R. C., 1987. The accuracy of Ross™ pH combination electrodes in dilute sulphuric acid standards. Analyst, v. 112, no. 10, in press.

Orion Research Incorporated, 1983. Instruction Manual - Model 611 pH/millivolt meter. Orion, Cambridge, Massachusetts.

20.0 DETERMINATION OF pH (OPEN SYSTEM)

20.1 OVERVIEW

The pH is defined as the negative logarithm of the activity of hydrogen ions (H^+). The H^+ activity is a measure of the "effective" concentration of hydrogen ions in solution; it is always equal to or less than the true concentration of hydrogen ions in solution. Values usually range from pH 1 to pH 14, with pH 1 being most acidic, pH 7 neutral (at 25 °C), and pH 14 most alkaline. Each pH unit represents a tenfold change in H^+ activity, i.e., a pH 4 solution is 10 times as acidic as a pH 5 solution.

When the pH of a sample solution is measured, the hydrogen ions come into equilibrium with the ion exchange surface (glass) of a calibrated pH electrode which creates an electrical potential. This voltage difference is measured by the pH meter in millivolts, then is converted and displayed as pH units.

20.1.1 Scope and Application

This method is applicable to the determination of pH in samples which are at equilibrium with respect to CO_2 gas transfer between the sample and the ambient atmosphere (e.g., snowpack and precipitation samples). For the AERP studies, pH is determined in the processing laboratory using an Orion Model 611 pH meter and an Orion Ross combination pH electrode. The method has been written assuming that the Orion meter and electrode are used (Orion, 1983). The method, however, can be modified for use with other instrumentation meeting equivalent specifications.

The applicable pH range is 3.0 to 11.0.

20.1.2 Summary of Method

The measurement is performed by immersing the electrode in a portion of the sample. The pH reading is considered stable when it does not vary more than 0.02 pH units in one direction throughout a one-minute interval.

20.1.3 Interferences

Atmospheric CO_2 will cause an interference if the sample is not at equilibria with the atmosphere or if the laboratory atmosphere is subject to variations in CO_2 concentration.

20.1.4 Safety

The calibration standards, sample types, and reagents used in this method pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses should be worn when handling sulfuric acid.

20.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples should be collected in clean, deionized water-washed containers. Approximately 60 mL of sample is needed. No special preservation procedures are necessary, although it is recommended samples be kept at 4 °C in the dark until analysis. Measurement of pH should be completed as soon as possible following sample collection, generally within 24 to 36 hours.

20.3 EQUIPMENT AND SUPPLIES

20.3.1 Apparatus and Equipment

1. Orion Model 611 pH meter or equivalent.

2. Orion Ross combination pH electrode or equivalent.

NOTE: Only combination electrodes are recommended.

3. Centrifuge tubes or small plastic beakers, deionized water-washed as described in Appendix C.

20.3.2 Reagents and Consumable Materials

1. pH Calibration Buffers (pH 4 and 7)--Commercially available pH calibration buffers (NBS-traceable) at pH values of 4 and 7.

2. Potassium Chloride (3M)--Dissolve 75 g KCl in 1 L of water.

3. pH 4.00 QC Solution (0.0001N H_2SO_4)--add 1.000 ml of 0.1N H_2SO_4 to a volumetric flask, dilute to 1 L. Prepare daily.

4. Water--Water used in all preparations should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).

20.4 PREPARATION

20.4.1 Instrument Preparation

Instrument preparation is identical to that described for closed system pH measurements (see Section 19.4.1).

20.4.2 Calibration and Standardization

Calibration is identical to that described for closed system pH measurements (see Section 19.4.2).

20.4.3 Maintenance

Maintenance is identical to that described for closed system pH measurements (see Section 19.4.3).

20.4.4 pH Meter Electronic Checkout

The pH meter electronic checkout is identical to that described for closed system pH measurements (see Section 19.4.4).

20.4.5 Electrode Etching Procedure

The electrode etching procedure is identical to that described for closed system pH measurements (see Section 19.4.5).

20.4.6 Sample Preparation

NOTE: Sample preparation should be done in the clean air station by an analyst wearing a lab coat and sterile gloves.

Obtain two 50-mL centrifuge tubes (deionized water-washed) which additionally have been leached in deionized water for at least 24 hours. Swirl the contents of the sample container and mix. Rinse the tubes three times with sample (if the sample volume is low, rinse twice with deionized water and a third time with sample). Swirl the sample container and pour approximately 25 mL of sample into each tube. Cap the tubes and label one "R" to be used as a rinse of the electrode.

20.5 PROCEDURE

NOTE: Refer to Figures 20-1 and 20-2.

20.5.1 Initial Quality Control Check

1. Rinse and fill two beakers with pH 4.00 QC solution.

2. Rinse the electrode by swirling it in the rinse beaker for 15 to 30 seconds.

3. Insert electrode into the measurement beaker.

4. Turn the knob to "pH" and start the stopwatch. Record the initial pH, temperature, and time (0:00) in the pH logbook.

5. Wait until the reading seems fairly consistent, then note the time and pH values on a loose sheet of paper. If the pH reading does not vary by more than 0.02 pH units in one direction after a 1-minute interval, the reading is considered stable. Record the

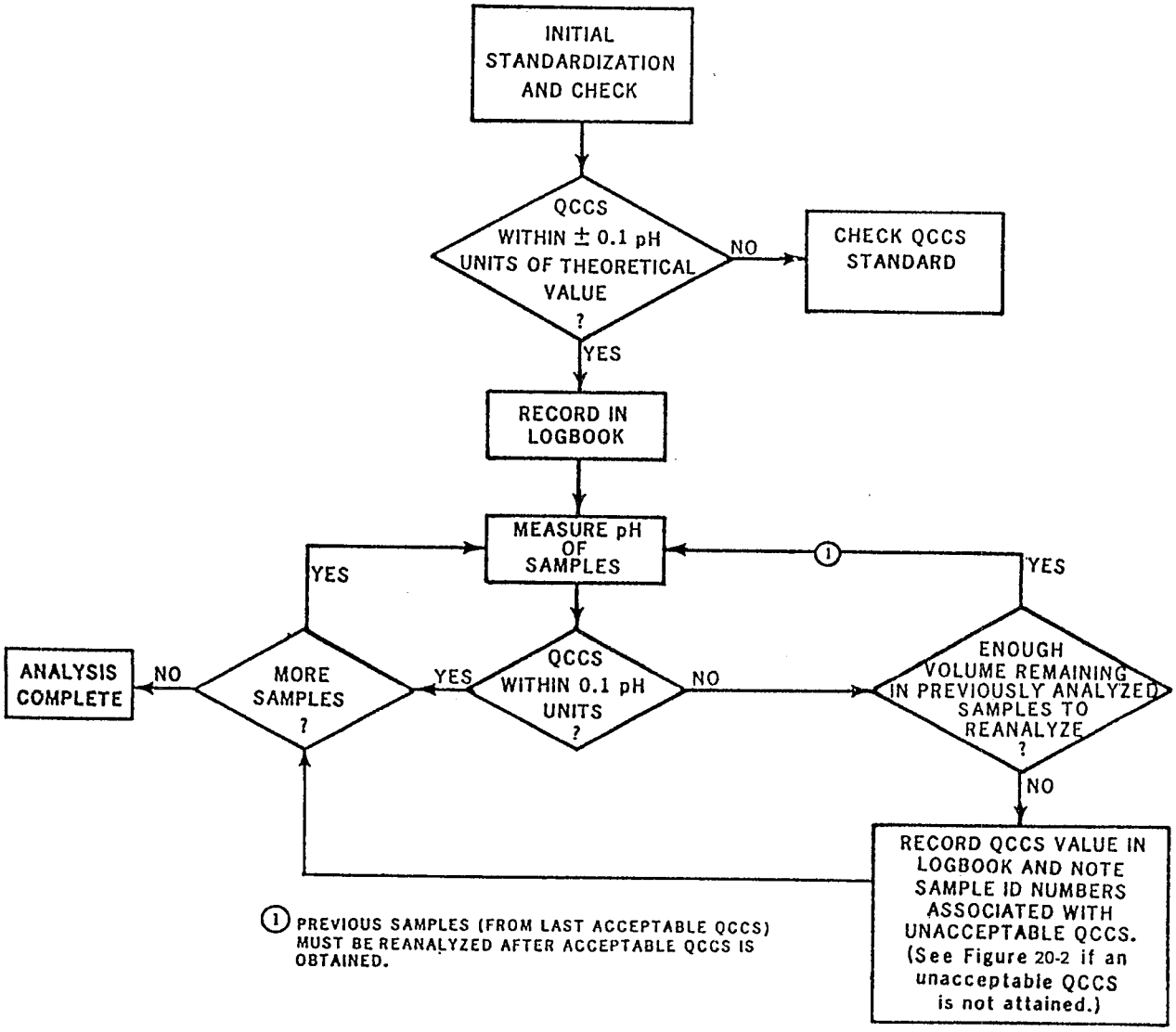


Figure 20-1. Flowchart for pH determination.

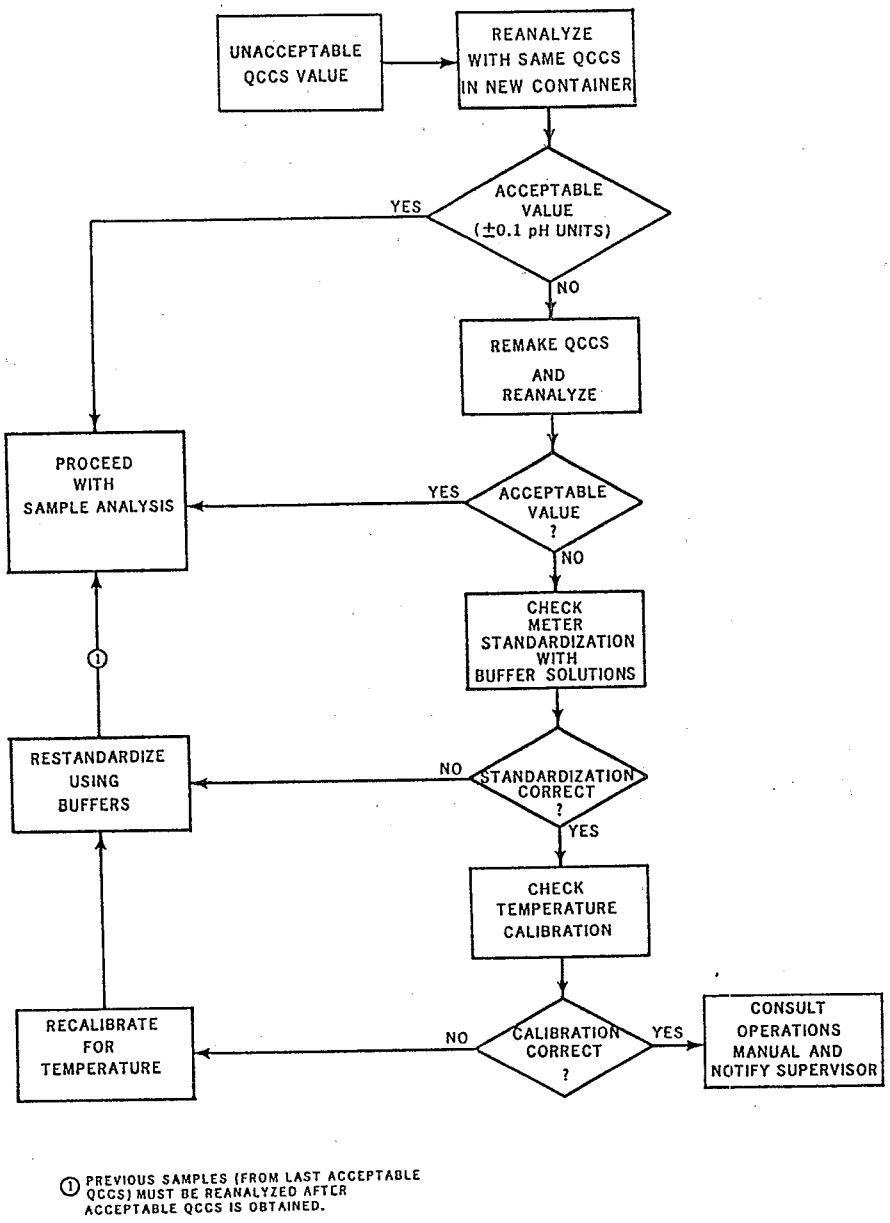


Figure 20-2. Troubleshooting flowchart for pH determination.

stable pH and temperature readings and the total elapsed time in the logbook.

20.5.2 Sample Measurement

1. Rinse the electrode copiously with deionized water, then rinse it in the sample tube marked "Rinse."

2. Determine sample pH by following the instructions in Section 20.5.1.

20.5.3 Routine Quality Control Check

NOTE: The pH 4.00 QC solution is analyzed at the beginning of a batch and at the end of a batch. The QCCS also is analyzed at intervals within the batch as specified by the quality assurance program.

1. Measure and record the QC solution by following the instructions in Section 20.5.1.

2. If the measured QC solution pH is acceptable (pH 4.00 ± 0.10), proceed with routine sample pH determinations.

3. If the QC solution pH is not acceptable, follow the steps below until an acceptable value is obtained:

   a. Repour the pH 4.00 QC solution into a beaker and reanalyze.

   b. Remake the pH 4.00 QC solution (see Section 20.3.2) and reanalyze.

   c. Repeat the standardization steps (see Section 20.4.3) and reanalyze the QC solution.

4. If the pH meter requires recalibration to obtain an acceptable QC reading, make a notation in the pH logbook. Determine which samples should be reanalyzed. Reanalyze all samples back to the last acceptable QC check.

20.5.4 Cleanup

1. Copiously rinse the electrode and glassware with deionized water.

2. Cover the fillhole of the electrode with the plastic sleeve and store the electrode in 3M KCl.

3. Make sure the meter is on "STD BY."

20.6 QUALITY ASSURANCE AND QUALITY CONTROL

See Section 19.6.

20.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagaent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

Orion Research Incorporated, 1983. Instruction Manual - Model 611 pH/millivolt meter. Orion, Cambridge, Massachusetts.

21.0 DETERMINATION OF TOTAL PHOSPHORUS

21.1 OVERVIEW

21.1.1 Scope and Application

This method may be used to determine concentrations of total phosphorus in natural surface waters in the range from 0.001 to 0.200 mg L^{-1} P.

NOTE: Samples preserved with $HgCl_2$ should not be analyzed using this method.

21.1.2 Summary of Method

All forms of phosphorus, including organic phosphorus, are converted to orthophosphate by an acid-persulfate digestion.

Orthophosphate ions react with ammonium molybdate in acidic solution to form phosphomolybdic acid, which upon reduction with ascorbic acid produces an intensely colored blue complex. Antimony potassium tartrate is added to increase the rate of reduction (Skougstad, et al., 1979; Gales, et al., 1966; Murphy and Riley, 1962).

21.1.3 Interferences

Barium, lead, and silver interfere by forming a precipitate. There is a positive interference from silica when the silica-to-total-phosphorus ratio exceeds about 400:1 (Table 21-1).

TABLE 21-1. PERCENT RECOVERY OF TOTAL PHOSPHORUS IN THE PRESENCE OF SILICA (Skougstad, et al., 1979)

	SiO_2 (mg L^{-1})				
Total P mg L^{-1}	20	15	10	5	1
0.200	98	100	100	102	101
0.100	103	---	---	---	103
0.050	104	104	102	102	102
0.010	144	133	122	111	100
0.005	160	140	120	120	100
0.002	550	350	250	250	100

$HgCl_2$-NaCl-preserved samples give inconsistent results and, therefore, should not be used.

21.1.4 Safety

The calibration standards, sample types, and most reagents used in this method pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses should be worn when handling concentrated sulfuric acid. Use proper care when operating the autoclave. Follow manufacturer's safety precautions.

21.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples are preserved with concentrated H_2SO_4 until the pH is less than 2. Samples are stored at 4 °C in the dark when not in use.

21.3 EQUIPMENT AND SUPPLIES

21.3.1 Equipment Specifications

1. Technicon AutoAnalyzer II or equivalent, consisting of sampler, cartridge manifold, proportioning pump, heating bath, colorimeter, voltage stabilizer, recorder, and printer. With this equipment the following operating conditions have been found satisfactory for the range from 0.001 to 0.200 mg L^{-1} P:

   Absorption cell--50 mm
   Wavelength--880 nm
   Cam--30 h^{-1} (1:1)
   Heating bath temperature--37.5 °C.

21.3.2 Apparatus

1. Autoclave.

2. Glass tubes with plastic caps, disposable--18 mm by 150 mm.

21.3.3 Reagents and Consumable Materials

All reagents should be ACS reagent grade or equivalent.

1. Ammonium Molybdate Solution (35.6 g L^{-1})--Dissolve 40 g of ammonium molybdate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$ in 800 mL of water and dilute to 1 L.

2. Ascorbic Acid Solution (18 g L^{-1})--Dissolve 18 g of ascorbic acid ($C_6H_8O_4$) in 800 mL of water and dilute to 1 L.

3. Antimony Potassium Tartrate Solution (3 g L^{-1})--Dissolve 3.0 g of antimony potassium tartrate [$K(SbO)C_4H_4O_6 \cdot 1/2H_2O$] in 800 mL of water and dilute to 1 L.

4. Combined Working Reagent--Combine reagents in the order listed below. (This reagent is stable for about 8 hours. The stability is increased if kept at 4 °C):

50 mL	Sulfuric acid, 2.45M
15 mL	Ammonium molybdate solution
30 mL	Ascorbic acid solution
5 mL	Antimony potassium tartrate solution

5. Phosphate Stock Standard Solution (100 mg L^{-1} P)--Dissolve 0.4394 g of potassium acid phosphate (KH_2PO_4, dried for 12 to 16 hours over concentrated H_2SO_4, sp. gr. 1.84) in water and dilute to 1.000 L.

6. Phosphate Standard Solution I (10.00 mg L^{-1} P)--Quantitatively dilute 100.0 mL phosphate stock standard solution to 1.000 L with water.

7. Phosphate Standard Solution II (1.000 mg L^{-1} P)--Quantitatively dilute 10.00 mL phosphate stock standard solution to 1.000 L with water.

8. Dilute Phosphate Working Standards--Prepare a blank and 1.000 L each of a series of working standards by appropriate quantitative dilution of phosphate standard solutions I and II. For example:

Phosphate standard solution II (mL)	Phosphate standard solution I (mL)	Total P concentration in working standard (mg L^{-1})
0.0	0.0	0.000
1.00	-	0.001
5.00	-	0.005
10.00	-	0.010
-	5.0	0.050
-	10.0	0.100
-	20.0	0.200

9. Potassium Persulfate Solution (4 g L^{-1})--Dissolve 4.0 g potassium of persulfate ($K_2S_2O_8$) in water and dilute to 1 L.

10. Sulfuric Acid (2.45M)--Slowly, and with constant stirring and cooling, add 136 mL of concentrated sulfuric acid (sp. gr. 1.84) to 800 mL water. Cool and dilute to 1 L with water.

11. Sulfuric Acid (0.45M)--Slowly, and with constant stirring and cooling, add 25.2 mL of concentrated sulfuric acid (sp. gr. 1.84) to 800 mL water. Cool and dilute to 1 L with water.

12. Sulfuric Acid-Persulfate Reagent (1 + 1)--Mix equal volumes of 0.45M sulfuric acid and potassium persulfate solution.

13. Water Diluent--Add 1.0 mL Levor IV (Technicon No. 21-0332 or equivalent) to 1 L water.

14. Water--Water should meet the specifications for Type I reagent grade water (ASTM, 1984).

21.4 PREPARATION

21.4.1 Calibration and Standardization

Analyze the series of total P standards as described in Section 21.5. Prepare a calibration curve by plotting the peak height versus standard concentration.

21.5 PROCEDURE

21.5.1 Standard Operating Procedure

NOTE: It is critical that the colorimeter is optically peaked prior to first analysis.

1. Mix each sample, pipet a volume of it containing less than 0.002 mg total P (10.0 mL maximum) into a disposable glass tube, and adjust the volume to 10.0 mL.

2. Prepare blank solutions and several standards bracketing the expected concentration range and adjust the volume of each to 10.0 mL.

3. Add 4.0 mL acid-persulfate reagent to samples, blanks, and standards.

4. Place plastic caps gently on top of tubes, but do not push down. Autoclave for 30 minutes at 15 psi pressure and 121 °C. After the samples have cooled, the caps may be pushed down.

5. Set up manifold as shown in Figure 21-1.

6. Allow the colorimeter, recorder, and heating bath to warm up for at least 30 minutes or until the temperature of the heating bath reaches 37.5 °C. Zero the recorder baseline while pumping all reagents through the system.

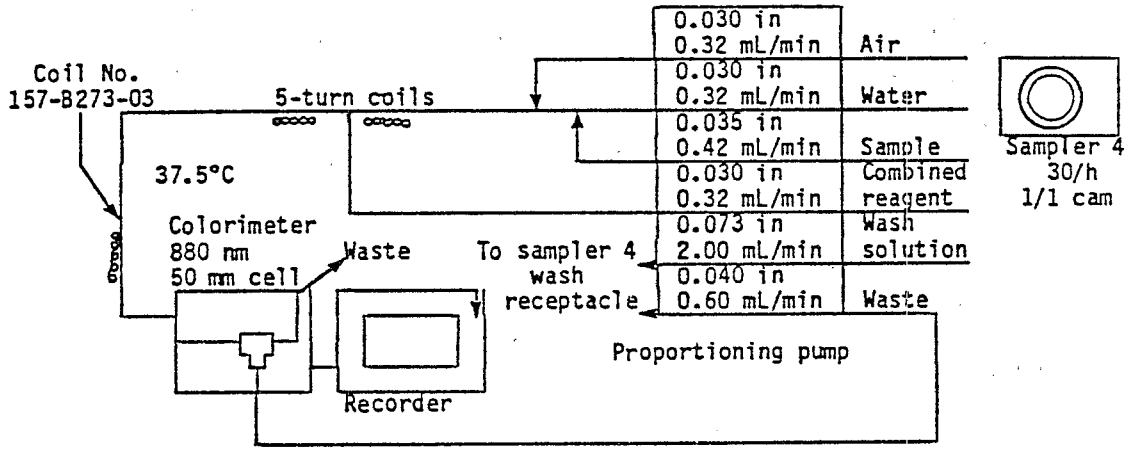


Figure 21-1. Total Phosphorus Manifold.

7. Beginning with the most concentrated standard, place a complete set of standards in the first positions of the first sample tray with blank solution between each standard. Fill remainder of each tray alternately with unknown samples and blank solution.

8. Begin analysis. When the peak from the most concentrated standard appears on the recorder, adjust the "STD CAL" control until the flat portion of the peak reads full scale. Using the baseline control, adjust each blank in the tray to read zero as it is analyzed.

9. Dilute and reanalyze samples with a total P concentration exceeding the calibrated range.

21.5.2 Calculations

Compute the concentration of total P in each sample by comparing its peak height to the calibration curve. Report results as mg L^{-1} P.

21.6 QUALITY ASSURANCE AND QUALITY CONTROL

21.6.1 Precision and Accuracy

Data for the determination of the precision and accuracy of the method are given in Tables 21-2 and 21-3.

TABLE 21-2. PRECISION AND ACCURACY OF THE PHOSPHORUS METHOD FOR NATURAL WATER SAMPLES (Skougstad, et al., 1979). (All data in mg L^{-1} P)

Sample	n	Mean	Std. Dev.	Rel. Std. Dev. (%)
4-065070	10	0.0347	0.0012	3.34
4-065080	10	0.1435	0.0031	2.16
4-066060	10	0.0902	0.0027	2.99

TABLE 21-3. PRECISION AND ACCURACY OF THE PHOSPHORUS METHOD FOR ANALYST-PREPARED STANDARDS (Skougstad, et al., 1979). (All data in mg L^{-1} P)

Sample	n	Mean	Std. Dev.	Rel. Std. Dev. (%)
0.040	9	0.0424	0.0007	1.71
0.030	10	0.0322	0.0006	1.96
0.020	10	0.0172	0.0004	2.45
0.004	9	0.0033	0.0007	21.21
0.001	9	0.0013	0.0005	37.5

It is estimated that the percent relative standard deviation (%RSD) (coefficient of variation) of this method is 38 percent at 0.001 mg L^{-1}, 2.5 percent at 0.020 mg L^{-1}, and 2.2 percent at 0.144 mg L^{-1}.

21.6.2 Quality Control Checks

The required quality control procedures are described in Appendix G.

21.7 REFERENCES

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22.0 DETERMINATION OF DISSOLVED SILICA

22.1 OVERVIEW

22.1.1 Scope and Application

This method is applicable for the determination of dissolved silica in natural surface waters in the concentration range from 0.1 to 10 mg L^{-1}.

22.1.2 Summary of Method

The procedure utilizes automated technology and is based on existing methodology (Skougstad, et al., 1979).

Silica reacts with molybdate reagent in acid media to form a yellow silicomolybdate complex. This complex is reduced by ascorbic acid to form the molybdate blue color. The silicomolybdate complex may form either as an alpha or beta polymorph, or as a mixture of both. Because the two polymorphic forms have absorbance maxima at different wavelengths, the pH of the mixture is kept below 2.5, a condition which favors formation of the beta polymorph (Govett, 1961; Mullen and Riley, 1955; Strickland, 1962).

A 1-hour digestion with 1.0M NaOH is required to ensure that all the silica is available for reaction with the molybdate reagent.

22.1.3 Interferences

Interference from phosphate, which forms a phosphomolybdate complex, is suppressed by the addition of oxalic acid. Hydrogen sulfide should be removed by boiling the acidified sample prior to analysis. Large amounts of iron interfere; however, neither hydrogen sulfide nor iron is expected in appreciable quantities in natural surface water samples.

22.1.4 Safety

The calibration standards, samples, and most reagents used in this method pose no hazard to the analyst. Protective clothing (lab coat and gloves) and safety glasses should be worn when handling concentrated sulfuric acid and performing sample digestions.

22.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples are collected and filtered using only deionized water-washed containers and apparatus. Sample containers are completely filled (i.e., no headspace) and are stored at 4 °C in the dark when not in use.

22.3 EQUIPMENT AND SUPPLIES

22.3.1 Equipment Specifications

1. Technicon AutoAnalyzer II or equivalent consisting of sampler, cartridge manifold, proportioning pump, colorimeter, voltage stabilizer, recorder, and printer.

With this equipment, the following operating conditions are recommended:

Absorption cell--15 mm

Wavelength--660 nm

Cam 60 $hour^{-1}$--(6/1)

22.3.2 Reagents and Consumable Materials

1. Ammonium Molybdate Solution (9.4 g L^{-1})--Dissolve 10 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) in 0.05M H_2SO_4 and dilute to 1 L with 0.05M H_2SO_4. Filter and store in an amber plastic container.

2. Ascorbic Acid Solution (17.6 g L^{-1})--Dissolve 17.6 g of ascorbic acid ($C_6H_8O_6$) in 500 mL of water containing 50 mL acetone. Dilute to 1 L with water. Add 0.5 mL Levor IV solution. The solution is stable for 1 week if stored at 4 °C.

3. Hydrochloric Acid (50 percent v/v)--Slowly add 500 mL of concentrated HCl to 500 mL water.

4. Hydrochloric Acid (2 percent v/v)--Add 10 mL of concentrated HCl to 490 mL water.

5. Hydrofluoric Acid (HF, ACS reagent grade).

6. Levor IV Solution--Technicon No. 21-0332 or equivalent.

7. Oxalic Acid Solution (50 g L^{-1})--Dissolve 50 g of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$) in water and dilute to 1 L.

8. Silica Standard Solution (500 mg L^{-1} SiO_2)--Dissolve 2.366 g of sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$) in water and dilute to 1.000 L. The concentration of this solution should be verified by standard gravimetric analysis (described in Section 22.4). Store in a plastic bottle.

9. Silica Working Standards--Prepare a blank and 500 mL each of a series of silica working standards by appropriate quantitative dilution of the silica stock standard solution. The following series is suggested:

Silica stock standard solution (mL)	Silica concentration in working standard (mg L^{-1})
0.0	0.0
0.200	0.200
0.500	0.500
1.00	1.00
5.00	5.00
10.0	10.0

10. Sodium Hydroxide Solution (1.0M NaOH)--Dissolve 4 g of sodium hydroxide (NaOH) in water and dilute to 1 L.

11. Sulfuric Acid Solution (0.05M H_2SO_4) (50% v/v H_2SO_4)--Cautiously add 2.8 mL of concentrated sulfuric acid (H_2SO_4, sp. gr. 1.84) to water and dilute to 1 L. Cautiously and slowly add 500 mL H_2SO_4 to 500 mL of water. Beware of excessive heat buildup.

12. Water--Water should meet the specifications for Type I reagent grade water (ASTM, 1984).

22.4 PREPARATION

22.4.1 Calibration and Standardization

Verify the concentration of the silica stock standard solution using the gravimetric procedure detailed in steps 1 through 7 (APHA, 1980).

1. Sample Evaporation--Add 5 mL of 50 percent v/v HCl to 200.0 mL silica stock standard. Evaporate to dryness in a 200-mL platinum evaporating dish, in several portions if necessary, on a water bath or suspended on an asbestos ring over a hot plate. Protect against contamination by atmospheric dust. During evaporation, add a total of 15 mL of 50 percent HCl in several portions. Evaporate sample to dryness and place dish with residue in a 110 °C oven or over a hot plate to bake for 30 minutes.

2. First Filtration--Add 5 mL of warm 50 percent HCl and add 50 mL of hot water. While hot, filter sample through an ashless medium-texture filter paper, decanting as much liquid as possible. Wash dish and residue with hot 2 percent HCl, then with a minimum volume of water until washings are chloride-free. Save all washings. Set aside filter paper with its residue.

3. Second Filtration--Evaporate filtrate and washings from the above operations to dryness in the original platinum dish. Bake residue in a 110 °C oven or over a hot plate for 30 minutes. Repeat step 2. Use a separate filter paper and a rubber policeman to aid in transferring residue from dish to filter.

4. Ignition--Transfer the two filter papers and residues to a covered platinum crucible, dry at 110 °C, and ignite at 1,200 °C to constant weight. Avoid mechanical loss of residue when first charring and burning off the paper. Cool in desiccator, weigh, and repeat ignition and weighing until constant weight is attained. Record weight of crucible and contents.

5. Volatilization with HF--Thoroughly moisten weighed residue with water. Add 4 drops of 50 percent v/v H_2SO_4 followed by 10 mL of concentrated HF, measuring the latter in a plastic graduated cylinder or pouring an estimated 10 mL directly from the reagent bottle. Slowly evaporate to dryness over an air bath or hot plate in a hood, and avoid loss by splattering. Ignite crucible to constant weight at 1,200 °C. Record weight of crucible and contents.

6. Blank--Repeat steps 1 through 5 with a blank sample.

7. Perform the following calculations for both the standard and blank samples:

   X = weight of crucible plus contents before HF treatment (mg)
   Y = weight of crucible plus contents after HF treatment (mg)
   Z = weight of silica in sample (mg) = X - Y

8. Calculate the silica concentration in the stock standard by:

$$\frac{\text{mg } SiO_2}{\text{L}} = \frac{\text{Z (standard) - Z (blank) mg}}{\text{0.200 L}}$$

9. Analyze the series of silica standards as described in Section 22.5 (including digestion).

10. Prepare a calibration curve by plotting the peak height versus standard concentration.

22.5 PROCEDURE

22.5.1 Standard Operating Procedure

1. Set up the manifold as shown in Figure 22-1.

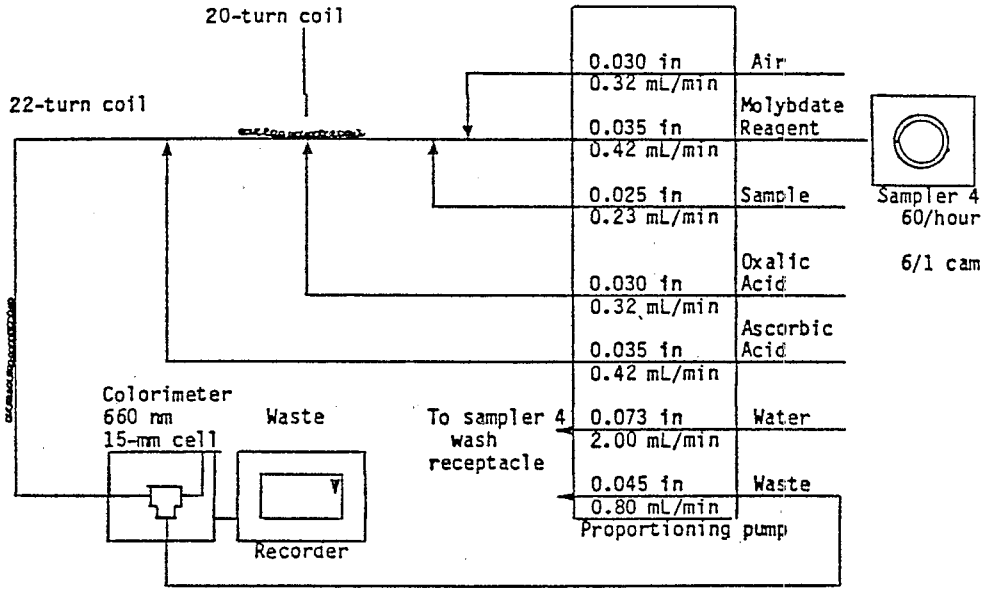

Figure 22-1. Silica manifold.

2. Allow colorimeter and recorder to warm up for at least 30 minutes. Zero the recorder baseline while pumping all reagents through the system.

3. Add 5.00 mL of 1.0M NaOH to 50.00 mL of sample. Digest for one hour.

4. Beginning with the most concentrated working standard, place a complete set of standards in the first positions of the first sample tray, followed by a blank. Fill remainder of each sample tray with unknown and QC samples.

5. Begin analysis. When the peak from the most concentrated working standard appears on the recorder, adjust the "STD CAL" control until the flat portion of the curve reads full scale.

6. Dilute and reanalyze any sample with a concentration exceeding the calibrated range.

22.5.2 Calculations

Compute the silica concentration of each sample by comparing its peak height to the calibration curve. Any baseline drift that may occur should be taken into account when computing the height of a sample or standard peak. Report results as mg L^{-1} SiO_2.

22.6 QUALITY ASSURANCE AND QUALITY CONTROL

22.6.1 Precision and Accuracy

In a multiple laboratory study using 111 lake samples containing 0.05 to 10 mg L^{-1} SiO_2 the duplicate relative standard deviation was 1.6 percent (note that this is the overall within-laboratory precision).

In a multiple laboratory study using two synthetic, simulated lake samples containing 10.7 and 1.07 mg L^{-1} SiO_2, respectively, recoveries obtained were 88 (n=21) and 95 (n=21) percent, respectively.

22.6.2 Quality Control Checks

The required QC is described in Appendix G.

22.7 REFERENCES

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23.0 DETERMINATION OF SPECIFIC CONDUCTANCE

23.1 OVERVIEW

Specific conductance is a measure which often can be linearly correlated with the ionic strength of a solution. Conductivity can be used to generate a synthetic ionic balance which can be used as a check of measured ionic concentrations.

23.1.1 Scope and Application

This method is applicable to natural surface waters of low ionic strength. Most freshwater lakes sampled in the AERP stuides have a specific conductance in the range 10 to 100 $\mu S\ cm^{-1}$; AERP-sampled streams generally have a specific conductance in the range 10 to 500 $\mu S\ cm^{-1}$.

23.1.2 Summary of Method

The specific conductance in samples is measured using a conductance meter and conductivity cell. The meter and cell are calibrated using potassium chloride standards of known specific conductance (U.S. EPA, 1983). Standards and samples are analyzed at 25 °C. A temperature-controlled water bath is recommended to maintain a constant temperature.

23.1.3 Interferences

Temperature variations represent the major source of potential error in specific conductance determinations. To minimize this error, calibration standards and samples should be measured at the same temperature. A temperature-controlled water bath is recommended.

Natural surface waters contain substances (humic and fulvic acids, suspended solids, etc.) which may build up on the conductivity cell. Such a buildup interferes with the operation of the cell and should be removed periodically, following the cell manufacturer's recommendations.

23.1.4 Safety

The calibration standards and sample types pose no hazard to the analyst.

23.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Samples are collected in clean, deionized water-washed containers. The container washing procedure is described in Appendix C. Specific conductance should be determined as close to sample collection time as possible, generally within 24 hours. Samples should be kept at 4 °C until analysis.

23.3 EQUIPMENT AND SUPPLIES

23.3.1 Equipment Specifications

Digital specific conductance meter with the following minimum specifications:

Range: 0.1 to 1,000 µS cm^{-1}
Readability: 0.1 µS cm^{-1}
Maximum Error: 1 percent of reading
Maximum Imprecision: 1 percent of reading

23.3.2 Apparatus

1. Conductivity Cell--High-quality glass cell with a cell constant of 1.0 or 0.1. Cells containing platinized electrodes are recommended.

2. Thermometer--NBS-traceable thermometer with a range of 0 to 40 °C and divisions of 0.1 °C.

3. Constant Temperature Water Bath (Optional)--Controlled to a temperature of 25.0 ± 0.1 °C.

4. Centrifuge Tubes--50 mL, deionized water-washed. Soak in deionized water for a minimum of 24 hours between uses.

23.3.3 Reagents and Consumable Materials

1. Potassium Chloride Stock Solution (1M KCl)--

NOTE 1: Prepare as needed and refrigerate at 4 °C.

NOTE 2: This stock solution is used to make the following standards:

147 µS cm^{-1} calibration standard
14.7, 74, 147 µS cm^{-1} QC standards.

NOTE 3: This stock solution should be made up in at least 1-L batches to minimize weighing and dilution errors. The 1M KCl stock solution has a theoretical specific conductance of 111,900 µS cm^{-1} at 25 °C. This value should be verified by measuring at least three 35-mL samples contained in 50-mL centrifuge tubes.

a. Fill a clean 1-L volumetric flask with approximately 500 mL of deionized water. Water should meet the specifications for Type I reagent grade water (ASTM, 1984).

b. Weigh 74.553 g of potassium chloride (KCl, ultrapure, dried for 2 hours at 105 °C and ampulated).

c. Completely dissolve the KCl in deionized water and dilute to the 1-L mark. Mix again thoroughly.

d. Store the stock solution in 500-mL bottles (deionized water-washed) which have been rinsed three times with the 1M KCl solution. Label the bottles "1M KCl Stock Solution" and refrigerate at 4 °C.

2. Calibration Blank-- Rinse two clean, labeled 50-mL centrifuge tubes three times with deionized water, then fill with 30 to 40 mL of deionized water.

NOTE 1: Two centrifuge tubes (leached in deionized water for a minimum of 24 hours) are needed for each calibration, QC and blank solution. Label accordingly and designate one of each set as the rinse.

NOTE 2: It cannot be assumed that the deionized water has a negligible conductivity; therefore, the blank conductivity value is subtracted from all standards.

NOTE 3: Be consistent in obtaining deionized water. Obtain deionized water from the same source from which all standards are made.

3. Calibration Standard - 147 µS cm^{-1}--

NOTE: Prepare daily

a. Fill a clean, labeled 1-L volumetric flask with approximately 500 mL of deionized water. Obtain a 50-mL disposable beaker, rinse three times with 1M KCl stock solution and pour 5 to 10 mL of stock solution. Use this stock solution to make calibration and QC solutions.

b. Use a calibrated 100- to 2,000-µL pipet (rinse pipet tip one time with solution) to deliver 1.000 mL of stock solution to the 1-L flask. Mix and dilute to 1-L mark and mix again.

c. Rinse two clean labeled 50-mL centrifuge tubes three times with calibration standard and pour 30 to 40 mL in each tube.

4. Quality Control Standards - 14.7, 74, 147 µS cm^{-1}--

NOTE: Prepare daily.

a. Fill three clean, labeled 500-mL volumetric flasks with approximately 250 mL deionized water.

b. Use the 1M KCl stock solution to prepare the following solutions:

1) 14.7 µS cm^{-1}--Use a calibrated 40- to 200-µL pipet to deliver 0.050 mL of stock solution to the volumetric flask labeled "14.7 µS cm^{-1} QC Standard". Alternately, weigh 50 mg of stock solution. Low-range standards can generally be prepared more accurately by weight than by volume.

2) 74 µS cm^{-1}--Use a calibrated 200- to 1,000-µL pipet to deliver 0.250 mL of stock solution to the volumetric flask labeled "74 µS cm^{-1} QC Standard".

3) 147 µS cm^{-1}--Use a calibrated 200- to 1,000-µL pipet to deliver 0.500 mL of stock solution to the volumetric flask labeled "147 µS cm^{-1} QC Standard".

c. Mix and dilute each of the three standards to the 500-mL mark and mix again.

d. Rinse each clean, labeled centrifuge tube three times with the appropriate standard (2 centrifuge tubes for each standard, with one tube designated as rinse). Fill each tube with its corresponding standard (30 to 40 mL).

e. Cap and store each standard and all poured centrifuge tubes at room temperature.

23.4 PREPARATION

NOTE: See Figure 23-1.

23.4.1 Electronics Check

1. Unscrew both of the leads connecting the probe to the conductance meter to break the circuit and to prevent capacitance shunting between calibrating resistors and probe.

2. Check the electronic function of the conductance meter by plugging in the resistors and reading the specific conductance at these ranges:

Resistor Value (µmho)	Range (µmohms = µ℧)
1.000	2 µ℧
10.00	200 µ℧
100.0	2 µ℧

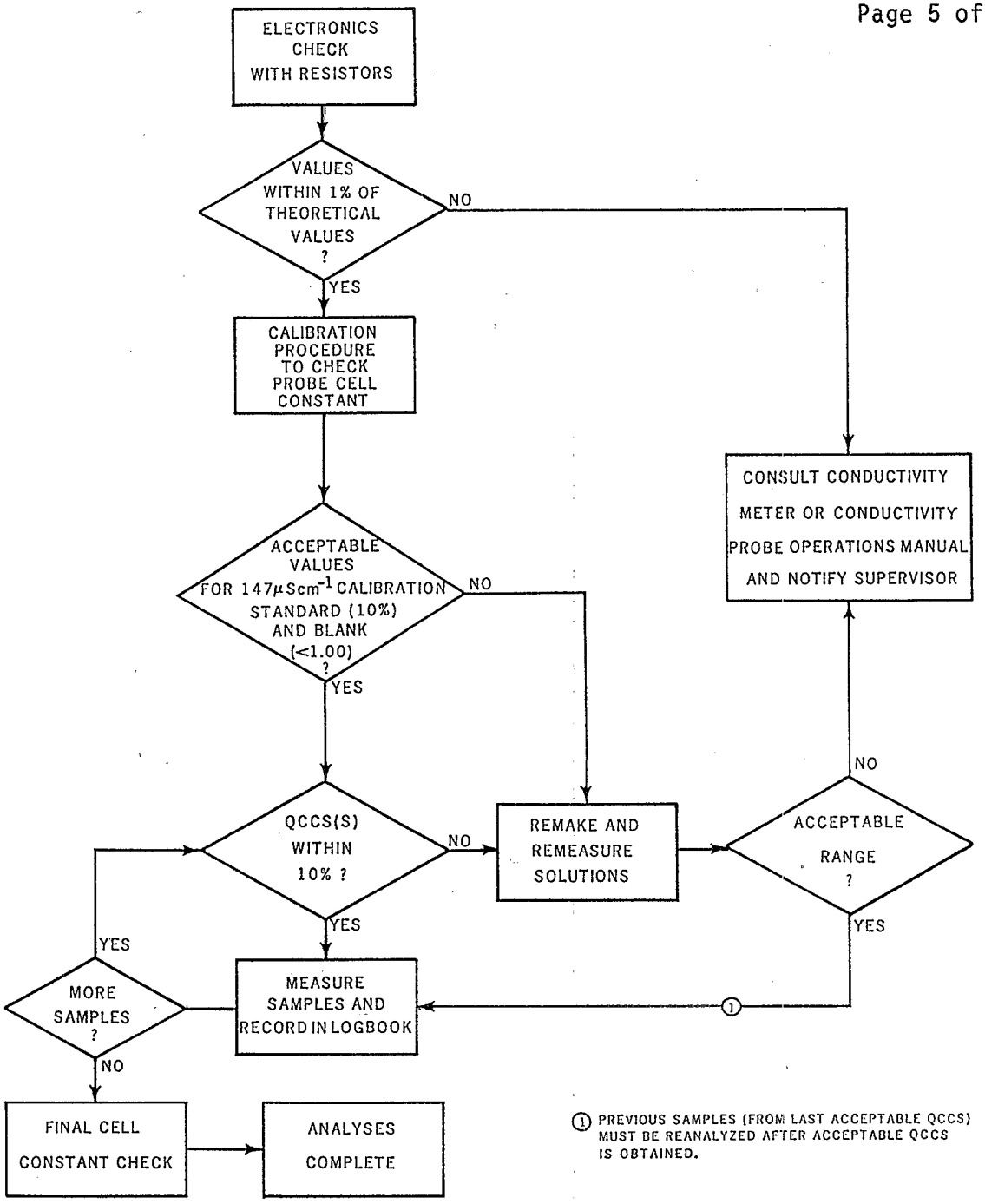


Figure 23-1. Flowchart for specific conductance measurement.

Readings should be within 1.0 percent of the theoretical value. Record the values in the logbook. If the values are not within 1.0 percent, consult the manufacturer's guide.

23.4.2 Conductivity Cell Calibration Check

NOTE 1: Turn conductance meter "OFF" when removing probe from solution.

NOTE 2: Gently mix all solutions in the centrifuge tubes by inverting them three times.

NOTE 3: When measuring the specific conductance of a solution, do not allow the probe to touch the sides or the bottom of the plasticware. Hold the cell upright. Be sure the vent holes are covered by solution and that there are no air bubbles around the probe.

NOTE 4: Rinse the probe and NBS-traceable thermometer in deionized water between each measurement.

NOTE 5: Measure the blank first.

1. Make sure the probe is connected properly.

2. Obtain a NBS-traceable thermometer. (A temperature probe may be substituted when approved and calibrated against an NBS-traceable thermometer).

3. Rinse the probe in the blank rinse solution for 10 to 15 seconds. Place the probe in the blank solution to be measured. The range should be set at 2μ .

4. Following manufacturer's directions, determine the specific conductance of the calibration blank. Allow the reading to stabilize. Record values in the logbook. The value should be less than 1.000 at this range; if not, repour and measure it again.

5. Rinse the NBS-traceable thermometer in the blank rinse and measure the temperature of the solution to the nearest 0.1 °C. Record the value in the logbook.

6. Measure the specific conductance of the 147 $\mu S/cm^{-1}$ calibration standard as described for the blank. The range should be set at 200 μ℧.

7. The cell constant (K) of the conductivity cell is a commercially certified standard which is checked daily in the laboratory. For samples with a specific conductance greater than 20 $\mu S\ cm^{-1}$, use a cell with a theoretical cell constant value of 1.0. For samples

with a specific conductance of less than 20 μS cm^{-1}, use a cell with a theoretical cell constant value of 0.1.

8. The cell constant is checked using the blank and 147-μS cm^{-1} calibration standard measurements. All theoretical values for the specific conductance standards are at 25 °C. A temperature correction table (Table 23-1) with the appropriate muiltiplication factors permits the conversion of a measured value at any temperature (° C) to the value at 25 °C. The chart is read to the nearest 0.1 °C. Calculate the cell constant by:

$$K = \frac{147\ (\text{Theoretical Value of Standard at 25 °C})}{\left(\begin{array}{c}\text{Measured Value}\\ \text{of Calibration}\\ \text{Standard}\end{array} \times \begin{array}{c}\text{Temperature}\\ \text{Correction}\\ \text{(from Table}\\ \text{23-1)}\end{array}\right) - \left(\begin{array}{c}\text{Measured}\\ \text{Value of}\\ \text{Blank}\end{array} \times \begin{array}{c}\text{Temperature}\\ \text{Correction}\\ \text{(from Table}\\ \text{23-1)}\end{array}\right)}$$

Record this value as K_i, the initial cell constant, showing all calculations.

23.4.3 Quality Control Check

1. Measure the specific conductance of the three QC solutions as described in Section 23.4.2, steps 3 through 6.

2. To complete the temperature corrected conductance value, use the following equation:

$$\begin{array}{c}\text{Temperature}\\ \text{Correction}\\ \text{Value}\end{array} = \begin{array}{c}\text{Meter}\\ \text{Reading}\end{array} \times \begin{array}{c}\text{Cell}\\ \text{Constant}\\ \text{Value } (K_i)\end{array} \times \begin{array}{c}\text{Temperature}\\ \text{Correction}\\ \text{Value (from}\\ \text{Table 23-1)}\end{array}$$

Do this calculation for the 14.7, 74, and 147 μS cm^{-1} and the blank solutions. Subtract the temperature-corrected blank value from each of the temperature-corrected QC check values and determine if the final values fall within the specified ranges:

QC Standard (μS cm^{-1})	Range (μS cm^{-1})
14.7	13.23-16.17 (10%)
74	70.30-77.70 (5%)
147	139.65-154.35 (5%)

TABLE 23-1. TEMPERATURE CORRECTION FACTORS TO COMPUTE SPECIFIC CONDUCTANCE VALUES AT 25.0 °C (from Dobos, 1975)

	Multiplication factor									
° C	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
4	1.521	1.518	1.515	1.512	1.509	1.506	1.503	1.500	1.497	1.494
5	1.491	1.488	1.485	1.482	1.479	1.476	1.474	1.471	1.468	1.465
6	1.462	1.459	1.456	1.453	1.450	1.447	1.444	1.442	1.439	1.436
7	1.433	1.430	1.427	1.424	1.421	1.419	1.416	1.413	1.410	1.407
8	1.405	1.402	1.399	1.396	1.393	1.391	1.388	1.385	1.382	1.380
9	1.377	1.374	1.371	1.369	1.366	1.363	1.360	1.358	1.355	1.352
10	1.350	1.347	1.344	1.341	1.339	1.336	1.333	1.331	1.328	1.325
11	1.323	1.320	1.318	1.315	1.312	1.210	1.307	1.304	1.302	1.299
12	1.297	1.294	1.292	1.289	1.286	1.284	1.281	1.279	1.276	1.274
13	1.271	1.268	1.266	1.263	1.261	1.258	1.256	1.253	1.251	1.248
14	1.246	1.243	1.241	1.238	1.236	1.234	1.231	1.229	1.226	1.224
15	1.221	1.219	1.216	1.214	1.212	1.209	1.207	1.204	1.202	1.199
16	1.197	1.195	1.192	1.190	1.188	1.185	1.183	1.180	1.178	1.176
17	1.173	1.171	1.169	1.166	1.164	1.162	1.159	1.157	1.155	1.153
18	1.150	1.148	1.146	1.143	1.141	1.139	1.136	1.134	1.132	1.130
19	1.129	1.125	1.123	1.121	1.118	1.116	1.114	1.112	1.110	1.107
20	1.106	1.103	1.101	1.099	1.096	1.094	1.092	1.090	1.088	1.085
21	1.084	1.081	1.079	1.077	1.075	1.073	1.070	1.068	1.066	1.064
22	1.062	1.060	1.058	1.056	1.053	1.051	1.049	1.047	1.045	1.043
23	1.041	1.039	1.037	1.035	1.033	1.031	1.028	1.026	1.024	1.022
24	1.020	1.018	1.016	1.014	1.012	1.010	1.008	1.006	1.004	1.002
25	1.000	0.998	0.996	0.994	0.992	0.990	0.988	0.986	0.984	0.982
26	0.980	0.978	0.976	0.974	0.972	0.971	0.969	0.967	0.965	0.963
27	0.961	0.959	0.957	0.955	0.953	0.951	0.949	0.947	0.946	0.944
28	0.942	0.940	0.938	0.936	0.934	0.932	0.931	0.929	0.927	0.925
29	0.923	0.921	0.919	0.918	0.916	0.914	0.912	0.910	0.908	0.907
30	0.905	0.903	0.901	0.899	0.898	0.896	0.894	0.892	0.890	0.889

23.4.4 Maintenance

1. Never acid-wash any containers used for specific conductance measurement. Rinse the containers three times with deionized water or soak them in deionized water overnight before use.

2. Store the conductivity cell in fresh deionized water daily. Substances which build up on the probe (e.g., humic and fulvic acids and suspended solids) should be removed periodically according to the manufacturer's recommendations.

3. Probe replatinization is required periodically. Consult the instruction manual for the proper method.

23.5 PROCEDURE

Follow manufacturer's instructions for the operation of the meter and cell.

1. Place the calibration standards, QC solutions, and samples in the constant-temperature water bath (25.0 ± 0.1 °C) to allow the samples and standards to equilibrate to 25.0 °C.

2. Rinse the cell thoroughly with water.

3. Rinse the cell with a portion of the sample to be measured. Immerse the electrode in a fresh portion of sample and measure its conductance.

4. Rinse the cell thoroughly with water after use. Store cell in water.

NOTE: If the readings become erratic, the cell may be dirty or may need replatinizing. Consult the manufacturer's operating manual for guidance.

5. Compute specific conductance by the equation given in Section 23.4.3, step 2.

23.6 QUALITY ASSURANCE AND QUALITY CONTROL

23.6.1 Precision and Accuracy

Forty-one analysts in seventeen laboratories analyzed six synthetic samples containing increments of inorganic salts, with the following results (U.S. EPA, 1983):

Increment, as Specific Conductance ($\mu S\ cm^{-1}$)	Precision, as Standard Deviations ($\mu S\ cm^{-1}$)	Accuracy	
		Bias (%)	Bias ($\mu S\ cm^{-1}$)
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
1,640	106	-5.36	-87.9
1,710	119	-5.08	-86.9

In a single laboratory (EPA-Cincinnati) using surface-water samples with an average conductance of 536 μS cm^{-1} at 25 °C, the standard deviation was 6 μS cm^{-1} (U.S. EPA, 1983).

23.6.2 Quality Control Checks

1. Use the three QC solutions described previously (see Section 23.3.3). These solutions should be measured before sample analysis, following sample analysis, and at intervals in between as recommended by the quality assurance program.

2. Measure one sample in duplicate (i.e., prepare four centrifuge tubes). The routine and duplicate values should agree to within ±10 percent. If they do not, prepare new sample portions and reanalyze.

23.7 REFERENCES

American Society of Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

Dobos, D., 1975. Electrochemical Data: A Handbook for Electrochemists in Industries and Universities. Elsevier Scientific Publishing Company, Amsterdam, The Netherlands.

U.S. Environmental Protection Agency, 1983 (revised). Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020. U.S. Environmental Protection Agency, Cincinnati, Ohio.

24.0 DETERMINATION OF TRUE COLOR

24.1 OVERVIEW

The amount of color in natural surface waters has been demonstrated to be closely correlated to the amount of dissolved organic carbon (DOC). The major sources of DOC are (1) photosynthetic products of algae and macrophytes, (2) humic compounds of terrestrial origin, and (3) excretions from zooplankton and larger animals. Thus a lake with a large pool of DOC might be a productive lake and would probably be more highly colored than an unproductive lake. Color analysis, in conjunction with other analytical methods, can yield information concerning the productivity of the water system.

Dissolved organic compounds also act as chelators for metals like aluminum. A colored lake may have high concentrations of aluminum, but the concentration of toxic forms is reduced due to the formation of alumino-organic complexes.

24.1.1 Scope and Application

This method is applicable to the determination of true color in natural surface waters. For AERP studies, true color is measured in the processing laboratory using a Hach Model CO-1 color test kit. The value obtained through the scale window is the sample's apparent color. This method has been written assuming that the Hach Color Determination Kit is used. However, the method may be modified for use with other instrumentation meeting equivalent specifications.

The applicable color range is 0 to 1000 APHA platinum-cobalt color units (PCUs) (APHA, 1985; U.S. EPA, 1983).

24.1.2 Summary of Method

The true color is determined after centrifuging a sample and comparing its color to APHA color standards.

24.1.3 Interferences

No interferences are known.

24.1.4 Safety

The sample types pose no hazard to the analyst. Tinted glass should not be worn when performing color measurements.

24.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

The sample portion for true color determination is taken from the sample container used to prepare aliquots. It is raw (unfiltered) sample.

24.3 EQUIPMENT AND SUPPLIES

24.3.1 Apparatus and Equipment

1. Hach Model CO-1 Color Determination Kit or equivalent.
2. Sample cuvettes.
3. Centrifuge.

24.3.2 Reagents and Consumable Materials

1. Water--Water used to rinse cuvettes should conform to ASTM specifications for Type I reagent grade water (ASTM, 1984).

2. Centrifuge tubes, 50-mL.

24.4 PREPARATION

24.4.1 Sample Preparation

1. Rinse a labeled 50-mL plastic centrifuge tube and lid with three 5-mL portions of sample from the appropriate sample container.

2. Fill the tube with 50-mL sample. Prepare additional centrifuge tubes for laboratory duplicates or replicates.

3. Place four sample tubes in the centrifuge at a time. If there are less than four samples, balance the centrifuge by placing a 50-mL centrifuge tube filled with deionized water into the empty port.

4. Centrifuge samples for 10 minutes with the centrifuge dial set at a medium speed (40).

5. Repeat steps 1 through 4 above for all samples in the batch. Make sure tubes are labeled with sample identification (ID) numbers.

6. Rinse a color-viewing tube with three 2-mL portions of supernatant from the centrifuge tube.

7. Fill the rinsed color-viewing tube to the top with supernatant and cover with Parafilm. Label the Parafilm with the sample ID.

8. Repeat steps 6 and 7 for all samples in the batch and for any duplicates prior to beginning color determinations.

24.4.2 Color Kit Preparation

Fill a tube with deionized water. Insert the plastic stopper in such a way as to expel air bubbles. Secure the stopper with a piece of scotch tape around the mouth of the tube. This tube is a blank which is used as a comparison. The color wheel is adjusted so that the color selected on the wheel along with the blank matches the color of the sample.

24.5 PROCEDURE

24.5.1 Low Range Sample Color Determination

NOTE: If the sample exceeds 100 color units, remove tubes from the comparator, cover, and set aside until low range samples have been analyzed.

1. Place lengthwise viewing adapter in comparator as shown in instruction manual for color determination kit.

2. Insert the plastic stopper into the top of the sample tube, making sure that no air bubbles are created. Insert the sample tube into the opening nearest the center on the back of the comparator. Rotate and push downward to prevent breakage. Be sure the outside of the tube is clean and dry.

3. Insert tube containing deionized water into the other opening on the back of the comparator.

4. Hold the comparator up to a white background and view through the openings of the comparator. (NOTE: To keep source constant, a piece of white Benchkote should be taped to the wall; the comparator should be viewed using it as a backdrop.) View the comparator at the same height above the floor each time.

5. Rotate the disc until a color on the dial matches the sample color.

6. Record the reading obtained by viewing through the scale window to the nearest 5 units. Record the value in the color logbook. Reading is expressed as APHA PCU's. (1000 platinum-cobalt units = color from mixing 2.492 g K_2PtCl_6 + 2 g $CoCl_2 \cdot 6H_2O$ + 200 mL HCl (conc) + 800 mL H_2O). If reading exceeds 100 platinum cobalt units, proceed with high range determination as described below.

24.5.2 High Range (100-500 PCU) Sample Color Determination

1. Using a disposable Pasteur pipet, extract the sample to the bottom line of each color tube. Also extract the deionized water in the comparison tube to the bottom line of the color tube.

2. Remove lengthwise viewing adapter from the comparator.

3. Insert the tube containing the sample vertically into the top right opening of comparator, as shown in the instruction manual for the color determination kit.

4. Insert the tube containing deionized water into the top left opening of comparator.

5. Proceed as in Section 24.5.1 steps 4 through 6, for low-range determinations.

6. Multiply the reading obtained by five and record the results in the color logbook. Be sure to include the calculations.

24.5.3 High Range (500-1000 PCU) Sample Color Determination

1. Be sure the volume of both the sample and the deionized water is level with the bottom line on the color tubes.

2. Fill another color tube to the bottom line with deionized water. Add this to the sample and invert the tube to mix (cover the tube with Parafilm). Using a disposable pipet, extract the solution to the bottom line of the color tube. Also extract the deionized water in the comparison tube to the bottom line of the color tube.

3. Proceed as in Section 24.5.1, steps 4 through 6.

4. Multiply the value by ten and record in the logbook. Record the initial value and document which procedure was followed.

25.5.4 Cleanup

1. When all samples have been analyzed for color, rinse the color viewing tubes and caps copiously with deionized water. Place the tubes upside down in a tube rack and allow them to dry in the clean air station.

2. Rinse all dirty centrifuge tubes and caps copiously with deionized water. Set all tubes upside down in tube rack and allow to dry in the clean air station (tubes are reusable). The caps can be placed upside down on Kimwipes and allowed to dry.

3. Place the color disc in its plastic cover and store it.

24.6 QUALITY ASSURANCE AND QUALITY CONTROL

24.6.1 Precision and Accuracy

As no calibration or QC standards are used in this method, precision and accuracy information are not available.

24.6.2 Quality Control Checks

1. Laboratory Duplicate--One sample per batch is measured in duplicate (two samples poured and analyzed from the same sample container). The duplicate sample is analyzed at the end of the batch; the value of the duplicate sample should agree within 10 color units of the routine sample. If the values do not agree within 10 color units, reanalyze both tubes.

24.7 REFERENCES

American Public Health Association, 1985. Standard Methods for the Examination of Water and Wastewater, 16th ed. APHA, Washington, D. C.

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

U.S. Environmental Protection Agency, 1983 (revised). Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency, Cincinnati, Ohio.

25.0 DETERMINATION OF TURBIDITY

25.1 OVERVIEW

Turbidity is a measure of suspended organic and inorganic material in the water column which affects light transmission. High turbidity may be the result of phytoplankton bloom or sediment from watershed runoff. Acidified bodies of water may be less productive and are often less turbid.

25.1.1 Scope and Application

This method is applicable to the determination of turbidity in natural surface waters. For AERP studies, turbidity is determined in the processing laboratory using a Monitek Model 21 nephelometer. As a result, the method has been written assuming that the Monitek nephelometer is used (Monitek, 1977). The method may be modified for use with other instrumentation meeting equivalent specifications.

The applicable turbidity range is 0 to 200 nephelometer turbidity units (NTUs).

25.1.2 Summary of Method

The nephelometer measuring system works by projecting an optical beam through the unfiltered sample contained in a special optical cuvette. A photodetector measures the intensity of the light scattered by particles in the solution. A ditigal reading is displayed which is proportional to the concentration of particles in the solution.

25.1.3 Interferences

Air bubbles in the sample cuvette interfere with the determination and cause a positive bias.

25.1.4 Safety

The calibration standards and sample types pose no hazard to the analyst.

25.2 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

The sample portion for turbidity determination is taken from the sample container used to prepare aliquots. It is raw (unfiltered) sample which is permitted to warm to room temperature.

25.3 EQUIPMENT AND SUPPLIES

25.3.1 Equipment and Apparatus

1. Monitek Model 21 nephelometer or equivalent
2. Sample cuvettes

25.3.2 Reagents and Consumable Materials

1. Certified Turbidity Calibration Standards--Commercially available certified turbidity standards with values 5.0, 10.0, and 20.0 NTU. Additional certified turbidity standards with values of 50.0, 100, and 200 NTU may be required for high range calibration (See Section 25.5.2). Repour all standards weekly; refrigerate between uses.

Prepare standards as follows:

NOTE 1: A 5 NTU standards is used as the QC check for the 20.0 NTU range. Prepare two cuvettes from two separate stock bottles. Label one as the QC sample.

NOTE 2: Allow all standard cuvettes to warm to room temperature before using.

NOTE 3: Stock bottles containing NTU standards should always be stored in the refrigerator.

a. Obtain a clean, scratch-free cuvette.

b. Rinse the cuvette three times with 5 mL of the appropriate standard. Cap cuvette and gently invert so that the rinse contacts all surfaces. The cuvettes used for the standards are screw cap cuvettes. If this type is unavailable, use a Parafilm cover.

c. Fill the cuvette with standard and cover or cap tightly. Dry the exterior with a Kimwipe to remove fingerprints or liquid.

d. Using a permanent marker pen, label the cap with the appropriate NTU value.

2. Prepared Turbidity Standards--In addition to the certified standards, above, standards with values of 2.0 NTU and 175 NTU (high range only) can be prepared as follows:

a. 2.0 NTU - Place 5.0 mL of the 10.0 NTU standard in a 100-mL graduated cylinder. Add 20.0 mL of deionized water and mix. Rinse the cuvette three times prior to filling the cuvette with

the 2.0 NTU standard. Check the value on the calibrated nephelometer. Replace this standard weekly as with all other standards.

b. 175 NTU - Place 12.5 mL of deionized water into a 100-mL volumetric flask. Fill to the 100-mL mark with the 200 NTU standard. Remake solution weekly.

3. Water--All water used in preparing reagents, in dilutions, and in cleaning labware should meet the specifications for Type I reagent grade water (ASTM, 1984).

25.4 PREPARATION

25.4.1 Daily Calibration

NOTE 1: The following procedure is written for the 0 to 20.0 NTU range.

NOTE 2: All cuvettes should be checked for optical comparability prior to use for both standards and samples (see Section 25.4.2).

1. If the instrument has been off, allow it to warm up for at least 15 minutes before using.

2. With the lamp on and the cuvette well empty, set the "RANGE" switch to 2 NTU.

3. Set the instrument display to read 0.00 by adjusting the "ZERO" control knob. The minus sign will flash on and off.

4. Insert the 10.0 NTU cuvette and align the index marks.

5. Set the instrument "RANGE" switch to the 20-NTU position. Adjust the "STANDARDIZE" control knob so the display reads 10.0 (or the standard value if another standard is used).

6. Insert the 2.0, 5.0, and 20.0 NTU standards in succession. Record each reading in the turbidity logbook. Do not adjust the instrument in any way to read these samples. Measure the 2.0 and 5.0 NTU on range 20 and the 20.0 NTU on range 200.

7. The measured values should be 2.0 ± 0.2, 5.0 ± 0.5, and 20.0 ± 2.0 NTU. If the measured values are unacceptable, wipe the cuvettes with a clean Kimwipe and reanalyze. If the values are still unacceptable, repeat steps 2 through 7. If the reanalyzed values are not within the acceptable range, repour the standards and repeat steps 2 through 7.

25.4.2 Maintenance

1. Cuvette Comparability--

   a. Clean all cuvettes by rinsing them three times with deionized water.

   b. Rinse the cuvette to be checked three times with the 10.0 NTU standard. Fill with approximately 25 mL of the standard.

   c. Follow Section 25.4.1, steps 3 through 5, using the original cuvette containing the 10.0 NTU standard to be sure the nephelometer is calibrated.

   d. Now place the cuvette to be compared into the cuvette well. Observe the reading and slowly rotate cuvette to obtain a reading which is as close as possible to the value obtained using the standard cuvette.

   e. The value should be 10.0 ± 0.2 NTU for a given location on the cuvette. Once the best acceptable alignment has been located, place an index mark on the cuvette at the index mark on the instrument.

   f. The cuvette is now considered comparable to the standard cuvette. Turbidity readings should be taken only with the index marks aligned.

25.5 PROCEDURE

NOTE 1: Refer to Figure 25-1 for the flowchart for turbidity measurements.

NOTE 2: Avoid rigorous agitation to minimize introduction of air bubbles which interfere with measurement. Allow sample containers to come to room temperature prior to pouring samples for turbidity analysis.

NOTE 3: Samples with numerous, large particulates will have readings which decrease as the particles settle out. Record the highest stable reading as the sample value.

25.5.1 Low Turbidity Samples

1. Gently swirl the sample container to evenly distribute any settled particles.

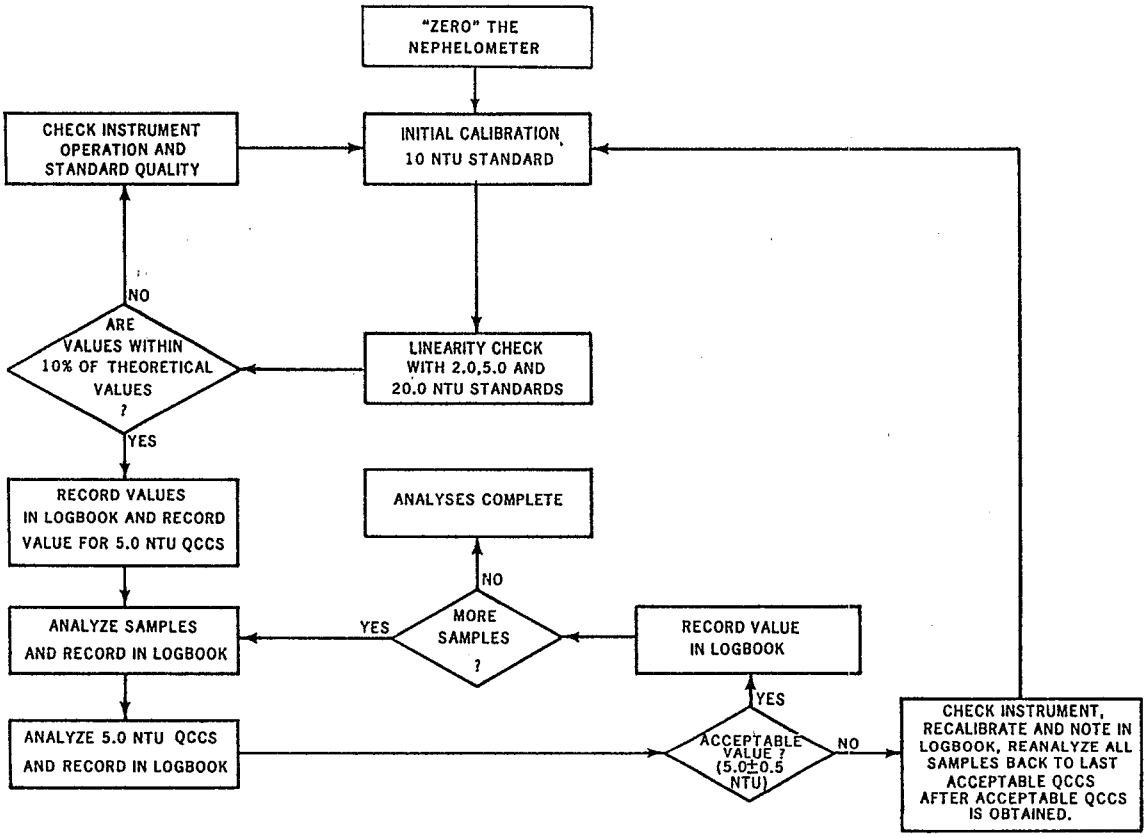


Figure 25-1. Flowchart for turbidity.

2. While wearing disposable, dust-free gloves, rinse the cuvette with three 5-mL portions of sample. It is not necessary to swirl the container between rinses.

3. Cap the sample container and swirl. Immediately fill the cuvette. Cover with Parafilm and label with the sample identification number. Wipe the cuvette with a Kimwipe to remove any liquid or fingerprints.

4. Invert the cuvette three times and place the sample into the calibrated nephelometer. Measure all values on range 20 unless the reading exceeds 20 NTU; then see steps 6 and 7. Record results in the turbidity logbook. If the reading is not stable, remove the cuvette and gently invert. Check for air bubbles, wipe, and reinsert into

the nephelometer. Record the stable reading or flag the unstable readings if a stable reading cannot be obtained.

5. Analyze the 5.0 NTU QC standard after every 10 samples or at intervals recommended by the quality assurance program. If the measurement is within acceptable limits, record the value in the logbook and continue sample analysis. If the measurement is not within acceptable limits, wipe the cuvette with a clean Kimwipe and reanalyze. If the reanalyzed value still is not within the acceptable range, see Section 25.4.1, step 7.

6. If the sample readings exceed 20 NTU, switch the "RANGE" selection knob to the 200 position and measure a 50-NTU standard. If the 50 NTU value is within 5 percent (47.5-52.5) of the theoretical value, remeasure the sample and record the value in the logbook along with the reading for the 50-NTU standard. Note in the logbook that no recalibration was required.

7. If the 50 NTU is not within acceptable limits, remove the high sample and set aside. Analyze the rest of the samples using the above procedure, setting aside all the samples with readings that exceed 20 NTU. After all low turbidity samples have been read, and the final QC sample has been analyzed, the nephelometer should be recalibrated for high turbidity samples.

25.5.2 <u>High Turbidity Samples</u>

1. If a sample reading exceeds 20 NTU and the 50-NTU value is not within acceptable limits (Section 25.5.1, steps 6 and 7), the nephelometer should be recalibrated as follows:

   a. DO NOT make any adjustments using the "ZERO" knob.

   b. Set the "RANGE" selector to 200. Place the 100-NTU standard into the cuvette well and adjust the "STANDARDIZE" knob until the display reads 100.

   c. Check the 20, 50, and 175 NTU standards and record the values in the logbook. If the values are not within range (20 ± 2.0, 50 ± 5.0, 175 ± 17.5) go back to Step 1b.

   d. Measure all high turbidity samples at one time. Run a QC check before, in the middle, or after every 10 samples, and at the end of a set. Analyze one high sample in duplicate, i.e., pour a second cuvette. Results should agree within 10 percent or reanalyze the pair.

e. Choose the QC sample value according the the chart below. Do not use the same standard that was used to calibrate the meter; pour a second standard from a separate stock bottle.

Sample Values (NTU)	QC solution (NTU)
20-50	20
50-175	50
175-199	175

2. If any sample reading exceeds 200 NTU, a dilution should be made. Obtain some fresh filtered sample by using a 60-mL disposable sterile syringe and an Acrodisc or equivalent syringe filter. Filter a small amount of sample into a clean cuvette and rinse thoroughly. Rinse the cuvette a total of three times.

3. Filter approximately 25 to 30 mL of sample into a cuvette. Place in the nephelometer and read the turbidity value. Record the value in the logbook noting the range used.

4. Rinse a clean 50-mL centrifuge tube three times with a small amount of unfiltered sample. Fill the tube with exactly 5.0 mL of unfiltered sample. Using a disposable pipet, add exactly 45 mL of filtered sample by filling the tube to the 50.0 mL mark (previously filtered sample from the cuvette may be used). Invert the centrifuge tube three times to mix thoroughly. Place a small amount of this diluted sample into a clean cuvette and rinse three separate times.

5. Fill the cuvette with the diluted sample. Read the turbidity value and record the value in the logbook noting the range used.

6. Calculate the actual turbidity value using the following equation:

$$\text{Actual Turbidity} = 10\left(\frac{\text{Turbidity of Diluted Sample}}{10}\right) - 9\left(\frac{\text{Turbidity of Filtered Sample}}{10}\right)$$

Record the actual turbidity value and the calculations in the logbook.

25.5.3 Cleanup

1. Clean the turbidity cuvettes by rinsing copiously with deionized water. Place upside down in the tube rack in the clean work station to dry.

2. Turn off the lamp. Leave the power on if the nephelometer is used daily. Follow the manufacturer's instructions for complete care.

25.5.4 General Precautionary Notes for Procedure

1. Check the optical comparability of all cuvettes monthly using the procedure in Section 25.4.2. Always align index marks when making readings.

2. Always wear gloves when handling cuvettes. Check that all cuvettes are clean and free of fingerprints or smudges. Avoid handling in the region of light path.

3. If the standards or samples have been cooled, allow them to warm to room temperature before analysis to avoid cuvette fogging.

4. If air bubbles are present in the sample, allow the sample to sit uncovered for about 20 minutes; gently tap the sides of the cuvette after covering it to release the bubbles. Mix gently prior to reading.

5. Perform a linear calibration check at least every 6 months.

6. To minimize the contamination of a standard, do not introduce any object into a standard bottle or pour the used standard back into the bottle. The foil lid on a bottle may fall into the standard but will not harm the solution in any way. Do not remove it.

25.6 QUALITY ASSURANCE AND QUALITY CONTROL

25.6.1 Precision and Accuracy

No information available at this time.

25.6.2 Quality Control Checks

1. Routine QC check--

   a. Insert the 5.0-NTU standard into the calibrated nephelometer.

   b. The measured value should be 5.0 ± 0.5 NTU. Record the acceptable value in the logbook and continue with sample measurement.

   c. If the value is not acceptable, wipe the cuvette with a clean Kimwipe and reanalyze. If the value is still not acceptable, refill the cuvette with a fresh portion of 5.0-NTU standard and

repeat Section 25.4.1, steps 3 through 6. Reanalyze all samples back to the last acceptable QCCS value. Record procedure and reanalyzed values in the logbook.

2. Laboratory Duplicate--One sample per batch is analyzed in duplicate (two cuvettes from the same sample container). The cuvette designated as the duplicate is analyzed at the end of the sample analysis before the final QC check. The values obtained from the routine and duplicate samples should agree within 10 percent. If they do not, repour both the routine and duplicate cuvettes and reanalyze.

25.7 REFERENCES

American Society for Testing and Materials, 1984. Annual Book of ASTM Standards, Vol. 11.01, Standard Specification for Reagent Water, D 1193-77 (reapproved 1983). ASTM, Philadelphia, Pennsylvania.

Monitek, Inc., 1977. Model 21 Laboratory Nephelometer, Preliminary Operating and Maintenance Instructions. Monitek, Hayward, California.

APPENDIX A

NATIONAL SURFACE WATER SURVEY MOBILE LABORATORY SPECIFICATIONS

A commercially available, insulated, heavy-duty cargo trailer shell (Wells Cargo) was modified and outfitted to produce a field laboratory facility for the Eastern Lake Survey. The design of the trailer took several factors into consideration. The trailers would be towed by 1-ton pickup truck driven by a non-professional driver. Electrical and water requirements had to be within the supply capabilities of utility companies located in rural or remote areas. The laboratory had to support field sampling and sample processing operations with cold storage space and reagent grade water. Workspace was required for four to five scientists and specific analytical instrumentation. Storage space was required for equipment, reagents, and supplies. A work area which minimized contamination (from metals, anions) was needed to conduct sample processing. Standard laboratory safety features for storing chemicals and protecting personnel were necessary.

Schematic drawings of the trailer are shown in Figures 1 and 2. Each trailer was 9.4 m long, 2.4 m wide, and 3.9 m high. These dimensions required a fifth-wheel type hitch configuration (i.e., where the hitch is attached to the bed of the tow vehicle over the rear axle, rather than to the rear bumper) to ensure road safety and stability. There were 146 m^3 of compartment storage. The interior work space of each trailer was 6.1 m long, 2.3 m wide, and 2.3 m high. Each trailer contained approximately 5.5 linear m of counterspace. Each trailer required both 110 V and 220 V alternating current, single-phase 80-amp electrical power, a minimum feed water pressure of 40 psi, and access to a sewer drain or leach field.

A 1.8 m-wide laminar flow hood (Continental Control Systems) was installed at the rear of each trailer to provide a contamination-free work area. The hood contained a high efficiency purification apparatus (HEPA filters, 0.3-um pore size) capable of delivering Class 100 air (as defined by Federal Standard 20913, 1973, Government Services Administration) into the work area. The hood also had adjustable flow vents that allowed a static, positive, or negative pressure to be maintained within the work area.

A reverse osmosis and deionization system provided high-quality water for each trailer. Tap water was pretreated by filtration (5-µm pore size) and reverse osmosis (Millipore Milli-RO, 4-L per hour output), and was stored in a 95-L reservoir. Water from the reservoir was deionized on demand (Millipore Milli-Q system). The end product at the point of use met ASTM Type I specifications for reagent grade water (ASTM, 1984).

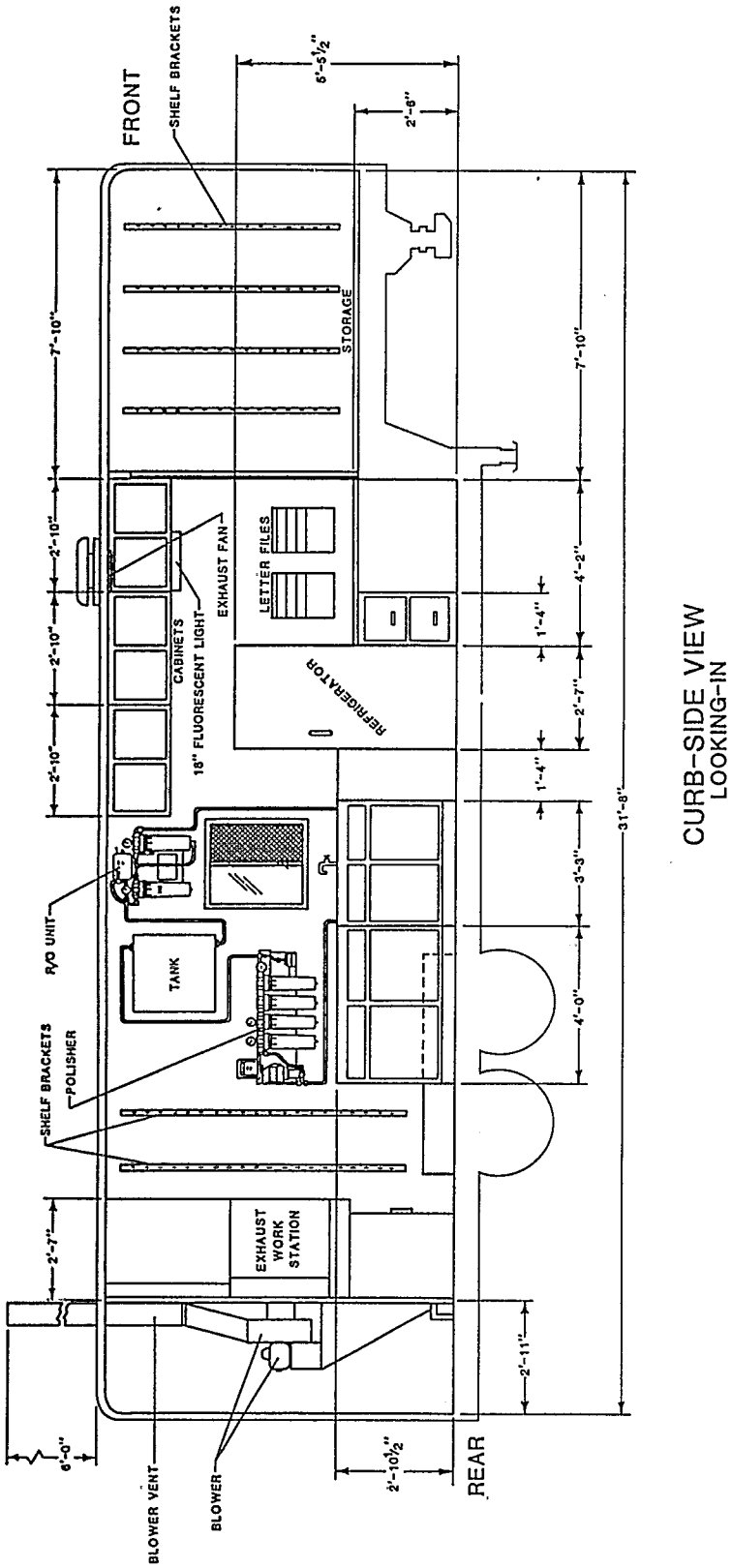
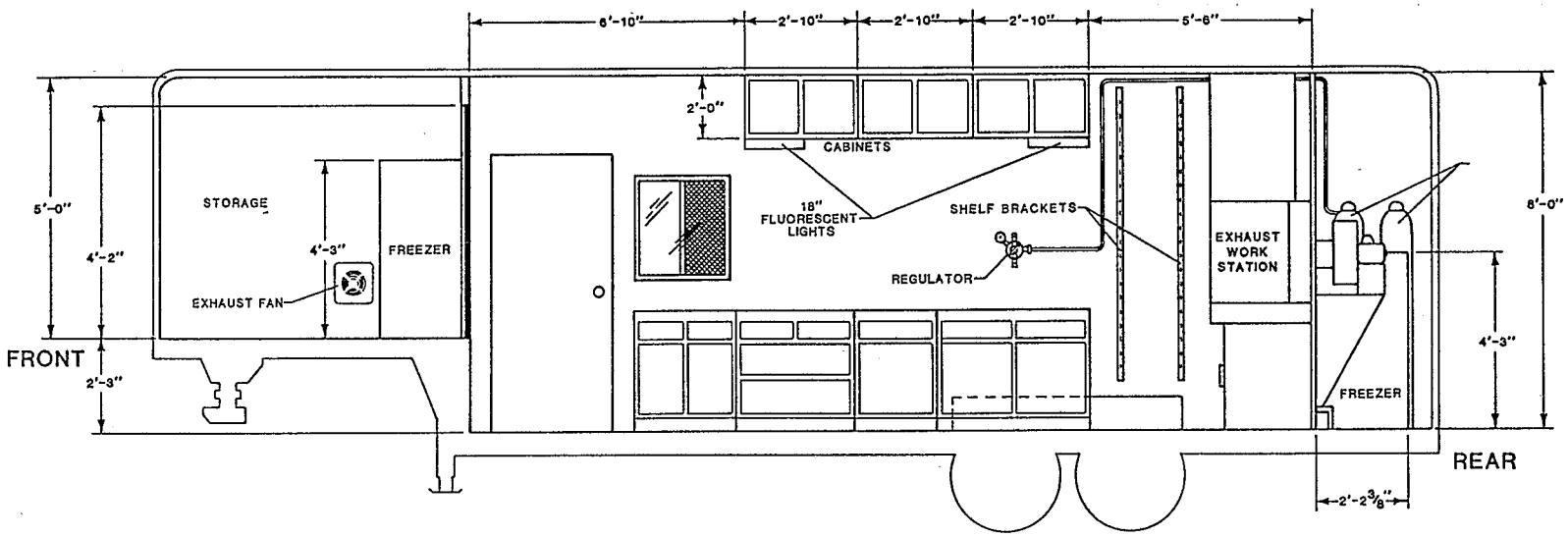


Figure A-1. Schematic drawing of laboratory trailer and features, curbside view.



ROAD-SIDE VIEW
LOOKING-IN

Figure A-2. Schematic drawing of laboratory trailer and features, roadside view.

Each trailer was equipped with two 2.4 m^3 freezers and one 9.1 m^3 combination refrigerator and freezer. Two roof-mounted heater/air conditioning units (5,000 BTU heating capacity and 13,200 BTU cooling capacity per unit) provided temperature control for the laboratory interior. Laboratory safety features included an eyewash station, first aid kit, two Halon fire extinguishers, a vented storage cabinet for flammable chemicals, racks for compressed gas cylinders, and a safety shower mounted outside the trailer door. A steel cabinet under the laminar flow hood provided vented storage for acids and other corrosive chemicals.

Each mobile laboratory trailer was equipped with identical instrumentation to allow for standardized sample processing and analysis. This instrumentation included a total carbon analyzer (0.001 mg L^{-1} C resolution) a pH meter with combination electrode (± 0.01 pH unit resolution), an electronic balance (± 0.001 g resolution), a nephelometer (± 0.1 NTU resolution), a benchtop centrifuge, a color test kit (± 5 APHA platinum-cobalt unit resolution), and vacuum filtration equipment. Specific instruments used in the trailers are described in Linthurst et al. (1986). Other laboratory equipment and supplies used are listed in Appendix B.

Once on site, a mobile laboratory trailer could be made operational within 48-72 hours. During field operations, water samples were analyzed and processed using standardized methods and quality assurance procedures (Hillman et al., 1986, and Drousé et al., 1986). Dissolved inorganic carbon (DIC), pH, turbidity, and true color were measured at the field laboratory. Water samples were processed in the laminar flow hood into several distinct aliquots and were preserved. They were shipped to an analytical laboratory the following day. Where appropriate, the processing procedure included filtration, preservation with ultrapure acid, and the preparation of a dissolved monomeric aluminum fraction. The laboratory also provided reagent grade water, frozen chemical refrigerant packs, and reagents for use by the field sampling crews.

Researchers who require the use of a trailer similar to that described here should consider several aspects of the design. Power, water, and drainage requirements preclude operating these trailers as truly self-sufficient units. Advance arrangements with utility companies may be necessary to prepare a site for setting up a mobile laboratory trailer. The internal environment of these trailers could not always be maintained during extreme weather conditions (i.e., subfreezing temperatures or high humidity). Alternative heating and air-conditioning units should be investigated if trailers are to be set up in areas subject to extremes in temperature and humidity.

The work and storage areas of the trailers were well organized and permitted four to five people to work comfortably without undue interference from each other. For extended periods in the field, additional on-site storage space for backup equipment and supplies is recommended.

Trailers involved in supporting a large operation, consisting of many field teams or a large daily sample load, may require larger-capacity water purification systems than those used during the Eastern Lake Survey. Each trailer used in the Eastern Lake Survey supported one or two helicopter teams and processed 20 to 30 samples per day. The amount of reagent grade water needed for this effort was just within the supply capability of the system in most locations.

Finally, the location of the trailer is important in terms of the proper operation of the laminar flow hood. Areas subjected to gusty winds and blowing dust, or heavy vehicular traffic should be avoided to reduce the possbility of sample contamination from particulate matter or organic vapors.

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APPENDIX B

PROCESSING LABORATORY EQUIPMENT LIST

1. Mobile processing laboratory facilities and supplies

   a. Electrical and water inputs

   b. Water outlet

   c. Source of water capable of meeting ASTM specifications for Type I reagent grade water (such as Barnstead NANOpure/ROpure 40 or Millipore Milli-RO/Super-Q System)

   d. Heating and cooling system

   e. Freezer/Refrigerator

   f. Laminar flow hood capable of delivering class 100 (Federal Standard 209 B 1973, Government Services Administration) air

   g. Solvent storage cabinet

   h. Standard laboratory countertops and sink

   i. Analytical balance (±0.001 g) and plastic weighing boats

   j. Vacuum pump

   k. Centrifuge (capable of holding four 50-mL tubes)

   l. Field data forms, shipping forms, batch forms, logbooks

   m. Class 100 air filtration filters

   n. Spare water treatment cartridges

   o. Coolers

   p. Clean 20-L Cubitainers with spigots

(continued)

PROCESSING LABORATORY EQUIPMENT LIST (Continued)

2. Total extractable aluminum supplies

   a. Clean 50-mL graduated centrifuge tubes with sealing caps

   b. Clean 10-mL centrifuge tubes

   c. Clean sealing caps for 10-mL centrifuge tubes

   d. HPLC-grade methyl isobutyl ketone (MIBK)

   e. Sodium acetate

   f. 8-hydroxyquinoline (99^+ percent purity)

   g. NH_4OH (30 percent - Baker Instra-Analyzed grade or equivalent)

   h. Clean 1-L, 500-mL, and 100-mL volumetric flasks

   i. Glacial acetic acid (Baker Instra-Analyzed grade or equivalent)

   j. Hydrochloric acid (12 M - Baker Instra-Analyzed grade or equivalent)

   k. Phenol-red indicator solution (0.04 percent w/v)

   l. 2.00-mL Repipet dispensers

   m. 3.00-mL Repipet dispensers top for 1-gallon bottle

   n. 5.00-mL Repipet dispensers

   o. 100-mL reagent bottles with droppers

   p. Polystyrene graduated cylinders (25-, 100-, 250-mL sizes)

3. PCV-Reactive Aluminum Supplies

   a. Clean 250-mL beakers

   b. Clean 100-mL beakers

(continued)

PROCESSING LABORATORY EQUIPMENT LIST (Continued)

c. Flow-injection analyzer

d. Micropipets, variable-volume, 1-5 µL

e. Micropipets, variable-volume, 40-200 µL

f. Micropipets, variable-volume, 200-1,000 µL

g. Disposable micropipet tips, 1-200 µL

h. Disposable micropipet tips, 600-1,000 µL

i. Disposable micropipet tips, 1-5,000 µL

j. Polyethylene bottles, 1-L capacity

k. Volumetric flasks, 100-mL capacity

l. Filter paper, Whatman GF/C or equivalent

m. Cation-exchange resin (Amberlite IR-120, 14-50 mesh or equivalent)

n. Hydrochloric acid, concentrated (Baker Ultrex grade or equivalent)

o. Ammonium hydroxide, concentrated (Baker InstraAnalyzed grade or equivalent)

p. Hydroxylammonium chloride

q. 1,10-phenanthroline

r. Hexamethylene tetramine

s. Stock Al calibration standard solutions, 1,000 mg L^{-1}

t. Stock Al QC solution (1,000 mg L^{-1}), certified standard from different source than the calibration standard solution

u. Sodium chloride (ACS reagent grade)

v. Pyrocatechol violet

(continued)

PROCESSING LABORATORY EQUIPMENT LIST (Continued)

4. True color determination supplies
   a. Color determination kit
   b. Color determination kit spare supplies
      1. Color discs
      2. Color viewing tube
      3. Hollow polyethylene stoppers
   c. Color-blindness test kit

5. Filtration apparatus and supplies
   a. Membrane filters, 0.45 µm, 47 mm diameter
   b. Teflon or plastic forceps
   c. Filtrators - low form (Fisher or equivalent)
   d. Acrylic vacuum chambers (custom made)
   e. Clean filter holders
   f. Spare rubber stoppers
   g. Vacuum pump with regulator
   h. Clean polyethylene amber wide-mouth bottles (125-, 250-, and 500-mL sizes)
   i. Disposable gloves (talc-free)
   j. Digital micropipets (5-40 µL, 40-200 µL, 200-1,000 µL, 1,000 - 5,000 µL)

(continued)

PROCESSING LABORATORY EQUIPMENT LIST (Continued)

k. Micropipet metal-free pipet tips (in four sizes corresponding to micropipet sizes in [j] above)

l. Indicating pH paper (Type CS, range 1.8 to 3.8)

m. HNO_3 and H_2SO_4 (Baker Ultrex grade or Seastar Ultrapure grade)

n. Frozen freeze gel packs

o. Styrofoam-lined shipping containers

6. DIC determination supplies

a. Dohrmann DC-80 carbon analyzer or equivalent

b. 50-mL polypropylene syringes

c. Syringe valves (Mininert or equivalent)

d. Zero-grade nitrogen gas

e. Anhydrous Na_2CO_3 (ACS Primary Standard Grade)

f. Syringe membrane filters (Gelman Acrodisc 4218, 0.45 µm or equivalent)

g. Spare carbon analyzer parts (nuts, ferrules, tubing)

7. pH determination supplies

a. pH meter (Orion Model 611 or equivalent)

b. Orion Ross epoxy body combination pH electrode

c. 3M KCl filling solution for combination pH electrode

d. pH sample chamber (custom made)

e. 0.100N H_2SO_4

f. Ringstand (to hold pH apparatus) and clamps

(continued)

PROCESSING LABORATORY EQUIPMENT LIST (Continued)

g. NBS-traceable pH buffers (pH 4 and 7)

h. 50-mL disposable beakers

8. Turbidity determination supplies

a. Nephelometer (Monitek Model 21 or equivalent)

b. 5-, 10-, 20-, 50-, 100-, 200-NTU standards

c. Cuvettes

9. Snowpack and bulk precipitation supplies

a. Racks--to hold buckets during sample-melting process

b. Sample buckets

c. Syringes (60 mL, plastic)

d. Syringe valves (Luer-Lok or equivalent)

e. Scale, capable of accurate measurement within ±1 g

APPENDIX C

GENERAL LABORATORY PROCEDURES

NOTE 1: Logbooks should be kept for each procedure or instrument. The lab supervisor should review and sign the logbook at the completion of daily analyses. There should also be an instrument calibration logbook including a record of the dates that new chemicals are opened.

NOTE 2: If an error is made in a logbook, use one line to cross out and initial. Always use ink when recording in the logbook.

NOTE 3: Gloves, lab coats, and lab glasses should be worn when contacting sample, acids, or hazardous materials.

C.1 ELECTRONIC BALANCE

C.1.1 Balance Standardization

NOTE 1: Check standardization weekly.

NOTE 2: Be sure to check the calibration over range for which the balance is used.

1. While wearing gloves, use the calibration weights to standardize the balance. Do not touch the weights with anything but the forceps included in the weight set. Tare the balance and record the reading for each weight in the instrument calibration logbook.

2. If weight values and balance readings do not agree, consult the manufacturer's guide for adjustments.

C.1.2 Weighing Procedures

NOTE 1: Use a dry Teflon spatula. Do not use same spatula for successive weighings of different chemicals.

NOTE 2: Remember to weigh the substance with the lid on the balance if it had been originally tared with the lid on the balance.

1. While wearing gloves, pour the approximate amount of substance needed into a weighboat. Do not put the spatula into the bottle.

2. Obtain a second weighboat, place it on the balance and tare.

3. Using a spatula, transfer the substance to the weighboat on the balance until the desired amount is obtained.

4. Dispose of any unused substance; do not return to bottle.

C.1.3 Cleanup

1. Rinse the spatula with deionized water after use and allow to air dry.

2. Do not leave any spilled chemicals on the balance. Use a damp Kimwipe to clean the balance pan and wipe dry.

C.2 MICROPIPET

NOTE 1: The following instructions refer to the Finn continuous volume micropipets used in AERP studies. These procedures can be modified for use with other digital micropipets.

NOTE 2: Keep pipet vertical at all times to prevent contamination.

C.2.1 Pipet Calibration

Note: Check the calibration of each pipet weekly and record data in the instrument calibration logbook. Each procedure requires a daily calibration check of all pipets used. This data is recorded in the corresponding procedural logbook.

1. Calibrate pipets as follows, setting the volume as instructed below:

Pipet Volume Range	Set Volume to:	Permitted Ranges
40-200 µL	50 µL	0.049-0.051 g
200-1000 µL	1000 µL	0.990-1.010 g
1000-5000 µL	2000 µL	1.990-2.010 g

2. While wearing gloves, place the appropriate size pipet tip onto the end of the pipet.

3. Using the balance, set for low range (0-30g), place a weighboat on the balance and tare. Use fresh deionized water and pipet the specified volume into weighboat. Weigh deionized water a total of five times and average to the nearest 0.1 mg and compare this average with the permissible ranges in step 1. Record all measurements in the instrument calibration logbook.

4. If the mean in step 3 lies outside of the permitted range, the volume setting is adjusted as follows: remove the cap of the micropipet to expose the adjustment dial. If the adjustment dial is turned in

the same direction as the thicker side of the arrow marking on the cap, the volume delivered by the micropipet will increase. Turn the dial toward the thinner side of the arrow marking to decrease the delivered volume.

C.2.2 Pipet Operation - Forward Technique

NOTE 1: Operate the thumb button slowly and steadily. Do not let the thumb button snap back. Deliver the volume smoothly.

1. While wearing gloves, place a clean pipet tip on pipet.

2. Keep the pipet as vertical as possible during up-take of solution. Depress thumb button to first stop. Dip the pipet tip slightly below the solution surface and slowly release the thumb button.

3. Deliver the liquid by gently depressing the thumb button to the first stop. Touch the pipet tip to side of the container (except when preserving aliquots) while simultaneously depressing the thumb button to the second stop. Slowly release the thumb button.

4. Remove the used pipet tip by pressing the tip ejector down. Dispose of the pipet tip in a proper waste receptacle.

C.2.3 Pipet Operation - Reverse Technique

NOTE: Use this technique to pipet viscous liquids.

1. Apply a pipet tip and depress thumb button to second stop. Dip the pipet tip slightly below the surface of the solution and slowly release the thumb button.

2. Deliver the liquid by gently depressing the button to the first stop. Release the thumb button. Remove and dispose of the pipet tip.

C.2.4 Care of Pipets

1. If any liquid is sucked into the barrel, immediately consult the manufacturer's guide and clean as directed. Do not use pipet until it is cleaned.

2. For leakage or inaccuracies refer to the manufacturer's trouble-shooting guide.

C.3 REPIPET DISPENSER INSTRUCTIONS

NOTE 1: These instructions are specific to the Reference Lab Industries' Repipet used in the AERP studies. These procedures may be modified for use for other equivalent apparatus.

NOTE 2: Do not include the drops that are dispensed when pulling up on the Repipet dispenser in the delivery volume.

C.3.1 Calibration of 2.0-mL and 5.0-mL Repipet Dispensers

NOTE: Use clean air station.

1. Set the Repipet to dispense 2.0 or 5.0 mL of solution as directed by manufacturer.

2. Place the weighboat on the balance and tare. Fill the Repipet bottle with deionized water and dispense 2.0 or 5.0 mL of deionized water into the weighboat.

3. Return the weighboat to the balance and weigh contents. Acceptable limits are:

Repipet	Limits
2.0 mL	2.0 ± 0.02 g
5.0 mL	5.0 ± 0.05 g

If weight is outside range, adjust dispenser as directed by manufacturer and recheck.

C.3.2. Calibration of 10.0-mL Repipet

NOTE: The 10.0-mL Repipet is used only for MIBK in AERP studies; it should always remain under the hood.

1. Set the Repipet to dispense 10.0 mL as directed by the manufacturers's manual.

2. Obtain a 50-ml centrifuge tube with its cap and place on balance and tare.

3. Dispense 10.00 mL of MIBK into the centrifuge tube and weigh. The acceptable range is 7.98 ± 0.05 g at 20 °C. If the weight is not within acceptable limits, adjust the dispenser as directed by the manufacturer and recheck.

C.4 PREPARATION OF 5 PERCENT NITRIC WASH

NOTE 1: Use Baker-Analyzed grade HNO_3 or equivalent for general glassware cleaning.

NOTE 2: The 5 percent HNO_3 wash is used both to clean glassware and in filtration to rinse the acid units.

1. Partially fill a 1-L volumetric flask with deionized water.

2. Add 50 mL of concentrated HNO_3 and mix well.

3. Dilute to the 1-L mark with deionized water. Mix and transfer to a 1-L wash bottle labeled "5% HNO_3".

C.5 SYRINGE FILTER PREPARATION

NOTE: These procedures are specific to the Nucleopore Swin-lok filter assemblies used in the AERP studies for syringe filtration of extractable aluminum and PCV-reactive aluminum samples. These procedures may be modified for use with other filter assemblies, except disposable types.

C.5.1 Cleaning

1. Used filter assemblies are cleaned by discarding the Nucleopore membrane, placing the parts in a beaker and rinsing the parts three times with deionized water.

C.5.2 Assembly

1. Refer to the Nucleopore Swin-lok filter instructions for part descriptions.

2. Place the disc in the filter base with the coarse grid down (away from filter). Place an O-ring in the groove around the disc, and seat the O-ring carefully.

3. Using gloves and clean Teflon forceps, obtain a Nucleopore membrane, discarding the blue separator papers. Dip the membrane into a beaker of deionized water and place it on top of the O-ring, being sure to center the membrane.

4. Obtain a filter stem and place a second disc into this part, being sure the coarse side is up (away from filter). The top and bottom discs are interchangeable but the smooth side of each filter should <u>always</u> face the membrane filter.

5. Place the filter stem on the filter base; aligning the notches to secure the membrane. Place the top onto the assembly and screw down tightly.

C.5.3 Acid-Wash Procedure

1. Attach an assembled filter onto a syringe containing deionized water. Inject approximately 2-3 mL through the filter. Check the filter for leaks.

2. Repeat the procedure using a syringe containing 5 percent nitric acid.

3. Repeat step 1, but rinse with three separate 2 to 3-mL portions of deionized water.

C.5.4 Filter Storage

1. Place wet filters in a large sealable bag and seal. This will prevent the membranes from drying out. The bag may then be stored in the refrigerator if desired.

2. Each filter may now be used to filter a sample syringe for MIBK extraction or FIA aluminum analysis.

C.6 CLEANING OF PLASTICWARE

Plasticware, depending on its use, is cleaned by either an acid-washing procedure or deionized water-washing procedure.

C.6.1 Acid-Washing Cleaning Procedure

All plasticware (with the exceptions given in Section C.6.2) is rinsed three times with deionized water, three times with 3N HNO_3 (prepared from Baker Instra-Analyzed HNO_3 or equivalent), and six times with deionized water. It is then filled with deionized water and allowed to stand for 48 hours. Next, it is emptied, dried in a laminar flow hood, delivering Class 100 air (when dry containers are necessary), and placed in clean plastic bags (bottles are capped first). The procedure in Section C.6.3 is used to check the cleaning procedure.

C.6.2 Deionized-Water-Leaching Cleaning Procedure

Plasticware to be used for pH, acidity, alkalinity, and anion determinations is rinsed three times with deionized water, filled with deionized water, allowed to stand for 48 hours, then emptied and sealed in clean plastic bags. The procedure in Section C.6.3 is used to check the cleaning procedure.

NOTE: The deionized water used in cleaning the plasticware should meet or exceed specifications for ASTM Type I reagent grade water.

C.6.3. Cleaning Procedure Quality Control Check

After the initial cleaning (by either procedure), 5 percent of the containers are checked to ensure that rinsing has been adequate. The check is made by first adding 500 mL (or the maximum amount) of deionized water to a clean container, sealing the container with a cap or Parafilm, and slowly rotating it so that the water touches all surfaces. The specific conductance of the water is then measured. It should be less than 1 $\mu S\ cm^{-1}$. If any of the containers fail the check, all of the containers are rerinsed and 5 percent are retested.

APPENDIX D

NATIONAL SURFACE WATER SURVEY BLANK DATA FORMS

The National Surface Water Survey forms shown in this appendix are facsimiles of the forms used in the processing and analytical laboratories.

Form Number	Form Title	Page
2/5	Batch/QC Field Data Form	2 of 21
3	Shipping	3 of 21
11	Summary of Sample Results	4, 5 of 21
13	ANC and BNC Results	6 of 21
14	QC Data for ANC and BNC Analyses	7 of 21
15	Conductivity	8 of 21
16	Anion-Cation Balance Calculation	9 of 21
17	IC Resolution Test	10 of 21
18	Detection Limits	11 of 21
19	Sample Holding Time Summary	12, 13 of 21
20	Blanks and QCCS	14, 15 of 21
22	Duplicates	16, 17 of 21
31	Summary of Analytical Results - Phytopigments	18 of 21
32	QC Results - Phytopigments Flurometry	19 of 21
33	QC Results - Phytopigments HPLC	20 of 21
34	QC Results - Phytopigments Time Line	21 of 21

NATIONAL SURFACE WATER SURVEY
BATCH/QC FIELD DATA FORM

DATE RECEIVED BY DATA MGT. _____
ENTERED _____
RE-ENTERED _____

☐ FORM 2 LAKES
OR
☐ FORM 5 STREAMS

BATCH ID _____
NO. SAMPLES IN BATCH _____

LAB TO WHICH BATCH SENT _____
DATE SHIPPED _____

DATE PROCESSED _____
AIR-BILL NO. _____

BASE SITE ID _____
LAB CREW ID _____
MOBILE LABORATORY SUPERVISOR _____

SPLIT	SAMPLE ID	LAKE OR STREAM ID	SITE OR TYPE	SAMPLE CODE	DIC (mg/L) QCCS LIMITS UCL — 2.2 LCL — 1.8		STATION pH QCCS LIMITS UCL — 4.1 LCL — 3.9		TURBIDITY (NTU) QCCS LIMITS UCL — 5.5 LCL — 4.5		COLOR (PC UNITS)	CONDUCTIVITY (uS cm $^{-1}$)		PCV ALUMINUM (ppm) UCL — LCL — DISSOLVED		PCV ALUMINUM (ppm) UCL — LCL — ORGANIC	
					VALUE	QCCS	VALUE	QCCS	VALUE	QCCS	VALUE	VALUE	QCCS	VALUE	QCCS	VALUE	QCCS
	QCCS			QCCS													
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	40																
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COMMENTS: DATA QUALIFIERS X, Y and Z ARE AVAILABLE FOR USE ON THIS FORM.

SAMPLE ID	QUALIFIER	COMMENT
	X	
	X	
	X	
	Y	
	Y	
	Y	
	Z	
	Z	
	Z	

WHITE — ORNL COPY YELLOW — FIELD COPY PINK — EMSL-LV COPY

GILLS (702) 342-7160 REVISION 1-86

NSWS Form 2/5

NATIONAL SURFACE WATER SURVEY
SAMPLE MANAGEMENT OFFICE
P.O. BOX 818
ALEXANDRIA, VA 22314

NSWS
FORM 3
SHIPPING

RECEIVED BY _____
IF INCOMPLETE IMMEDIATELY NOTIFY:
SAMPLE MANAGEMENT OFFICE
(703) 557-2490

PAGE _____ OF _____

FROM (STATION ID):	TO (LAB):	BATCH ID	DATE PROCESSED	DATE SHIPPED	DATE RECEIVED
			_____	_____ AIR-BILL NO. _____	_____

SAMPLE ID	ALIQUOTS SHIPPED (FOR STATION USE ONLY)								SPLITS	SAMPLE CONDITION UPON LAB RECEIPT (FOR LAB USE ONLY)
	1	2	3	4	5	6	7	8		
01										
02										
03										
04										
05										
06										
07										
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QUALIFIERS:
√: ALIQUOT SHIPPED
M: ALIQUOT MISSING DUE TO DESTROYED SAMPLE

WHITE — FIELD COPY PINK — LAB COPY YELLOW — SMO COPY GOLD — LAB COPY FOR RETURN TO SMO

GILL'S (702) 362-2100

NATIONAL SURFACE WATER SURVEY
FORM 11

SUMMARY OF SAMPLE RESULTS

LAB NAME _____ BATCH ID _____ LAB MANAGER'S SIGNATURE _____

	ALIQUOT ID											
	1						2	3				
SAMPLE ID:	Ca mg/L	Mg mg/L	K mg/L	Na mg/L	Mn mg/L	Fe mg/L	Total Extr. Al mg/L	Cl^- mg/L	SO_4^{2-} mg/L	NO_3^- mg/L	SiO_2 mg/L	ISE Total F^- mg/L
01												
02												
03												
04												
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NOTE: Approved data qualifiers and instructions for their use are listed in Table 3-10.

NATIONAL SURFACE WATER SURVEY
FORM 11

SUMMARY OF SAMPLE LIMITS

LAB NAME _____ BATCH ID _____ LAB MANAGER'S SIGNATURE _____

	ALIQUOT ID											
	4		5								6	7
			Measured									
SAMPLE ID:	DOC mg/L	NH_4^+ mg/L	Eq. pH	ANC Init. pH	BNC Init. pH	CO_2- BNC µeq/L	ANC µeq/L	COND. µS/cm	Eq. DIC mg/L	Init. DIC mg/L	Total P mg/L	Total Al mg/L
01												
02												
03												
04												
05												
06												
07												
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NOTE: Approved data qualifiers and instructions for their use are listed in Table 3-10.

NSWS Form 11 (Continued)

NATIONAL SURFACE WATER SURVEY
Form 13

ANC AND BNC RESULTS

Lab Name_____ Batch ID_____ Sample ID_____

Lab Manager's Signature_____ Analyst_____

RESULTS

[ANC] = _____ μeq/L Initial Sample Volume = _____ mL
[CO_2-BNC] = _____ μeq/L Blank ANC = _____ μeq/L

DATA

C_A = _____ eq/L DATE STANDARDIZED _____
C_B = _____ eq/L DATE STANDARDIZED _____

ACID TITRATION

VOLUME HCl (mL)	MEASURED pH'	CALCULATED pH
0.00		
0.00 (with KCl)		

BASE TITRATION

VOLUME NaOH (mL)	MEASURED pH'	CALCULATED pH
0.00		
0.00 (with KCl)		

NATIONAL SURFACE WATER SURVEY
Form 14*

QC DATA FOR ANC AND BNC ANALYSES

LAB NAME _____ BATCH ID _____

LAB MANAGER'S SIGNATURE _____

SAMPLE ID	ANC µeq/L	CO_2-BNC µeq/L	CALCULATED ANC		%X[b]
			RESULT	DIFFERENCE[a]	
01					
02					
03					
04					
05					
06					
07					
08					
09					
10					
11					
12					
13					
14					
15					
16					
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36					
37					
38					
39					
40					

*Form not required in data package but recommended for internal QC requirements.

[a]Difference = Calculated ANC-Measured ANC

[b]
$$\%X = \frac{\text{DIC (in µmoles/L)} - ([\text{ANC}] + [CO_2\text{-BNC}])}{\text{DIC}} \times 100$$

NATIONAL SURFACE WATER SURVEY
Form 15*

CONDUCTIVITY

LAB NAME _____ BATCH ID _____

LAB MANAGER'S SIGNATURE _____

Sample ID	SPECIFIC CONDUCTANCE (µS/cm) Calculated	Measured	%CD**	CALCULATED CONDUCTANCE FOR EACH ION µS/cm HCO_3^-	Ca^{+2}	CO_3^{-2}	Cl^-	Mg^{+2}	NO_3^-	K^+	Na^+	SO_4^{2-}		NH_4^+	H^+	OH^-
01																
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Conductance Factors of Ions [(µS/cm at 25°C) per mg/L]				0.715	2.60	2.82	2.14	3.82	1.15	1.84	2.13	1.54		4.13	3.5×10^5 (per mole/L)	1.92×10^5 (per mole/L)

*Form not required in data package but recommended for internal QC requirements

$$**\% \text{ Conductance Difference} = \frac{\text{Calculated Cond.} - \text{Measured Cond.}}{\text{Measured Conductance}} \times 100$$

NSWS Form 15

NATIONAL SURFACE WATER SURVEY
Form 16*

ANION-CATION BALANCE CALCULATION

LAB NAME _____ BATCH ID _____

LAB MANAGER'S SIGNATURE _____

		Ions (μeq/L)										
Sample ID	% Ion Difference**	Ca^{2+}	Cl^-	Mg^{2+}	NO_3^-	K^+	Na^+	SO_4^{2-}	F^-	NH_4^+	ANC	H^+***
01												
02												
03												
04												
05												
06												
07												
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09												
10												
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Factor to Convert mg/L to μeq/L		49.9	28.2	82.3	16.1	25.6	43.5	20.8	52.6	55.4	----	----

*Form not required in data package but recommended for internal QC requirements

$$**\% \text{ Ion Difference} = \frac{\text{ANC} + \Sigma \text{ Anions} - \Sigma \text{ Cations (except } H^+)}{\Sigma \text{ Anions} + \Sigma \text{ Cations} + \text{ANC} + 2[H^+]} \times 100$$

$***[H^+] = (10^{-pH}) \times 10^6$ μeq/L

NATIONAL SURFACE WATER SURVEY
Form 17

IC RESOLUTION TEST

LAB NAME _____

BATCH ID _____

LAB MANAGER'S SIGNATURE _____

IC Resolution Test

IC Make and Model: _____

Date: _____

Concentration: SO_4^{2-} _____ µg/mL, NO_3^- _____ µg/mL

Column Back Pressure (at max. of stroke): _____ psi

Flow Rate: _____ mL/min

Column Model: _____ Date of Purchase: _____

Column Manufacturer: _____

Column Serial No: _____

Is precolumn in system ____ Yes ____ No

(a) _____ cm (b) _____ cm

Percentage Resolution: 100 x (1-a/b) _____

The resolution must be greater than 60%

Test Chromatogram:

NATIONAL SURFACE WATER SURVEY
Form 18

DETECTION LIMITS

LAB NAME _____ BATCH ID _____

LAB MANAGER'S SIGNATURE _____

Parameter	Units	Contract Required Detection Limit	Instrumental Detection Limit	Date Determined (DD MMM YY)
Ca	mg/L	0.01		
Mg	mg/L	0.01		
K	mg/L	0.01		
Na	mg/L	0.01		
Mn	mg/L	0.01		
Fe	mg/L	0.01		
Al, total extractable	mg/L	0.005		
Cl^-	mg/L	0.01		
SO_4^{2-}	mg/L	0.05		
NO_3^-	mg/L	0.005		
SiO_2	mg/L	0.05		
F^-, total	mg/L	0.005		
NH_4^+	mg/L	0.01		
DOC	mg/L	0.1		
Specific Conductance	µS/cm	*		
DIC	mg/L	0.05		
P, total	mg/L	0.002		
Al, total	mg/L	0.005		

*Report the X, which must not exceed 0.9 µS/cm, of six nonconsecutive blanks.
Note: Report with four significant figures or down to IDL.

NATIONAL SURFACE WATER SURVEY
FORM 19

SAMPLE HOLDING TIME SUMMARY

LAB NAME _____ BATCH ID _____ LAB MANAGER'S SIGNATURE _____

DATE* PROCESSED _____ DATE* RECEIVED _____

Parameter	Ca	Mg	K	Na	Mn	Fe	Total Extr. Al	Cl^-	SO_4^{2-}	NO_3^-	SiO_2	ISE Total F^-
Holding Time	28	28	28	28	28	28	7	28	28	7	28	28
Holding Time Plus Date Sampled												
Sample ID:	Date*						Analyzed**					
01												
02												
03												
04												
05												
06												
07												
08												
09												
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11												
12												
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40												

*Report these dates as Julian dates (i.e., March 26, 1984 = 4086).
**If parameter was reanalyzed due to QA problems, report the last date analyzed.

NATIONAL SURFACE WATER SURVEY
FORM 19

SAMPLE HOLDING TIME SUMMARY

LAB NAME _____ BATCH ID _____ LAB MANAGER'S SIGNATURE _____

DATE* PROCESSED _____ DATE* RECEIVED _____

Parameter	DOC	NH_4^+	Eq. pH	ANC	BNC	Specific Conductance	Eq. DIC	Init. DIC	Total P	Total Al
Holding Time	14	28	7	14	14	14	14	14	28	28
Holding Time Plus Date Sampled										
Sample ID:			Date*			Analyzed**				
01										
02										
03										
04										
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34										
35										
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40										

*Report these dates as Julian dates (i.e., March 26, 1984 = 4086).
**If parameter was reanalyzed due to QA problems, report the last date analyzed.

NATIONAL SURFACE WATER SURVEY
FORM 20

BLANKS AND QCCS

LAB NAME _____ BATCH ID _____

LAB MANAGER'S SIGNATURE _____

Parameter	ALIQUOT ID 1						2	3				
	Ca mg/L	Mg mg/L	K mg/L	Na mg/L	Mn mg/L	Fe mg/L	Extr. Al mg/L	Cl mg/L	SO_4^{2-} mg/L	NO_3^- mg/L	SiO_2 mg/L	ISE Total F^- mg/L
Calibration Blank												
Reagent Blank	N	N	N	N	N	N	N	N	N	N		N
DL QCCS Theoretical												N
DL QCCS Measured												N
Low QCCS True Value												
Low QCCS Upper Control Limit												
Low QCCS Lower Control Limit												
Initial												
Continuing												
Continuing												
Continuing												
Continuing												
Continuing												
Final												
High QCCS True Value												
High QCCS Upper Control Limit												
High QCCS Lower Control Limit												
Initial												
Continuing												
Continuing												
Continuing												
Continuing												
Continuing												
Final												

Note: Approved data qualifiers and instructions for their use are listed in Table 3-10.

NATIONAL SURFACE WATER SURVEY
FORM 20

BLANKS AND QCCS

LAB NAME _____ BATCH ID _____

LAB MANAGER'S SIGNATURE _____

		ALIQUOT ID									
		4		Measured			5			6	7
Parameter		DOC mg/L	NH_4^+ mg/L	Eq pH	ANC pH	BNC pH	Spec. Cond. µS/cm	Eq. DIC mg/L	Init. DIC mg/L	Total P mg/L	Total Al mg/L
Calibration Blank				N	N	N					
Reagent Blank		N	N	N	N	N	N	N	N	N	
DL	theoretical			N	N	N	N				
QCCS	measured			N	N	N	N				
Low QCCS True Value											
Low QCCS Upper Control Limit											
Low QCCS Lower Control Limit											
Initial											
Continuing											
Continuing											
Continuing											
Continuing											
Continuing											
Final											
High QCCS True Value											
High QCCS Upper Control Limit											
High QCCS Lower Control Limit											
Initial											
Continuing											
Continuing											
Continuing											
Continuing											
Continuing											
Final											

Note: Approved data qualifiers and instructions for their use are listed in Table 3-10.

NATIONAL SURFACE WATER SURVEY
Form 22
DUPLICATES

LAB NAME _____ BATCH ID _____ LAB MANAGER'S SIGNATURE _____

Parameter	ALIQUOT ID 1						2	3				
	Ca mg/L	Mg mg/L	K mg/L	Na mg/L	Mn mg/L	Fe mg/L	Total Extr.Al mg/L	Cl^- mg/L	SO_4^{2-} mg/L	NO_3^- mg/L	SiO_2 mg/L	ISE Total F mg/L
Duplicate Sample ID												
Sample Result												
Duplicate Result												
% RSD												
Second Duplicate Sample ID												
Sample Result												
Duplicate Result												
% RSD												
Third Duplicate Sample ID												
Sample Result												
Duplicate Result												
% RSD												

Note: Approved data qualifiers and instructions for their use are listed in Table 3-10.

NATIONAL SURFACE WATER SURVEY
Form 22

DUPLICATES

LAB NAME _____ BATCH ID _____ LAB MANAGER'S SIGNATURE _____

Parameter	ALIQUOT ID											
	4		Measured			5					6	7
	DOC mg/L	NH_4^+ mg/L	Eq. pH	ANC Initial pH	BNC Initial pH	CO_2- BNC µeq/L	ANC µeq/L	Spec. Cond. µS/cm	Eq. DIC mg/L	Init. DIC mg/L	Total P mg/L	Total Al mg/L
Duplicate Sample ID												
Sample Result												
Duplicate Result												
% RSD*												
Second Duplicate Sample ID												
Sample Result												
Duplicate Result												
% RSD*												
Third Duplicate Sample ID												
Sample Result												
Duplicate Result												
% RSD*												

Note: Approved data qualifiers and instructions for their use are listed in Table 3-10.
*Report absolute difference rather than %RSD for pH determinations.

NATIONAL SURFACE WATER SURVEY
FORM 31

SUMMARY OF ANALYTICAL RESULTS - PHYTOPIGMENTS

BATCH ID: _____ LAB NAME: _____

LAB MANAGER'S SIGNATURE: _____

	SAMPLE ID	EXTRACT VOLUME (mL)	CHLa (µg/L) FLUOROMETRIC UNCORRECTED	CHLa (µg/L) HPLC	PHEa (µg/L) HPLC	OTHERS, COMMENTS
01						
02						
03						
04						
05						
06						
07						
08						
09						
10						
11						
12						
13						
14						
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NATIONAL SURFACE WATER SURVEY
FORM 32

QC RESULTS - PHYTOPIGMENTS
FLUOROMETRY

BATCH ID: _____ LAB MANAGER'S SIGNATURE: _____

DATE: _____

ITEM	RESULT*	COMMENTS
METHOD DETECTION LIMIT	_____µg/L CHLa*	
BLANK	_____µg/L CHLa*	
RESPONSE FACTORS		
X1		
X3		
X10		
X30		
CALIBRATION CHECK µg/L		
Standard conc. _____ CHLa*	_____µg/L CHLa*	
DUPLICATES		
SAMPLE ID _____		
a)	_____µg/L CHLa*	
b)	_____µg/L CHLa*	
MEAN	_____µg/L CHLa*	

*Calculate as for a 200-mL sample

NATIONAL SURFACE WATER SURVEY
FORM 33

QC RESULTS - PHYTOPIGMENTS
HPLC

ITEM	RESULT*	COMMENTS
METHOD DETECTION LIMIT	_____µg/L CHLa _____µg/L PHEa	
BLANK	_____µg/L CHLa _____µg/L PHEa	

RESPONSE FUNCTIONS
CHLa:
PHEa:

CALIBRATION CHECK

CHLa

Standard conc.	_____µg/L	_____µg/L	Standard conc.	_____µg/L	_____µg/L
Standard conc.	_____µg/L	_____µg/L	Standard conc.	_____µg/L	_____µg/L
Standard conc.	_____µg/L	_____µg/L	Standard conc.	_____µg/L	_____µg/L

PHEa

Standard conc.	_____µg/L	_____µg/L	Standard conc.	_____µg/L	_____µg/L
Standard conc.	_____µg/L	_____µg/L	Standard conc.	_____µg/L	_____µg/L
Standard conc.	_____µg/L	_____µg/L	Standard conc.	_____µg/L	_____µg/L

DUPLICATES

SAMPLE ID _____

a) _____µg/L CHLa* _____µg/L PHEa*
b) _____µg/L CHLa* _____µg/L PHEa*
MEAN: _____ _____

SAMPLE ID _____

a) _____µg/L CHLa* _____µg/L PHEa*
b) _____µg/L CHLa* _____µg/L PHEa*
MEAN: _____ _____

COMMENTS

*Calculate as for a 200-mL sample

NATIONAL SURFACE WATER SURVEY
FORM 34

QC RESULTS - PHYTOPIGMENTS
TIME LINE

RUN NUMBER	SAMPLE (STANDARD) ID	EXTRACTION TIME	FLUOR. ANALYSIS TIME	HPLC ANALYSIS TIME
01				
02				
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APPENDIX E

EXAMPLES OF CALCULATIONS REQUIRED FOR ACIDITY AND ALKALINITY DETERMINATIONS

E.1.0 HCl STANDARDIZATION (SECTION 5.4.1)

1.00 mL of 0.01038N Na_2CO_3, 4.00 mL of 1.0M KCl, plus 36.00 mL of CO_2-free deionized water are titrated with HCl titrant. The titration data are given below:

mL HCl added	pH	mL HCl added	pH	mL HCl added	pH
0.00	10.23	0.800	6.37	1.700	3.84
0.100	9.83	0.900	6.03	1.900	3.72
0.200	9.70	1.000	5.59	2.100	3.63
0.300	9.54	1.100	4.91	2.300	3.56
0.400	9.28	1.200	4.48	2.500	3.49
0.500	8.65	1.300	4.26		
0.600	7.20	1.400	4.11		
0.700	6.71	1.500	4.00		

F_{1b} is calculated for the data sets (V, pH) that are within the pH range 4 to 7 using the equation:

$$F_{1b} = (V_s + V)\left[\frac{V_s C}{(V_s + V)}\left(\frac{[H^+]K_1 + 2\,K_1\,K_2}{[H^+]^2 + [H^+]K_1 + K_1\,K_2}\right) + \frac{K_w}{[H^+]} - [H^+]\right]$$

where

V_s = initial sample volume (41.00 mL)
V = volume of HCl added (mL)
C = 1.266×10^{-4} = $(N_{Na_2CO_3})/(2 \times 41)$
$[H^+]$ = 10^{-pH}
K_1 = 7.079×10^{-7}
K_2 = 1.202×10^{-10}
K_w = 1.660×10^{-14}

The (V, F_{1b}) values are tabulated below:

V	F_{1b} ($\times 10^{-3}$)	V	F_{1b} ($\times 10^{-3}$)
0.700	3.57	1.100	-0.34
0.800	2.59	1.200	-1.33
0.900	1.60	1.300	-2.28
1.000	0.64	1.400	-3.26
		1.500	-4.23

The plot of F_{1b} versus V is shown in Figure E-1. The data lie on a straight line and are analyzed by linear regression to obtain the coefficients of the line $F_{1b} = a + bV$. From the regression:

$$r = 1.0000$$
$$a = 0.01038 \pm 0.00001$$
$$b = -0.009747 \pm 0.000012$$

Then $V_1 = -a/b = 1.065$ mL

and

$$N_{HCl} = \frac{N_{Na_2CO_3} \times V_{Na_2CO_3}}{V_1} = \frac{(0.01038)\ (1.00)}{1.065} = 0.009743 \text{ eq } L^{-1}$$

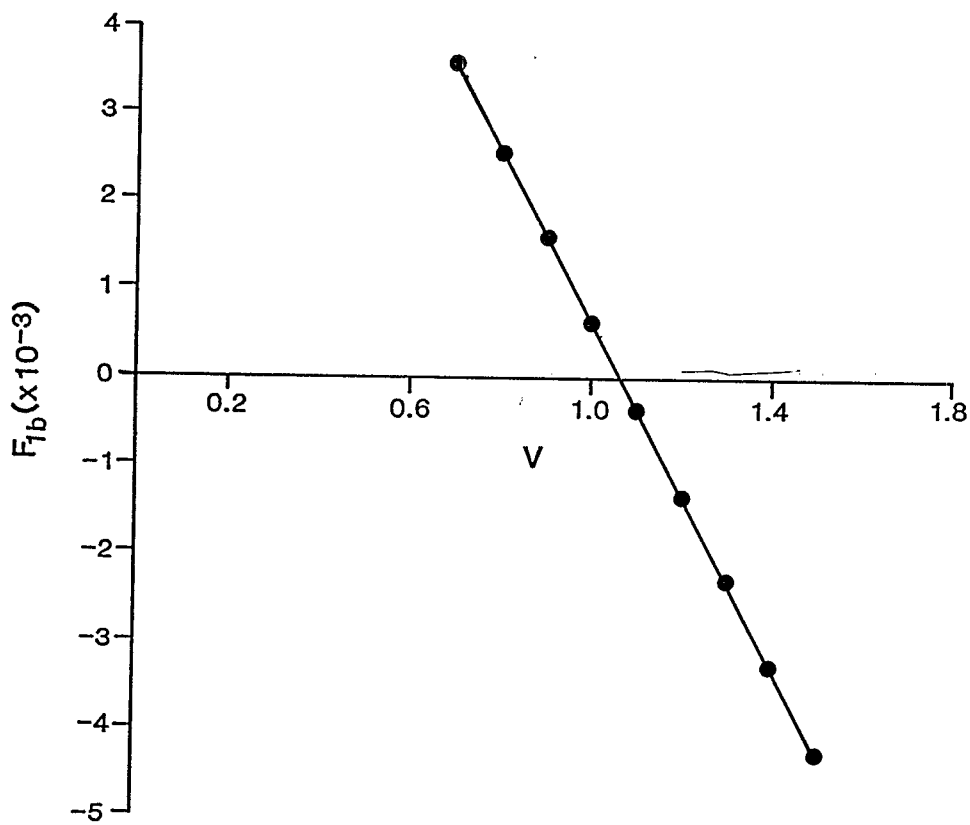

Figure E-1. Plot of F_{1b} versus V for HCl standardization.

E.2.0 INITIAL NaOH STANDARDIZATION WITH KHP (SECTION 5.4.2)

5.00 mL of 9.793×10^{-4}N KHP, 2.00 mL of 1.0M KCl, plus 18.0 mL of CO_2-free deionized water are titrated with approximately 0.01N NaOH. The titration data and appropriate Gran function values are given below:

Volume NaOH (mL)	pH	F_{3b} ($\times 10^{-3}$)
0.000	4.59	--
0.050	4.78	--
0.100	4.97	3.90
0.150	5.14	3.39
0.200	5.31	2.86
0.250	5.48	2.34
0.300	5.66	1.82
0.350	5.87	1.29
0.400	6.14	0.79
0.450	6.66	0.26
0.500	8.99	-0.25
0.550	9.26	-0.77
0.600	9.48	-1.28
0.700	9.95	-2.29
0.900	10.23	-4.40
1.100	10.39	--
1.300	10.51	--

The Gran function F_{3b} is calculated for data with pH 5 to 10. F_{3b} is calculated by:

$$F_{3b} = \left[(V_s + V) \left(\frac{V_s C}{(V_s + V)} \quad \frac{[H^+]K_1 + 2[H^+]^2}{([H^+]^2 + [H^+]K_1 + K_1K_2)} \right) + [H^+] - \frac{K_w}{[H^+]} \right]$$

V = volume NaOH added (mL)
V_s = initial sample volume (25.00 mL)
C = $N_{KHP}/5$ = 1.9586×10^{-4}
$[H^+]$ = 10^{-pH}
K_1 = 1.3×10^{-3}
K_2 = 3.9×10^{-6}
K_w = 1.660×10^{-14}

F_{3b} versus V is plotted in Figure E-2. The data lie on a straight line with the equation $F_{3b} = a + bV$. The coefficients are calculated by linear regression. From the regression:

$$r = 1.0000$$
$$a = 0.004931 \pm 0.000008$$
$$b = -0.01036 \pm 0.00002$$

From this, V_3 and N_{NaOH} are calculated by:

$$V_3 = -a/b = 0.4760 \text{ mL}$$

$$N_{NaOH} = \frac{N_{KHP} \times V_{KHP}}{V_3} = 0.01028 \text{ eq } L^{-1}$$

E.3.0 NaOH-HCl STANDARDIZATION CROSSCHECK (SECTION 5.4.3)

0.500 mL of 0.00921N NaOH, 2.50 mL of 1.0M KCl, plus 22.0 mL of CO_2-free deionized water are titrated with 0.0101N HCl (standardized with Na_2CO_3). The titration data and appropriate Gran function values are given below:

Volume HCl (mL)	pH	F_1 (x 10^{-3})
0.000	10.07	--
0.100	9.93	--
0.200	9.77	2.46
0.250	9.71	2.15
0.300	9.58	1.60
0.350	9.40	1.06
0.400	9.15	0.59
0.450	8.13	0.057
0.500	4.76	-0.44
0.550	4.44	-0.93
0.600	4.26	-1.41
0.650	4.12	-1.95
0.700	4.04	-2.34
0.800	3.88	--

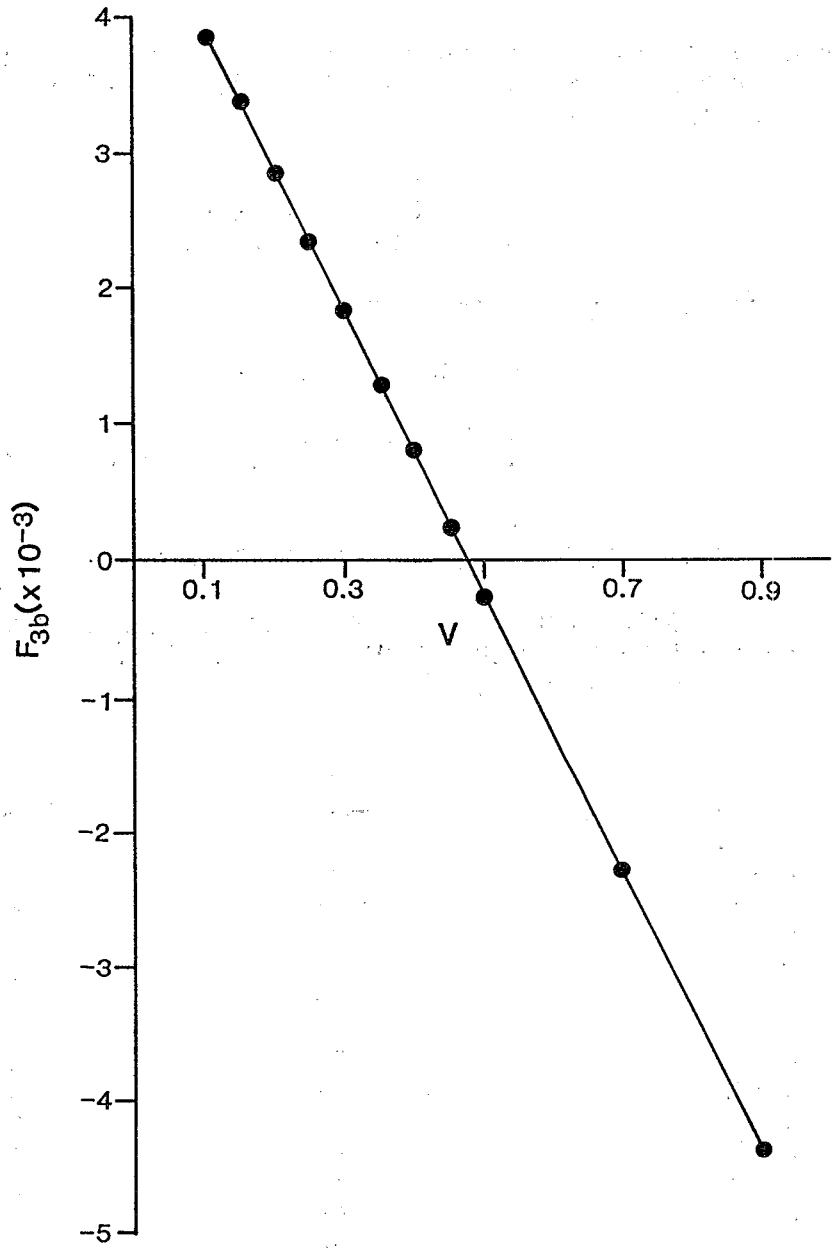

Figure E-2. Plot of F_{3b} versus V for initial NaOH standardization with KHP.

The Gran function F_1 is determined for data in the pH range 4 to 10. F_1 is calculated by:

$$F_1 = (V_s + V)\left(\frac{K_w}{[H^+]} - [H^+]\right)$$

where

V_s = initial sample volume = 25.0 mL
V = volume of HCl added
K_w = 1.660×10^{-14}
$[H^+]$ = 10^{-pH}

F_1 versus V is plotted in Figure E-3. The data are on a straight line with the equation $F_1 = a + bV$. The coefficients, determined by linear regression, are:

r = 0.999
a = 0.00453 ± 0.00005
b = -0.00990 ± 0.00010

From these values, V_1 and N'_{HCl} are calculated by:

$$V_1 = -a/b = 0.4576$$

$$N'_{HCl} = \frac{N_{NaOH} \times V_{NaOH}}{V_1} = 0.01006$$

Comparing this value for N'_{HCl} with the previously determined value of N_{HCl}, the absolute relative percent deviation (RPD) is:

$$\text{RPD in } N_{HCl} \text{ values} = \left|\frac{0.01006 - 0.0101}{0.5\ (0.01006 + 0.0101)}\right| \times 100 = 0.4\%$$

This RPD is acceptable because it is less than 5 percent.

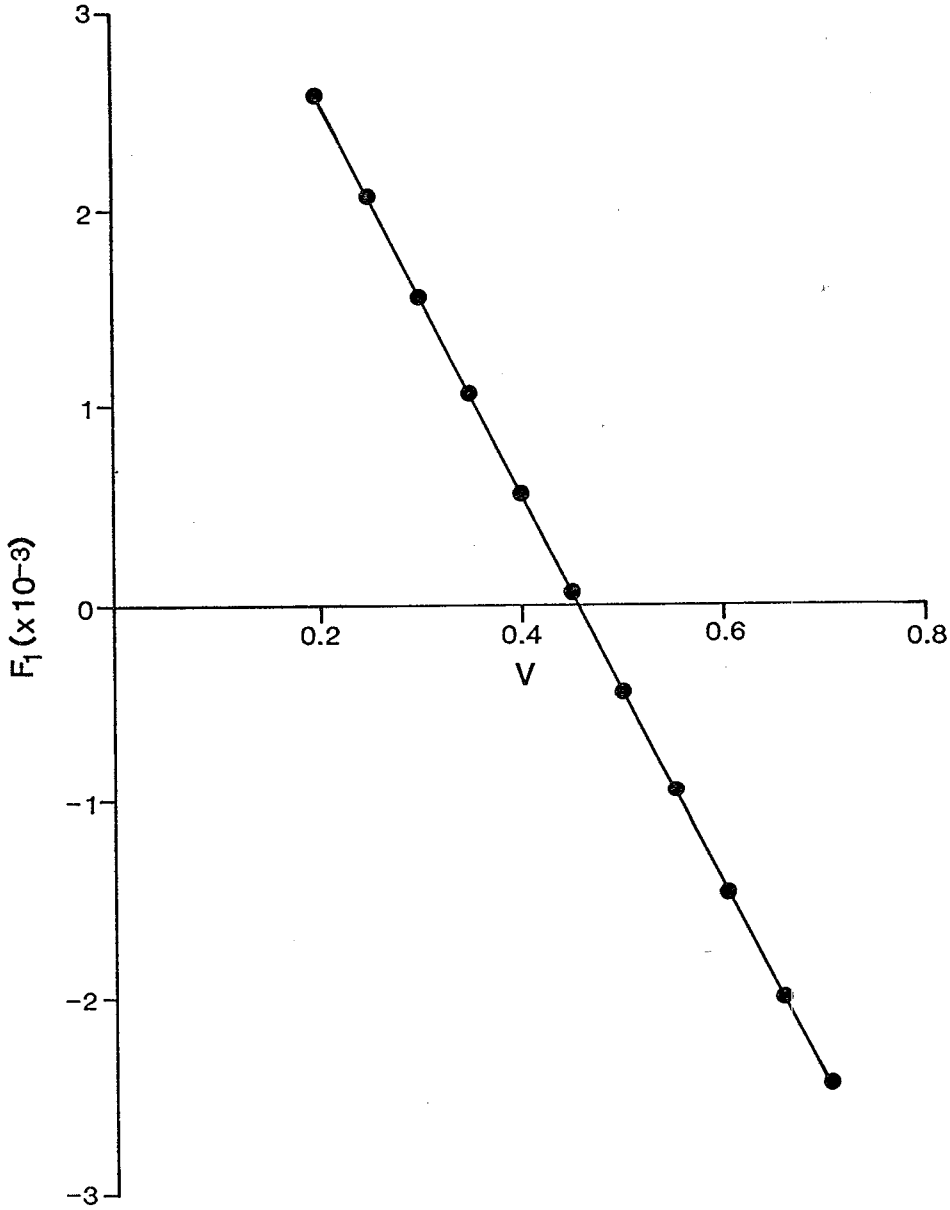

Figure E-3. Plot of F_1 versus V for NaOH-HCl standardization cross-check.

E.4.0 DAILY NaOH STANDARDIZATION WITH STANDARDIZED HCl (SECTION 5.4.4)

1.000 mL of an approximately 0.01N NaOH solution, 2.50 mL of 1.0M KCl, and 21.50 mL of CO_2-free deionized water are titrated with 0.009830N HCl. The titration data are given below:

mL HCl added	pH	mL HCl added	pH	mL HCl added	pH
0.00	10.24	0.750	5.44	1.200	3.78
0.200	10.10	0.800	4.65	1.400	3.62
0.400	9.90	0.850	4.37		
0.600	9.51	0.900	4.22		
0.650	9.32	1.000	4.02		
0.700	8.97	1.100	3.88		

F_1 is calculated for each data pair (V, pH) in the pH range 4 to 10 using the equation:

$$F_1 = (V_s + V)\left(\frac{K_w}{[H^+]} - [H^+]\right)$$

where

V_s = initial sample volume (25.00 + 1.00 = 26.00 mL)
V = volume of HCl added
K_w = 1.660×10^{-14}
$[H^+]$ = 10^{-pH}

The new data pairs (V, F_1) are tabulated below:

V	F_1 ($\times 10^{-3}$)	V	F_1 ($\times 10^{-3}$)
0.400	3.35	0.850	-1.10
0.600	1.38	0.900	-1.56
0.650	0.89	1.000	-2.48
0.700	0.40	1.100	-3.44
0.750	-0.093		
0.800	-0.58		

A plot of F_1 versus V is shown in Figure E-4. The data sets corresponding to volumes from V = 0.40 to V = 1.10 lie on a straight line with the equation $F_1 = a + bV$.

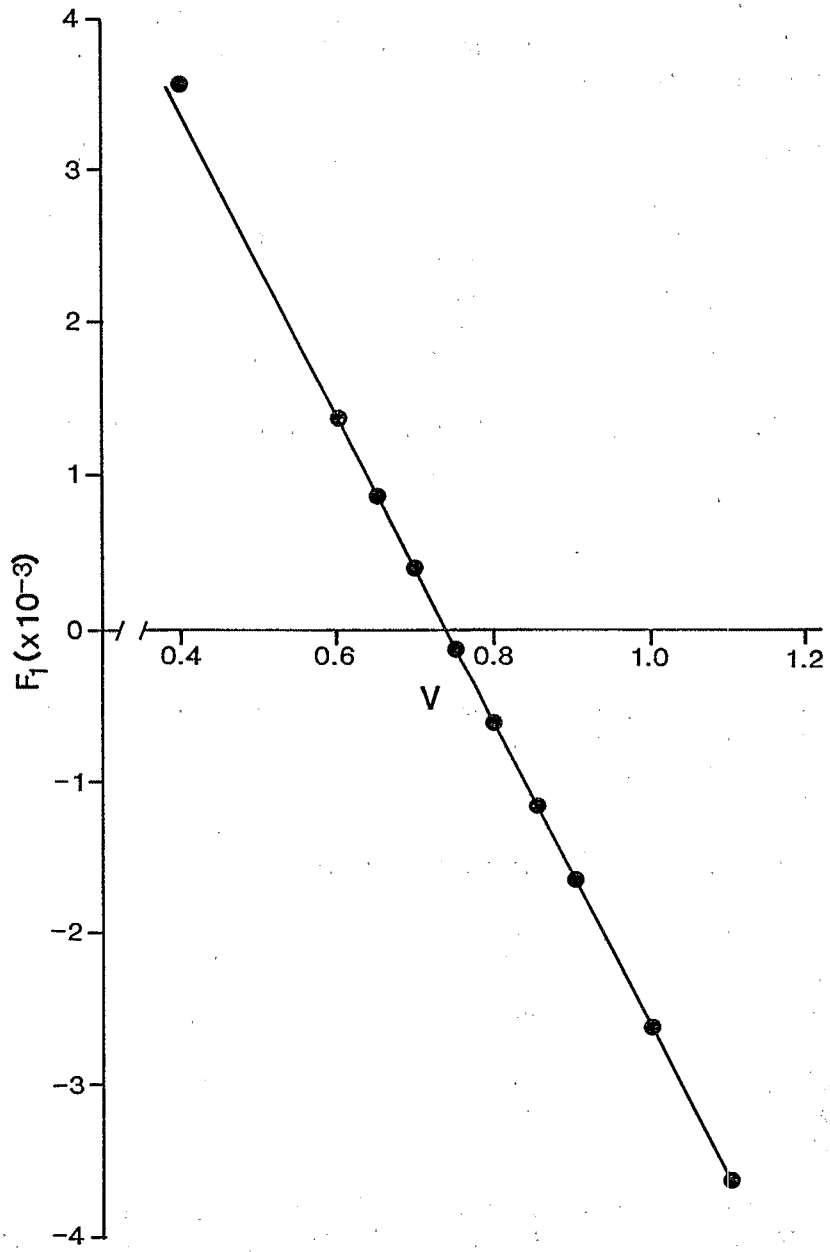

Figure E-4. Plot of F_1 versus V for daily NaOH standardization.

The coefficients are obtained by linear regression. The results are:

$r = 1.000$
$a = 0.00720 \pm 0.00004$
$b = -0.009710 \pm 0.00047$

From these results:

$$V_1 = -a/b = 0.741$$

and

$$N_{NaOH} = \frac{N_{HCl} \times V_1}{V_{NaOH}} = \frac{(0.009830)\ (0.741)}{1.000} = 0.00728$$

E.5.0 ELECTRODE CALIBRATION (SECTION 5.4.5)

This section describes the electrode calibration procedure. The tables below (E-1 and E-2) tabulate the titration data (V and pH), the calculated pH values (pH*), and the coefficients for the line pH = a + b pH*.

TABLE E-1. ACID TITRATION

V_s = 50.00 mL N_{HCl} = 0.00983

Volume HCl (mL)	pH	pH*	Volume HCl (mL)	pH	pH*
0.000	5.87	---	0.450	4.05	4.06
0.025	5.25	5.31	0.500	4.00	4.02
0.050	4.97	5.01	0.600	3.92	3.94
0.100	4.68	4.71	0.800	3.80	3.81
0.150	4.51	4.54	1.000	3.71	3.72
0.200	4.38	4.41	1.200	3.64	3.64
0.250	4.29	4.31	1.500	3.55	3.55
0.300	4.22	4.24	1.700	3.50	3.50
0.350	4.15	4.17	2.000	3.43	3.43
0.400	4.10	4.11			

r = 1.00 a = 0.10 ± 0.01 b = 0.971 ± 0.002

TABLE E-2. BASE TITRATION

V_S = 50.0 mL N_{NaOH} = 0.00804

Volume NaOH (mL)	pH	pH*	Volume NaOH (mL)	pH	pH*
0.000	6.66	---	0.820	9.87	9.89
0.050	8.67	8.68	0.940	9.93	9.95
0.200	9.28	9.29	1.080	9.99	10.01
0.300	9.34	9.46	1.200	10.04	10.06
0.400	9.40	9.58	1.300	10.07	10.09
0.500	9.66	9.68	1.400	10.11	10.12
0.600	9.74	9.76	1.500	10.13	10.15

r = 0.99 a = 0.08 ± 0.27 b = 0.99 ± 0.03

The data in Tables E-1 and E-2 are plotted in Figure E-5. Except for two points in the base titration (at V = 0.3 and 0.4), the data lie on a straight line. (NOTE: the lines calculated for each titration are essentially coincident as indicated by their coefficients.) Excluding these two points, the data are fit to the line with the equation pH = a + b pH*. The coefficients of the line (obtained by linear regression) are:

r = 1.0000
a = -0.014 ± 0.0011
b = 0.999 ± 0.002

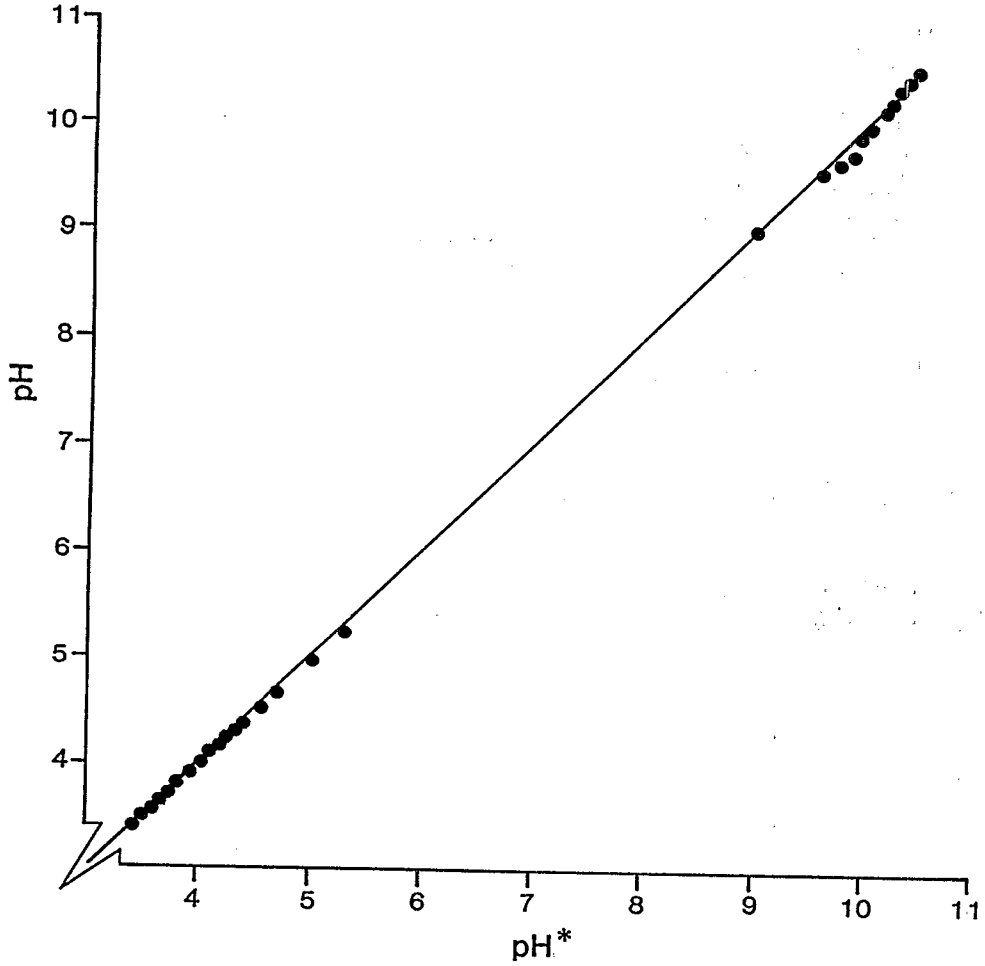

Figure E-5. Plot of pH* versus pH for electrode calibration.

E.6.0 BLANK ANALYSIS - ANC DETERMINATION (SECTION 5.6.5)

This section describes the determination of ANC in a blank solution. The blank is prepared by adding 4.00 mL of 0.10M NaCl to 36.00 mL of deionized water. It is titrated with 0.00983N HCl. The titration data are given below (both measured and calculated pH* values are included):

Volume HCl (mL)	pH	pH*	F_{1a}	Volume HCl (mL)	pH	pH*	F_{1a}
0.000	5.84	5.85	--	0.500	3.91	3.91	0.00498
0.080	4.69	4.70	--	0.600	3.84	3.84	0.00587
0.120	4.52	4.53	--	0.700	3.77	3.77	0.00691
0.200	4.31	4.32	0.00192	1.000	3.62	3.62	0.00984
0.300	4.14	4.14	0.00292	1.200	3.55	3.55	0.0116
0.400	4.01	4.02	0.00386	1.500	3.45	3.45	0.0147

The Gran function F_{1a}:

$$F_{1a} = (V_s + V)\ [H^+]$$

is calculated for pH* values less than 4.5; the values are included in the table.

F_{1a} versus V is plotted in Figure E-6. The data are linear and fit the line $F_{1a} = a + bV$ using linear regression. The resulting coefficients are:

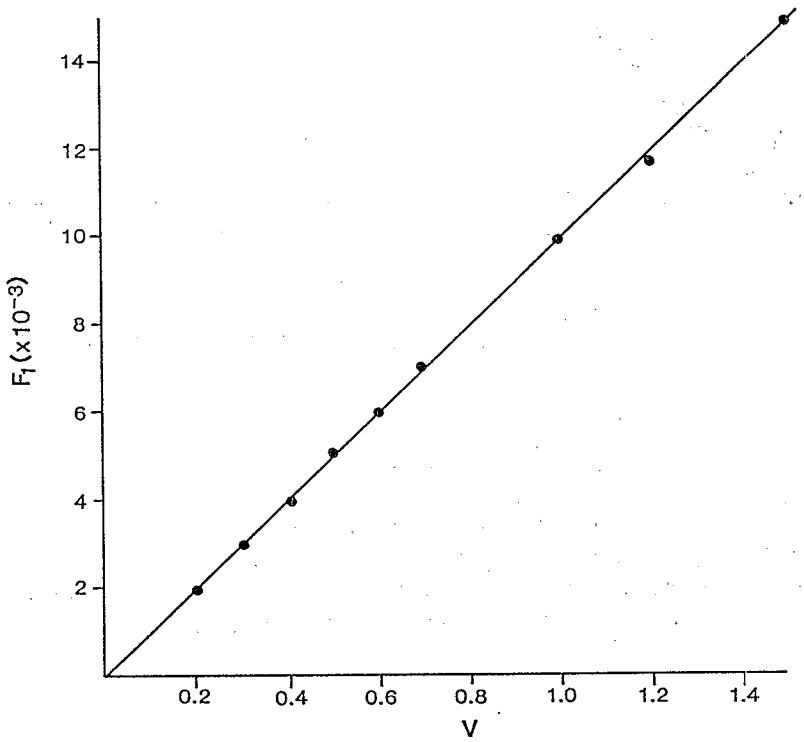

Figure E-6. Plot of F_{1a} versus V for ANC determination of blank.

$r = 0.9999$
$a = (-0.3 \pm 5.0) \times 10^{-5}$
$b = 0.009777 \pm 0.000061$

From this:

$$V_1 = -a/b = 3.07 \times 10^{-4} \text{ mL}$$

and

$$ANC = \frac{V_1 C_{HCl}}{V_{sa}} = 7.6 \times 10^{-8} \frac{eq}{L} = 0.08 \ \mu eq \ L^{-1}$$

This value for ANC is acceptable.

E.7.0 SAMPLE ANALYSIS

E.7.1 Titration Data (Section 5.5.)

A natural lake sample was titrated as described in Section 5.5. The titration data are given below. Also included are values for the calculated pH (pH*).

Acid Titration

V_{sa} = 36.00 mL V_{salt} = 4.00 mL

C_a = 0.00983 eq L^{-1}

V_a	pH	pH*	V_a	pH	pH*
0.000	5.10	5.11	0.460	3.99	3.99
0.040	4.89	4.90	0.550	3.91	3.91
0.080	4.71	4.72	0.650	3.84	3.84
0.120	4.56	4.57	0.750	3.77	3.77
0.140	4.50	4.51	0.900	3.69	3.69
0.160	4.44	4.44	1.100	3.61	3.61
0.260	4.24	4.24	1.400	3.50	3.50
0.280	4.21	4.21	1.700	3.42	3.42
0.380	4.08	4.08			

Base Titration

V_{sb} = 36.00 mL V_{salt} = 4.00 mL

C_b = 0.00702 eq L^{-1}

V_b	pH	pH*	V_b	pH	pH*
0.00	5.08	5.09	0.425	8.30	8.32
0.015	5.13	5.14	0.470	8.66	8.68
0.030	5.26	5.27	0.500	8.85	8.87
0.050	5.35	5.36	0.540	9.01	9.03
0.080	5.57	5.58	0.560	9.10	9.12
0.120	5.78	5.79	0.600	9.21	9.23
0.160	6.06	6.07	0.660	9.35	9.37
0.200	6.30	6.31	0.700	9.44	9.47
0.240	6.65	6.66	0.780	9.57	9.60
0.280	6.98	7.00	0.900	9.72	9.75
0.320	7.29	7.31	1.000	9.83	9.86
0.340	7.46	7.48	1.100	9.92	9.95
0.360	7.62	7.64	1.405	10.12	10.15
0.380	7.83	7.85	1.700	10.26	10.29
0.400	8.03	8.05	2.200	10.43	10.43
			2.500	10.51	10.54

E.7.2 Initial Estimate of V_1 (Section 5.5.4)

The Gran function F_{1a} is calculated for each data pair from the acid titration that has a pH* less than 4. The values are given below:

V_a	$F_{1a}(x10^{-3})^*$	V_a	$F_{1a}(x10^{-3})^*$
0.460	4.14	0.900	8.35
0.550	4.99	1.100	10.10
0.650	5.88	1.400	13.10
0.750	6.92	1.700	15.90

$^*F_{1a} = (V_s + V_a)\ [H^+]$

F_{1a} versus V_a is plotted in Figure E-7. A regression of F_{1a} on V_a is performed to fit the data to the line $F_{1a} = a + bV$. The resulting coefficients are:

$$r = 0.9999$$
$$a = -0.000241 \pm 0.000051$$
$$b = 0.009496 \pm 0.000050$$

From this, the initial estimate of V_1 is calculated by:

$$V_1 = -a/b = 0.0254 \text{ mL}$$

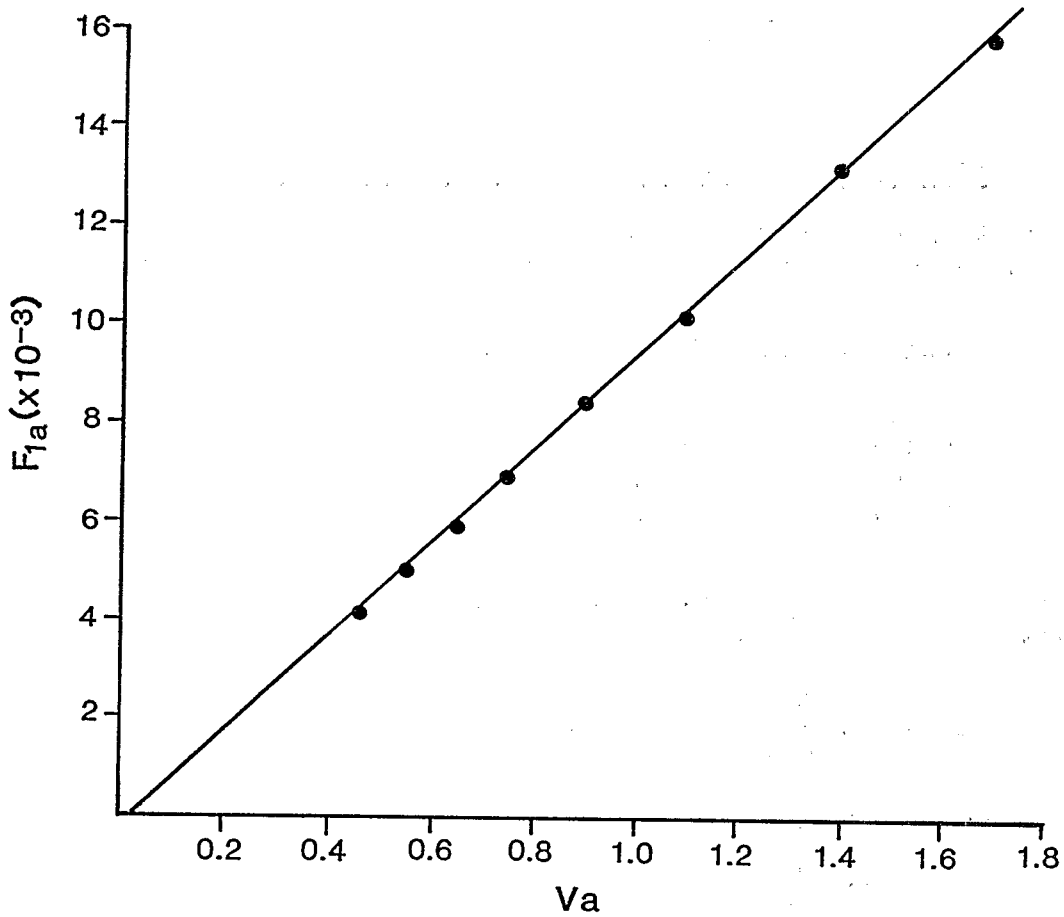

Figure E-7. Plot of F_{1a} versus V_a for initial determination of V_1.

Because $V_1 > 0$ and the initial sample pH* $\leq$ 7.6, calculation procedure B (section 5.5.4, step 3) is used to determine the ANC and BNC of the sample.

E.7.3 Initial Estimates of V_2, ANC, BNC, and C (Section 5.5.4)

From the base titration data, V_2 is estimated to be 0.40 mL (the first point with a pH* $\leq$ 8.2). Now that initial estimates of V_1 and V_2 have been obtained, estimates of ANC, BNC, and C can be calculated:

$$ANC = \frac{V_1 C_a}{V_{sa}} = 6.9 \times 10^{-6} \text{ eq } L^{-1}$$

$$BNC = \frac{V_2 C_b}{V_{sb}} = 7.80 \times 10^{-5} \text{ eq } L^{-1}$$

$$C = ANC + BNC = 8.49 \times 10^{-5} \text{ eq } L^{-1}$$

E.7.4 Refined Estimates of V_1 and V_2 (Section 5.5.4)

The Gran function F_{1c} (equation 5-1) is calculated for acid titration data with volumes across the current estimate of V_1. The values are given below:

V_a	$F_{1c}(x10^{-4})$	V_a	$F_{1c}(x10^{-4})$
0.000	-0.26	0.140	-11.6
0.040	-3.23	0.160	-13.9
0.080	-6.42	0.260	-22.8
0.120	-9.93	0.280	-24.4
		0.380	-33.3

F_{1c} versus V_a is plotted in Figure E-8. A regression of F_{1b} on V_a is performed. The regression results are:

$$r = 0.999$$
$$a = -0.000032 \pm 0.00019$$
$$b = -0.00882 \pm 0.0010$$

A new estimate of V_1 is:

$$V_1 = -a/b = -0.0036 \text{ mL}$$

Next the Gran function F_{2c} (Equation 5-2) is calculated from data sets from the base titration with volumes across the current estimate of V_2. The values are given below:

V_b	$F_{2c}(x\ 10^{-4})$	V_b	$F_{2c}(x\ 10^{-4})$
0.340	1.22	0.425	-2.01
0.360	0.61	0.470	-4.98
0.380	-0.087	0.500	-7.73
0.400	-0.78	0.540	-11.1

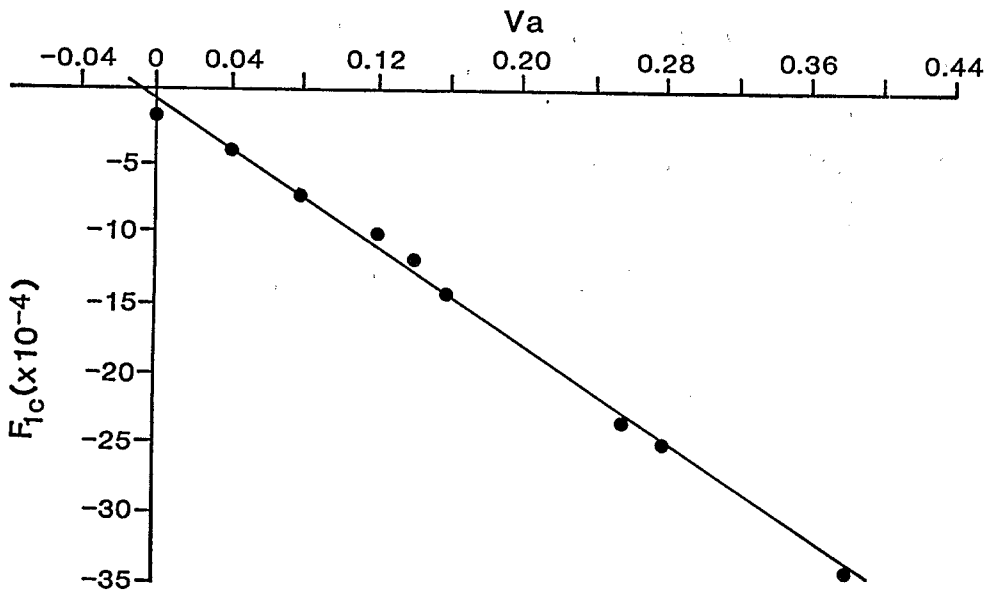

Figure E-8. Plot of F_{1c} versus V_a for V_1 determination.

F_{2c} versus V_b is plotted in Figure E-9. A regression of F_{2c} on V_b is performed. (NOTE: data with $V_b > 0.4$ are not used in the regression.) The regression results are:

$$r = 0.999$$
$$a = 0.00126 \pm 0.00003$$
$$b = -0.003348 \pm 0.000073$$

A new estimate of V_2 is:

$$V_2 = -a/b = 0.376 \text{ mL}$$

E.7.5 New Estimates of ANC, BNC, and C (Section 5.5.4)

From the new estimates of V_1 and V_2, new estimates of ANC, BNC, and C are calculated:

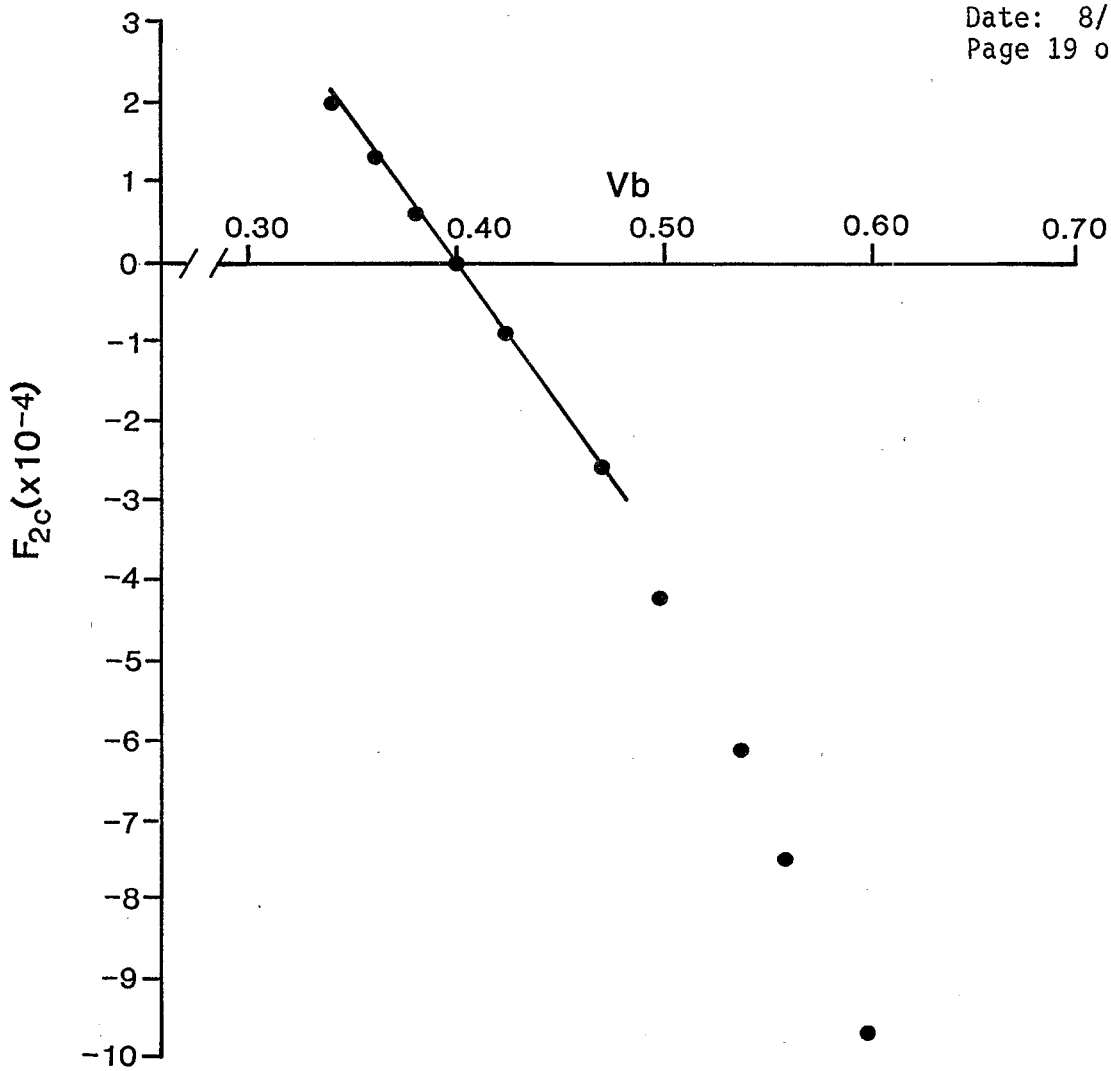

Figure E-9. Plot of F_{2c} versus V_b for V_2 determination.

$$ANC^* = \frac{V_1\ C_a}{V_{sa}} = 0.99 \times 10^{-6}\ eq\ L^{-1}$$

$$BNC^* = \frac{V_2\ C_b}{V_{sb}} = 7.36 \times 10^{-5}\ eq\ L^{-1}$$

$$C^* = ANC + BNC = 7.45 \times 10^{-5}\ eq\ L^{-1}$$

E.7.6 Comparison of Latest Two Estimates of Total Carbonate (Section 5.5.4)

$$\left|\frac{C - C^*}{C + C^*}\right| = 0.065 > 0.001$$

Because C and C* do not agree, a new C is calculated from their average:

$$C(new) = (C + C^*)/2 = 7.97 \times 10^{-5} \text{ eq } L^{-1}$$

The calculations in Sections E.7.4 through E.7.6 are repeated until successive iterations yield total carbonate values which meet the criteria given above. The results from each iteration (including those already shown) are given below. Note that all decimal values used are not shown.

Iteration	V_1(mL)	V_2(mL)	ANC (μeq L^{-1})	BNC (μeq L^{-1})	C (μeq L^{-1})	$\frac{C - C^*}{C + C^*}$	New C (μeq L^{-1})
1	0.0254	0.400	6.9	78.0	84.9	-	-
2	0.0036	0.377	0.99	73.6	74.5	0.065	79.7
3	0.0022	0.376	0.60	73.4	74.0	0.037	76.8
4	0.0014	0.376	0.40	73.3	73.7	0.021	75.2
5	0.0010	0.375	0.28	73.2	73.5	0.012	74.4
6	0.0008	0.375	0.22	73.2	73.4	0.007	73.9
7	0.0007	0.375	0.18	73.1	73.3	0.004	73.6
8	0.0006	0.375	0.16	73.1	73.3	0.002	73.4
9	0.0006	0.375	0.15	73.1	73.3	0.001	73.4
10	0.0005	0.375	0.15	73.1	73.3	0.0006	73.3

The final values for ANC and BNC are reported in a format similar to NSWS Form 11 (see Appendix D).

E.8.0 QUALITY CONTROL CALCULATIONS

Examples of the quality control calculations are described in this section.

E.8.1 Comparison of Calculated ANC and Measured ANC (Section 5.6.5)

For the sample analyzed in Section E.7.0, the following data were obtained:

Initial pH = 5.09 Air-equilibrated pH = 5.06

Dissolved Inorganic Carbon (DIC) = 59 mg L^{-1} Air-equilibrated DIC = 0.36

From these data, the calculated ANC values are computed using the equation:

$$[ANC]_c\ (\mu eq\ L^{-1}) = \left[\frac{DIC}{12{,}011}\left(\frac{[H^+]K_1 + 2\ K_1K_2}{[H^+]^2 + [H^+]K_1 + K_1\ K_2}\right) + \frac{Kw}{[H^+]} - [H^+]\right] \times 10^6$$

The results are:

$$[ANC]_{C1} = -4.2\ \mu eq\ L^{-1} \qquad [ANC]_{C2} = -6.4\ \mu eq\ L^{-1}$$

Then

$$|[ANC]_{C1} - [ANC]_{C2}| = 2.2\ \mu eq\ L^{-1} < 15\ \mu eq\ L^{-1}$$

Because $[ANC]_{C1}$ and $[ANC]_{C2}$ are in agreement, their average value is used for comparison to the measured value:

$$[ANC]_{C-avg} = -5.3\ \mu eq\ L^{-1} \qquad ANC = 0.15\ \mu eq\ L^{-1}$$

$$D = |ANC_C - ANC| = 5.4\ \mu eq\ L^{-1} < 15\ \mu eq\ L^{-1}$$

The calculated and measured ANC values agree, which backs up the assumption of a carbonate system.

E.8.2 Comparison of Calculated and Measured BNC (Section 5.6.3)

For the sample analyzed in Section E.7.0, the following data were obtained:

Initial pH = 5.09
DIC = 0.59 mg L^{-1}
BNC = 73.1 $\mu eq\ L^{-1}$

From these data, the BNC is computed using the equation:

$$[BNC]_C\ (\mu eq\ L^{-1}) = \left[\frac{DIC}{12{,}011}\left(\frac{[H^+]^2 - K_1K_2}{[H^+]^2 + [H^+]K_1 + K_1K_2}\right) + [H^+]\right] - \frac{K_w}{[H^+]} \times 10^6$$

The result is:

$$[BNC]_C = 53.3\ \mu eq\ L^{-1}$$

This value is compared to the measured value:

$$D = [BNC]_C - BNC = -19.8\ \mu eq\ L^{-1} < -10\ \mu eq\ L^{-1}$$

This value of D is indicative of other protolytes in the system which are contributing to the measured BNC. This might be expected because the sample also contains 3.2 mg L^{-1} dissolved inorganic carbon (DOC).

E.8.3 Comparison of Calculated Total Carbonate and Measured Total Carbonate (Section 5.6.4)

For the sample analyzed in Section E.7.0, the following data were obtained:

$$ANC = 0.15\ \mu eq\ L^{-1} = 0.15\ \mu mole\ L^{-1}$$

$$[ANC]_{C\text{-}avg} = -5.3\ \mu eq\ L^{-1} = -5.3\ \mu mole\ L^{-1}$$

$$BNC = 73.1\ \mu eq\ L^{-1} = 73.1\ \mu mole\ L^{-1}$$

$$[BNC]_C = 53.3\ \mu eq\ L^{-1} = 53.3\ \mu mole\ L^{-1}$$

From the DIC value, the total carbonate is calculated:

$$C_c = [ANC]_{C\text{-}avg} + [BNC]_{C\text{-}avg} = 48.0\ \mu mole\ L^{-1}$$

This calculated value is then compared to the measured value:

$$D = C_C - (ANC + BNC) = -25.2\ \mu mole\ L^{-1} < -10\ \mu mole\ L^{-1}$$

This value of D is indicative of other protolytes in the system. This might be expected because the sample also contains 3.2 mg L^{-1} DOC. Notice that the same conclusion was reached in the BNC comparison.

In general, noncarbonate protolytes are significant (i.e., contribute significantly to the total protolyte concentration), when indicated by one (or both) of the individual comparisons (ANC and BNC comparisons) and by the total carbonate comparison.

APPENDIX F

THE PHOTOIONIZATION DETECTOR FOR USE AS A METHYL ISOBUTYL KETONE DETECTION SYSTEM

NOTE: These procedures are specific to the AID photoionization detector installed in the NSWS mobile laboratories. These procedures can be modified for use with other photoionization detectors.

The AID photoionization detector should be used as a danger-level alert system for MIBK. This detector was field tested during the NSWS and was found to be useful in alerting laboratory personnel to the presence of potentially hazardous levels of MIBK in the work area. The full instructions for calibration are included with the detector, but an alternative weekly method is described below. The instrument should be calibrated to emit its warning signal at 25 ppm. MIBK odor is noticeable at levels much below 25 ppm.

The detector should be calibrated outside the laboratory and should be plugged into an AC outlet using the supplied adapter. Batteries may be used, but should be fully charged prior to use. Obtain a polyethylene bag and flush with standard MIBK gas. Empty the bag outside and refill with standard MIBK gas. The exact concentration of MIBK in the gas is shown on the certification tag attached to the tank. Turn on the meter and allow reading to stabilize with the inlet tube in the open air. Be sure that no higher than normal levels of organic vapors are present and adjust the reading to zero using a small screwdriver to turn the zero set screw on the rear of the meter.

Place the detector inlet tube into the bag filled with MIBK standard gas and turn on meter. Adjust the meter reading to the value which is recorded on the tank certification tag by turning the "SENS CAL" knob (bottom front). Be sure the reading has stabilized. Remove the tube from the bag and be sure the meter reading returns to near zero. Set the alarm level by adjusting the ppm set knob (top front) to 25 ppm on the scale of the knob. The meter reading should <u>not</u> change. Test the sensitivity by placing the inlet tube in the bag.

Place the calibrated instrument on the shelf near the clean workstation with the inlet tube placed near the sash opening. Record the calibration information in the MIBK logbook. If the ambient air level exceeds 25 ppm at the inlet site, the alarm will sound, warning personnel to put on respirator masks. If the indicator meter exceeds 50 ppm, the laboratory should be evacuated until the level drops to an acceptable limit. For specific questions concerning proper operation procedures, consult the instrument manufacturer's manual.

APPENDIX G

INTERNAL QUALITY CONTROL REQUIREMENTS

Quality control (QC) is an integral part of sample analysis. QC requirements common to all analytical methods are detailed in this section. QC requirements specific to a single method are detailed in the section for that method.

G.1 METHOD QUALITY CONTROL

Each method contains specific QC steps which should be performed to ensure data quality. Table G-1 is a brief summary of the required QC checks as well as control limits and corrective actions for QC checks outside control limits. QC steps common to all (or most) of the methods are detailed in Sections G.1.1 through G.1.4, while QC steps specific to a single method are detailed in the method section.

G.1.1 Calibration Verification QC Check Sample

After performing the calibration step for a method, verify the calibration (to ensure proper standard preparation) prior to sample analysis by analyzing a calibration QC check sample (QCCS). The QCCS is a known sample containing the analyte of interest at a concentration in the low- to mid-calibration range. Furthermore, the QCCS should be prepared from an independent source of that used for preparation of the calibration standards.

For each batch of samples, analyze the calibration QCCS immediately after calibration, after every 10 sample analyses or at intervals determined by the quality assurance program, and after the final sample analysis. Plot the measured analyte concentration of the QCCS on a control chart and develop the 95 percent and 99 percent confidence intervals. The 99 percent confidence interval should be within the limits given in Table G-2. (The limits in Table G-2 may be used as initial limits until enough data are obtained to generate a control chart.) If the 99 percent confidence interval is not within those limits, a problem exists with the experimental technique or with the QCCS itself.

The measured analyte concentration in the QCCS should be within the 99 percent confidence interval. An acceptable result should be obtained prior to continuing sample determinations. If unacceptable results are obtained, repeat the calibration step and reanalyze all samples analyzed since the last acceptably analyzed QCCS.

TABLE G-1. SUMMARY OF INTERNAL METHOD QUALITY CONTROL CHECKS

Parameter or Method	Quality Control Check	Control Limits	Corrective Action[a]
Acidity, Alkalinity, pH	1. Titrant standardization cross-check.	1. Relative difference <5%.	1. Restandardize titrants.
	2. Electrode calibration (Nernstian response check).	2. Slope = 1.00 ± 0.05.	2. Recalibrate or replace electrode.
	3. pH QCCS[b] (pH 4 and 10) analysis.	3. pH 4 = 4.00 ± 0.05. pH 10 = 10.00 ± 0.05.	3. Recalibrate electrode.
	4. Blank analysis (salt spike).	4. |Blank| $\leq$10 μeq L^{-1}.	4. Prepare fresh spike solution.
	5. Duplicate analysis.	5. %RSD[c] $\leq$10%.	5. Refine analytical technique. Analyze another duplicate.
	6. Protolyte comparison.	6. See method (Section 5).	6. See method (Section 5).
Ions (Cl^-, F^-, NH_4^+, NO_3^-, SO_4^{-2}), Metals (Al, Ca, Fe, K, Mg, Mn, Na), Silica, Total Phosphorus, Dissolved Inorganic Carbon (DIC), Dissolved Organic Carbon (DOC) Specific Conductance	1a. Initial QCCS[b] analysis (calibration and verification).	1a,b. The lesser of the 99% confidence interval or value given in in Table G-2.	1a. Prepare new standard and recalibrate.
	b. Continuing QCCS[b] analysis (every 10 samples).		b. Recalibrate. Reanalyze associated samples.
	2a. Detection limit (DL) determination (weekly).	2a. DL < values in method.	2a,b. Optimize instrumentation and technique.
	b. DL QCCS[b] analysis (daily, metals and total P only).	b. %Recovery = 100 ±20%.	
	3. Blank analysis.	3a. Blank $\leq$2 x DL (except sp. cond.). b. Blank $\leq$0.9 μS cm^{-1} (sp. cond. only).	3a,b. Determine and eliminate contamination source. Prepare fresh blank solution. Reanalyz associated samples.
	4. Duplicate analysis.	4. Duplicate precision (%RSD) $\leq$ values given in Method.	4. Investigate and eliminate source of imprecision. Analyze another duplicate.
	5. Matrix spike (except total ext. Al, DIC, and sp. cond.).	5. %Recovery = 100 ± 15%.	5. Analyze 2 additional spikes. If one or both outside control limits, analyze sample batch by method of standard additions.
	6. Resolution test (ion chromatography only).	6. Resolution $\geq$60%.	6. Clean or replace separator column. Recalibrate.

[a]Assuming QC check is outside control limits.
[b]QCCS = Quality control check sample.
[c]%RSD = percent relative standard deviation.

TABLE G-2. MAXIMUM CONTROL LIMITS FOR QUALITY CONTROL SAMPLES

Parameter	Maximum Control Limit for QC Sample (% Deviation from Theoretical Concentration of QC Sample)
Al, total extractable	±20%
Al, total	±20%
Ca	±5%
Cl^-	±5%
Dissolved Inorganic Carbon	±10%
Dissolved Organic Carbon	±10%
F^-, total	±5%
Fe	±10%
K	±5%
Mg	±5%
Mn	±10%
Na	±5%
NH_4^+	±10%
NO_3^-	±10%
P, total	±20%
SiO_2	±5%
SO_4^{-2}	±5%
Specific conductance	±2%

G.1.2 Detection Limit Determination and Verification

Determine the detection limit weekly for all parameters (except pH, alkalinity, acidity, and specific conductance for which the term detection limit does not apply). The detection limit is defined as three times the standard deviation of 10 nonconsecutive reagent or calibration blank analyses. In the case where a signal is not obtained for a blank analysis (such as in ion chromatographic analyses or autoanalyzer analyses), a low-concentration standard (concentration about three to four times the detection limit) is analyzed rather than a blank. Detection limits should not exceed the values listed in Table G-3. If a detection limit is not met, refine the analytical technique and optimize any instrumentation variables until the detection limit is achieved.

To verify the detection limit for the determination of metals, PCV-reactive aluminum, and total P, daily, analyze a detection limit QCCS after calibration and prior to sample analysis. The detection limit QCCS should contain the analyte of interest at two to three times the detection limit. The measured concentration should be within 20 percent of the true concentration. If it is not, the detection limit is questionable. Determine the detection limit as described above.

G.1.3 Blank Analysis

Once per batch analyze a calibration blank as a sample. The calibration blank is defined as a "0" mg L^{-1} standard (contains only the matrix of the calibration standards). The measured concentration of the calibration blank should be less than twice the instrumental detection limit. If not, the blank is contaminated or the calibration is in error at the low end. Prior to sample analysis, investigate and eliminate any contamination source and repeat the calibration.

Prepare and analyze a reagent blank for the three methods which require sample preparation (dissolved SiO_2, total P, and total Al). A reagent blank contains all the reagents (in the same quantities) used in preparing a real sample for analysis. Process in the same manner (digestions) as a real sample. The measured concentration of the reagent blank should be less than twice the required detection limit (Table G-3). If it is not, the reagent blank is contaminated. Investigate and eliminate the contamination source. Prepare and analyze a new reagent blank and apply the same criteria. Reanalyze all samples associated with the contaminated blank after the contamination is eliminated. Contact the laboratory supervisor or QA manager if a contaminated reagent blank problem cannot be rectified.

Prepare one reagent blank with each set of samples processed at one time. For example, if two sample batches are processed together, only one reagent blank is necessary. Report the concentration of the single reagent blank for both batches. On the other hand, if a sample batch is split into groups that

TABLE G-3. REQUIRED MINIMUM ANALYTICAL DETECTION LIMITS, EXPECTED RANGES, AND INTRALABORATORY RELATIVE PRECISION

Parameter[a]	Units	Required Detection Limit	Expected Range	Relative Intralababoratory Precision Goal (%)[b]
Acidity	$\mu eq\ L^{-1}$	-	10-150	10
Alkalinity	$\mu eq\ L^{-1}$	-	-100-1000	10
Al, Total Extractable	$mg\ L^{-1}$	0.005	0.005-1.0	10(Al>0.01),20(Al<0.01)
Al, PCV-reactive	$mg\ L^{-1}$	0.007	0.000-5.0	10(Al>0.01),20(Al<0.01)
Al, Total	$mg\ L^{-1}$	0.005	0.005-1.0	10(Al>0.01),20(Al<0.01)
Ca	$mg\ L^{-1}$	0.01	0.5-20	5
Cl^-	$mg\ L^{-1}$	0.01	0.2-10	5
DIC	$mg\ L^{-1}$	0.05	0.05-15	10
DOC	$mg\ L^{-1}$	0.1	0.1-50	5(DOC>5),10(DOC<5)
F^-, Total	$mg\ L^{-1}$	0.005	0.01-0.2	5
Fe	$mg\ L^{-1}$	0.01	0.01-5	10
K	$mg\ L^{-1}$	0.01	0.1-1	5
Mg	$mg\ L^{-1}$	0.01	0.1-7	5
Mn	$mg\ L^{-1}$	0.01	0.01-5	10
Na	$mg\ L^{-1}$	0.01	0.5-7	5
NH_4^+	$mg\ L^{-1}$	0.01	0.01-2	5
NO_3^-	$mg\ L^{-1}$	0.005	0.01-5	10
P, Total	$mg\ L^{-1}$	0.002	0.005-0.07	10(P>0.01),20(P<0.01)
pH, Processing Laboratory	pH units	-	3-8	±0.1[c]
pH, Laboratory	pH units	-	3-8	±0.05[c]
S_iO_2	$mg\ L^{-1}$	0.05	2-25	5
SO_4^{-2}	$mg\ L^{-1}$	0.05	1-20	5
Specific Conductance	$\mu S\ cm^{-1}$	[d]	5-1000	1
True Color	PCU[e]	0	0-200	±5[c]
Turbidity	NTU[f]	2	2-15	10

[a]Dissolved ions and metals are being determined, except where noted.
[b]Unless otherwise noted, this is the relative precision at concentrations above 10 times instrumental detection limits.
[c]Absolute precision goal is in terms of applicable units.
[d]Blank should be <0.9 $\mu S\ cm^{-1}$
[e]PCU = platinum-cobalt units.
[f]NTU = nephelometer turbidity units.

are processed at different times, a reagent blank is necessary for each group. In this case, report all reagent blank values for the batch.

G.1.4 Duplicate Sample Analysis

Prepare and analyze one sample per batch in duplicate. If possible, choose a sample for duplicate analysis containing analyte at a concentration greater than five times the detection limit. Calculate the percent relative standard deviation (%RSD) between duplicates. The duplicate precision (%RSD) should not exceed the value given in Table G-3. If duplicate %RSD values fall outside the values given in Table G-3, a problem exists (such as instrument malfunction, calibration drift). After finding and resolving the problem, analyze a second sample in duplicate. Acceptable duplicate sample results should be obtained prior to continuing sample analysis.

$$\% \text{ RSD} = \frac{S}{\bar{x}} \times 100$$

$$S = \left(\frac{\Sigma(\bar{x} - x)^2}{n-1} \right)^{1/2}$$

G.1.5 Matrix Spike Analysis

Prepare one matrix spike with each batch by spiking a portion of a sample with a known quantity of analyte. The spike concentration should be the larger of two times the endogenous level or ten times the required detection limit. Also, the volume of the spike added should be negligible (less than or equal to 0.001 of the sample aliquot volume). Calculate the percent recovery of the spike as follows:

$$\% \text{ spike recovery} = \frac{\left(\begin{array}{c}\text{measured}\\ \text{concentration}\\ \text{of sample}\\ \text{plus spike}\end{array} - \begin{array}{c}\text{measured}\\ \text{concentration}\\ \text{of unspiked}\\ \text{sample}\end{array}\right)}{(\text{actual concentration of spike added})} \times 100$$

The spike recovery should be 100 ± 15 percent. If the recovery is not acceptable, spike and analyze two additional, different samples. If either recovery is unacceptable, analyze the entire batch by the method of standard additions. The method of standard addition involves analyzing the sample,

the sample plus a spike at about the endogenous level, and the sample plus a spike at about twice the endogenous level.

NOTE: Matrix spikes for graphite furnace atomic absorption spectroscopy (GFAA) analyses may not be added directly in the furnace.

The concentration of the matrix spike should not exceed the instrument linear dynamic range. For this reason, the matrix spike concentration for furnace analyses should be chosen judiciously and may be different than suggested above.

Similarly, care should be taken to avoid exceeding the linear range when performing standard additions for GFAA analyses. The samples may be diluted and the spike levels may be adjusted so that the linear range is not exceeded.

G.2 OVERALL INTERNAL QUALITY CONTROL

Once each parameter in a sample has been determined, two procedures exist for checking the correctness of analyses. These procedures are outlined in Sections G.2.1 and G.2.2.

G.2.1 Anion-Cation Balance

Theoretically, the acid neutralizing capacity (ANC) of a sample equals the difference between the concentration (eq L^{-1}) of cations and the anions in a sample (Kramer, 1982). In practice, this is rarely true due to analytical variability and to ions that are present but not measured. For each sample, calculate the percent ion difference (%ID) as follows:

$$\text{\% Ion Difference} = \frac{\text{ANC} + \Sigma\text{ anions} - \Sigma\text{ cations}}{\text{TI}} \times 100$$

$$\text{TI (Total ion strength)} = \Sigma\text{ anions} + \Sigma\text{ cations} + \text{ANC} + 2[H^+]$$

$$\Sigma\text{ anions} = [Cl^-] + [F^-] + [NO_3^-] + [SO_4^{-2}]$$

$$\Sigma\text{ cations} = [Na^+] + [K^+] + [Ca^{+2}] + [Mg^{+2}] + [NH_4^+]$$

$$\text{ANC} \cong [\text{ALK}]$$

$$[H^+] = (10^{-pH}) \times 10^6\ \mu\text{eq } L^{-1}$$

All concentrations are expressed as microequivalents per liter (μeq L^{-1}). Table G-4 lists factors for converting mg L^{-1} to μeq L^{-1} for each of the parameters.

The %ID should not exceed the limits given in Table G-5. An unacceptable value for %ID indicates the presence of unmeasured ions or an analytical error in the sample analysis. For the surface waters sampled, the ions included in the %ID calculation are expected to account for 90 to 100 percent of the ions in a sample. Note that the ANC term in the calculation accounts for protolyte ions that are not specifically determined (such as organic acids and bases).

Examine the data from samples that do not meet the %ID criteria for possible causes of unacceptable %ID. Often, the cause is improper data reporting (misplaced decimal point, incorrect data reduction, switched sample identifications). After examining the data, redetermine any parameter that is suspect. If an explanation for the poor %ID cannot be found and the problem cannot be corrected, contact the laboratory supervisor or QA manager for further guidance.

TABLE G-4. FACTORS TO CONVERT mg L^{-1} TO μeq L^{-1}

Ion	Factor (μeq L^{-1} per mg/L^{-1})
Ca^{+2}	49.9
Cl^-	28.2
F^-	52.6
K^+	25.6
Mg^{+2}	82.3
Na^+	43.5
NH_4^+	55.4
NO_3^-	16.1
SO_4^{-2}	20.8

TABLE G-5. CHEMICAL REANALYSIS CRITERIA

A. Anion-Cation Balance

Total Ion Strength (μeq L^{-1})	Maximum % Ion Difference[a]
<50	60
≥50<100	30
≥100	15

B. Specific Conductance

Measured Conductance (μS cm^{-1})	Maximum % Conductance Difference[a]
<5	50
≥5<30	30
≥30	20

[a]If the absolute value of the percent difference exceeds these values, the sample should be reanalyzed. When reanalysis is indicated, the data for each parameter are examined for possible analytical error. Any suspect results are then redetermined and the above percent differences are recalculated (Peden, 1981). If the differences are still unacceptable or no suspect data are identified, the laboratory supervisor or QA manager should be contacted for guidance.

G.2.2 Conductivity Balance

Estimate the specific conductance of a sample by summing the equivalent conductances for each measured ion. Calculate the equivalent conductance for each ion by multiplying the ion concentration by the appropriate factor in Table G-4 (only major ions are included in the calculation). Calculate the percent conductance difference (%CD) as follows:

$$\% \text{ Conductance Difference} = \frac{\text{calculated cond.} - \text{measured cond.}}{\text{measured conductance}} \times 100$$

The %CD should not exceed the limits listed in Table G-5. As with the %ID calculation, an unacceptable value for %CD indicates either the presence of unmeasured ions or an analytical error in the sample analysis. For the surface waters sampled, the ions included in the %CD calculation are expected to account for 90 to 100 percent of the ions in a sample. However, in contrast to the %ID calculation, there is no term in the %CD calculation to account for protolytes not specifically determined.

Examine the data from samples that do not meet the %CD criteria for possible causes of the unacceptable %CD, such as improper data reporting or analysis. The presence or absence of unmeasured protolytes can be tested by the procedures described in Section 5. Note that the absence of unmeasured protolytes is positive evidence that the %CD exceeds the maximum difference due to analytical error. Redetermine any parameter that is identified as suspect. If an explanation for the poor %CD cannot be found and the problem cannot be corrected, contact the laboratory supervisor or QA manager for further guidance.

G.3 DATA REPORTING

Record the results from each method on the proper data form (blank NSWS data forms are included in Appendix D). Report results to the number of decimal places in the actual detection limit. However, report no more than four significant figures. Sample results from reanalyzed samples or from standard additions should be annotated. After the forms are completed, the laboratory supervisor should sign them, indicating he has reviewed the data and that the samples were analyzed in accordance with the method protocol.

G-4 References

Kramer, J. R., 1982. Alkalinity and Acidity. In R. A. Minear, L. H. Keith (eds.), Water Analysis. Vol. 1. Inorganic Species, Part 1. Academic Press, Orlando, Florida.

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