United States
Environmental Protection
Agency

Environmental Monitoring and Support
Laboratory
Cincinnati OH 45268

EPA-600/4-78-064
December 1978

Research and Development

McKee

Color
P-11

Automation of an Ultraviolet-Visible Spectrometer

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ENVIRONMENTAL MONITORING series. This series describes research conducted to develop new or improved methods and instrumentation for the identification and quantification of environmental pollutants at the lowest conceivably significant concentrations. It also includes studies to determine the ambient concentrations of pollutants in the environment and/or the variance of pollutants as a function of time or meteorological factors.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/4-78-064
December 1978

AUTOMATION OF AN ULTRAVIOLET-VISIBLE SPECTROMETER

by

Dennis P. Ryan
SouthWestern Ohio Regional Computer Center
University of Cincinnati
Cincinnati, Ohio 45220

Contract No. GS-05S-10458

Project Officer

William L. Budde
Physical and Chemical Methods Branch
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

DISCLAIMER

This report was reviewed by the Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid wastes.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water; and to determine the responses of aquatic organisms to water quality.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report was developed by the Advanced Instrumentation Section of the Environmental Monitoring and Support Laboratory in the interest of distribution of information to aid the advancement of laboratory techniques, and quality control through computerization.

Dwight G. Ballinger
Director
Environmental Monitoring and Support
Laboratory - Cincinnati

ABSTRACT

This report is an overview of the major features of an automated ultraviolet-visible spectrometer system.

Four functional software modules are described which include the chlorophyll analysis module, the color analysis module, the multi-option module, and the quality control module. The hardware interfacing is described in narrative fashion.

Finally, the general systems design methodology is discussed in relation to the ultraviolet-visible spectrometer in particular, and the laboratory automation effort in general.

This report is a result of work done in conjunction with the laboratory automation project, sponsored by the Environmental Monitoring and Support Laboratory of the Environmental Protection Agency. This work was accomplished over the period April, 1977 to June, 1978.

CONTENTS

ACKNOWLEDGMENTS

The author and the project officer wish to express their appreciation to a number of colleagues who provided assistance which was essential to the successful completion of this project. These colleagues included Dr. Cornelius Weber, who defined the requirements for the chlorophyll analyses methodology and provided a chlorophyll calculation and report generation program, originally written in FORTRAN IV; Larry Lobring, who assisted in the development of the color analysis module and the overall quality control requirements; Jack Teuschler, who designed the hardware interface to the Nova 840 minicomputer and provided assistance with the minicomputer hardware and software; David Fleck, who also assisted with the minicomputer hardware and software; Bruce Almich, who gave advice on the software design; and all of the laboratory personnel of EMSL who provided technical assistance during the testing phase of the project.

Appreciation is also extended to Louis Taber, Les Rigdor and George Barton of the Lawrence Livermore Laboratory for their lab automation software design efforts. Their functional designs served as a framework for some of the software developed for the UVVIS system.

Special appreciation is due to Dr. George Garland of the Dupont Company, who supplied several industrial dye samples and test results for a study using the color analysis module.

SECTION I

INTRODUCTION

The ultraviolet-visible spectrometer is a recent addition to the EPA laboratory automation system (1). In 1976, a hardware interface was designed to link a Coleman model 124 dual-beam, scanning ultraviolet-visible spectrometer to a Data General NOVA 840 minicomputer. This interface system, which resembled a two-way communication network, was designed to serve two functions. The first function of the interface was to control the scanning of the spectrometer. The system was designed so that signals sent from the computer could start and stop the scanning mechanism within the spectrometer. The second function of the interface was to transfer spectrometric signals from the instrument to the computer. Interface circuitry was designed so that two signals, representing relative values of wavelength and sample transmittance, could be sent simultaneously to the computer (See Part VI, Hardware Interfacing and System CALLs, for more information on this subject).

Once the hardware interface was constructed and installed on a suitable spectrometer, work began on the development of computer programs (software) which could make use of the automated spectrometer. The BASIC programming language was selected as the foundation for this software system because of its flexibility and versatility in an interactive laboratory environment (1). The first major software application utilizing the automated ultraviolet-visible spectrometer was the chlorophyll computation program. Tests were made in this initial phase to verify the speed and accuracy of the automated spectrometric analysis of chlorophyll samples (1). The success of these tests proved the usefulness of the automated instrument and pointed the way toward expanded software development utilizing the automated ultraviolet-visible spectrometer.

Starting in April of 1977 a major effort was undertaken to expand and unify the capabilities of the automated ultraviolet-visible spectrometric (UVVIS) system. The system was completed in June of 1978 and currently contains a number of analysis modules as well as a module to aid in controlling instrument performance (See sections II, III, IV and V of this report).

The purpose of this report is to describe, in moderate detail, the software and hardware components of the UVVIS system. However, this report is intended to do more than just introduce a new laboratory automation system. It is intended to show the great potential of developing computerized systems for the automation of even the most common and relatively inexpensive laboratory instruments.

The following overview of the UVVIS system will assist in defining conventions and processes referred to in the balance of the report. Figure 1 depicts the hardware and software components of the UVVIS system. The hardware components control scanning and facilitate the transfer of analog spectrometric signals representing wavelength and transmittance. These analog signals (voltages) are converted to digital equivalents (counts) in the analog to digital converter (A/D). These signals are then reduced to exact wavelength and transmittance values within the central processing unit (CPU) through the use of system software. It is important to note that the hardware interface presents only relative signals to the CPU for processing. It is the responsibility of the user, with the assistance of system software, to define the real-world equivalents of these signals. This is done through the process of instrument calibration which must be performed at the beginning of each computer-assisted analysis. In effect this process conditions the computer to translate digital signals to their corresponding equivalents of wavelength and transmittance. Wavelength signals are always converted to nanometer (nm) equivalents.

Transmittance values are useful in the evaluation of the color characteristics of solutions in conjunction with the International Commission on Illumination (CIE) method of color classification. In some cases, additional reductions may be required to convert transmittance (T) into absorbance (A) values. This is accomplished through the equation:

$$A = LOG_{10}(1/T)$$

Absorbance values are commonly used in the evaluation of concentration levels of certain environmental pollutants in conjunction with Beer's Law which states that, at well defined wavelengths, the analyte concentration is proportional to the measured absorbance of a sample.

The software components of the UVVIS system assist in the automated acquisition and reduction of spectrometric data for a number of analysis techniques, and the generation of formatted reports describing the outcome of each analysis. Control for the various analysis modules resides in an ultraviolet-visible spectrometric master program called UVVIS. The master program controls access to a number of program modules which facilitate the execution of the particular types of spectrometric analysis. Three analysis modules are currently available within the UVVIS package. The chlorophyll computation module assists in the determination of the concentrations of chlorophylls a, b, and c in extracts of algae and other plants. The color analysis module aids in the evaluation of the color characteristics and color levels in industrial waste water. The multi-option module assists in determining concentration levels of a number of environmental pollutants currently found in wastewater samples.

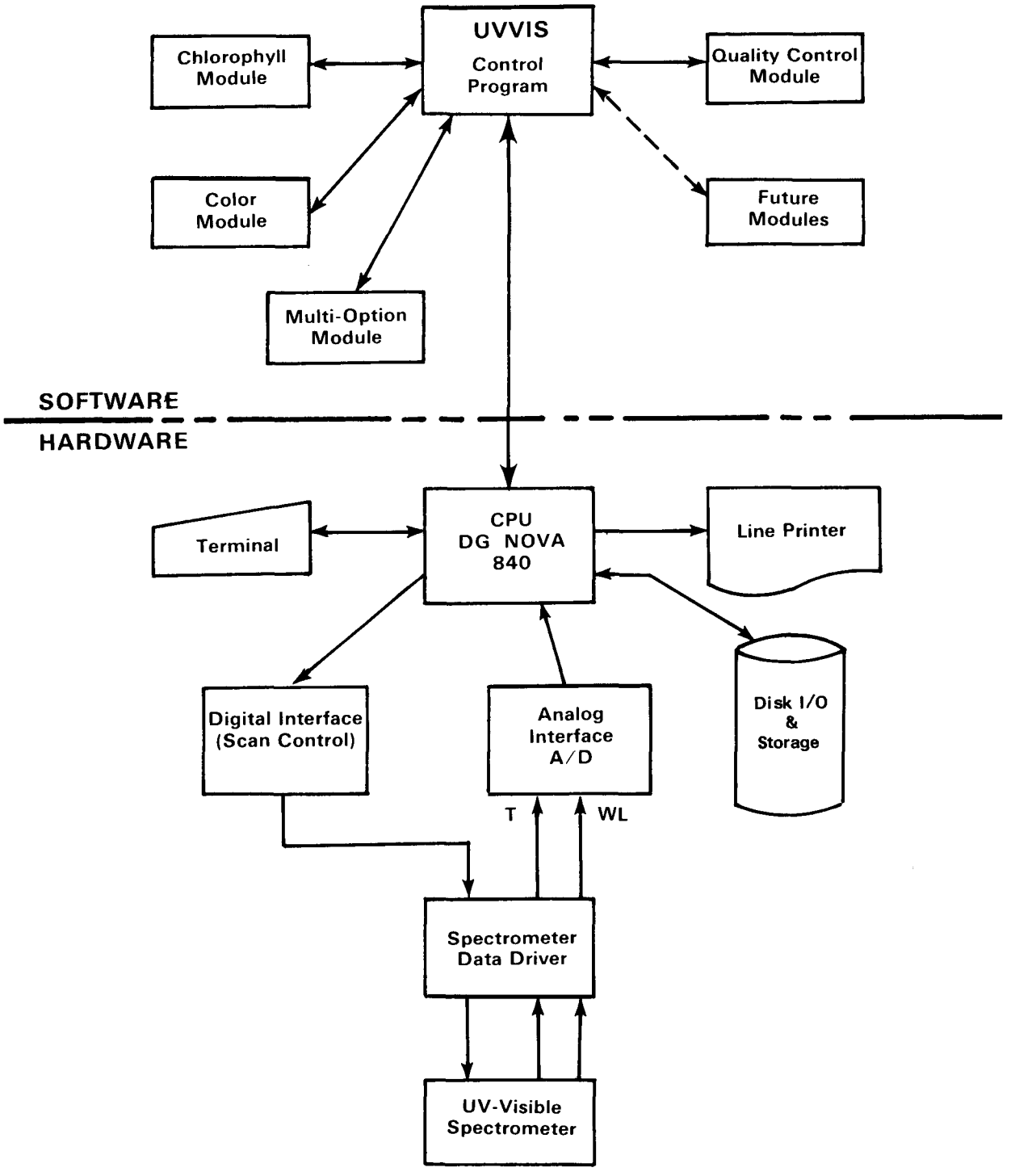

Figure 1. The hardware and software components of the Automated Ultraviolet-Visible Spectrometric System.

The UVVIS master program contains a set-up/check-out routine which directs the start-up procedures for any automated analysis, and which allows for on-line signal monitoring of the automated instrument.

Finally, the UVVIS package contains a quality control module. This very important component of the UVVIS system allows for a detailed analysis of spectrometric precision and accuracy using certified filters available from the National Bureau of Standards (NBS).

In time, other modules may be designed for inclusion into the UVVIS system, especially in the realm of ultraviolet analysis. This, however, does not preclude the fact that the automated ultraviolet-visible spectrometer is presently a valuable part of the EPA laboratory automation system.

SECTION II

CHLOROPHYLL ANALYSIS MODULE

The chlorophyll analysis module of the UVVIS software package facilitates the determination of the chlorophyll concentrations in environmental samples utilizing the spectrometric method (2,3).

The chlorophyll is extracted from algae or other plant materials. The absorbance of the extract is measured between 620 and 760 nm, and the data are reduced using trichromatic equations. In this way, sample concentrations of chlorphyll a, b, and c are determined quickly and accurately. Chlorophyll a is considered a useful index of algal standing crop. Measurable amounts of pheophytin a introduce interference errors in the evaluation of chlorophyll a concentrations. To correct for pheophytin a the sample is acidified and the absorbance is remeasured. The new peak absorbance and the previous peak absorbance are used in the monochromatic equations to calculate the concentration of pheophytin a and a "corrected" concentration of chlorophyll a described by CHL a'.

Five trichromatic computational methods have been developed in the past 25 years to determine concentrations of chlorophyll a, b, and c. Of these, only the three most recent methods are implemented in the chlorophyll analysis module. The newest method is the Jeffrey-Humphrey method (4), which is still in the development phase. The other two methods available in the chlorophyll analysis module are the UNESCO Method (2) and the Strickland-Parson method (5). Of these the most commonly used is the UNESCO method.

In the trichromatic method, concentrations of chlorophyll a, b, and c are determined utilizing the absorbance at approximately 663 (peak), 645 and 630 nm, respectively. The precise wavelengths vary with each method. The absorbance at 750 nm is subtracted from each to correct for sample turbidity. The adjusted absorbances obtained in these measurements are used in the chosen trichromatic equations to determine concentrations of CHL a, b, and c. The measurement of pheophytin a and the subsequent "correction" of the chlorophyll a concentration (CHL a') is accomplished by acidifying the sample and remeasuring the absorbance at 665 nm (peak). Once again the 750 nm measurement is used to correct for the turbidity of the acidified sample and the adjusted absorbance is used in the monochromatic equation to determine the concentration of pheophytin a and "corrected" chlorophyll a.

Table 1 shows the precise wavelengths at which absorbances are required for each method. In Table 1, the B indicates measurements taken before sample acidification and the A indicates measurements taken after sample acidification.

TABLE 1. WAVELENGTHS (NM) USED IN THE CHLOROPHYLL ANALYSIS COMPUTATIONAL METHODS

Method	CHL a (peak)	CHL b	CHL c	PHEO a (peak)
UNESCO	663B	645B	630B	665A
Strickland-Parson	665B	645B	630B	665A
Jeffrey-Humphrey	664B	647B	630B	665A

In the program, absorbances are acquired at the appropriate wavelengths and are immediately adjusted for turbidity. The values are then substituted into one of the following sets of equations:

UNESCO Method

$$\text{CHL } a = F*(11.64*A_{663B}-2.16*A_{645B}+0.10*A_{630B})$$
$$\text{CHL } b = F*(-3.94*A_{663B}+20.97*A_{645B}-3.66*A_{630B})$$
$$\text{CHL } c = F*(-5.53*A_{663B}-14.81*A_{645B}+54.22*A_{630B})$$
$$\text{PHEO } a = F*(26.73*(1.7*A_{665A}-A_{663B}))$$
$$\text{CHL } a' = F*(26.73*(A_{663B}-A_{665A}))$$

Strickland-Parsons Method

$$\text{CHL } a = F*(11.64*A_{665B}-1.31*A_{645B}-0.14*A_{630B})$$
$$\text{CHL } b = F*(-4.34*A_{665B}+20.7*A_{645B}-4.42*A_{630B})$$
$$\text{CHL } c = F*(-4.64*A_{665B}-16.30*A_{645B}+55.00*A_{630B})$$
$$\text{PHEO } a = F*(26.73*(1.7*A_{665A}-A_{665B}))$$
$$\text{CHL } a' = F*(26.73*A_{665B}-A_{665A}))$$

Jeffrey-Humphrey Method

$$\text{CHL } a = F*(11.85*A_{664B}-1.54*A_{647B}-0.08*A_{630B})$$
$$\text{CHL } b = F*(-5.43*A_{664B}+21.03*A_{647B}-2.66*A_{630B})$$
$$\text{CHL } c = F*(-1.67*A_{664B}-7.60*A_{647B}+24.52*A_{630B})$$
$$\text{PHEO } a = F*(26.73*(1.7*A_{664A}-A_{664B}))$$
$$\text{CHL } a' = F*(26.73*(A_{664B}-A_{664A}))$$

F is an analysis constant defined in the following way:

$$F = \frac{\text{Extract Volume} * \text{Dilution Factor}}{\text{Cell path Length (cm)} * \text{Sample Volume} * 1000}$$

Figure 2 shows the spectra from an unacidified and acidified standard chlorophyll sample. Note that each method required a "peak" reading at a different wavelength (Table 1). This dichotomy will be examined in more detail in the following description of the automated chlorophyll computation module.

Data acquisition, data reduction and report generation are the three major segments of the chlorophyll analysis module (Figure 3). Data acquisition is accomplished through on-line measurements of chlorophyll samples using the automated spectrometer. Precision may be established by allowing the user to accumulate data on up to five replicates of the sample. Line printer plots may be generated for each sample scan showing absorbance vs. wavelength from 620 to 760 nm.

Once the absorbance data has been acquired, the computation segment reduces the data to chlorophyll concentrations using one of the computational methods discussed above. Certain ratios and indexes are also calculated in this segment.

After the data is reduced, replicate statistics are generated. These statistics include the mean, standard deviation and relative standard deviation for each set of replicate parameters. The reduced data and the replicate statistics are then output to the lineprinter in the form of a final report. Once an automated run has been completed and the output report has been generated, the user may elect to delete one "bad" replicate and regenerate updated replicate statistics in another report.

Two additional features are available to the user who is making automated sample scans. The first of these is a simplified NBS filter test to determine the relative accuracy of the instrument, and the second feature is an optional peak search routine for determining the wavelength location of the CHL a peak absorbance.

The relative accuracy of the automated spectrometer may be checked through a specialized NBS filter test option. This test simply compares the absorbance values obtained from a scan of an NBS linearity filter (SRM930b, Filters 1-282,2-282 and 3-282) with those obtained from another "more accurate" instrument such as the Beckman ACTA-V or the Cary 14 spectrometer. The wavelengths sampled in this test correspond to those used in the particular computational method (663, 645, 630 and 750 nm), and as yet absorbances at these wavelengths have not been certified by the National Bureau of Standards for these filters. (See Section IV, Quality Control Module for more information on the evaluation of spectrometric precision and accuracy using NBS filters.)

It was previously noted that the three computation methods described above differ with respect to the exact location of the sample peak.

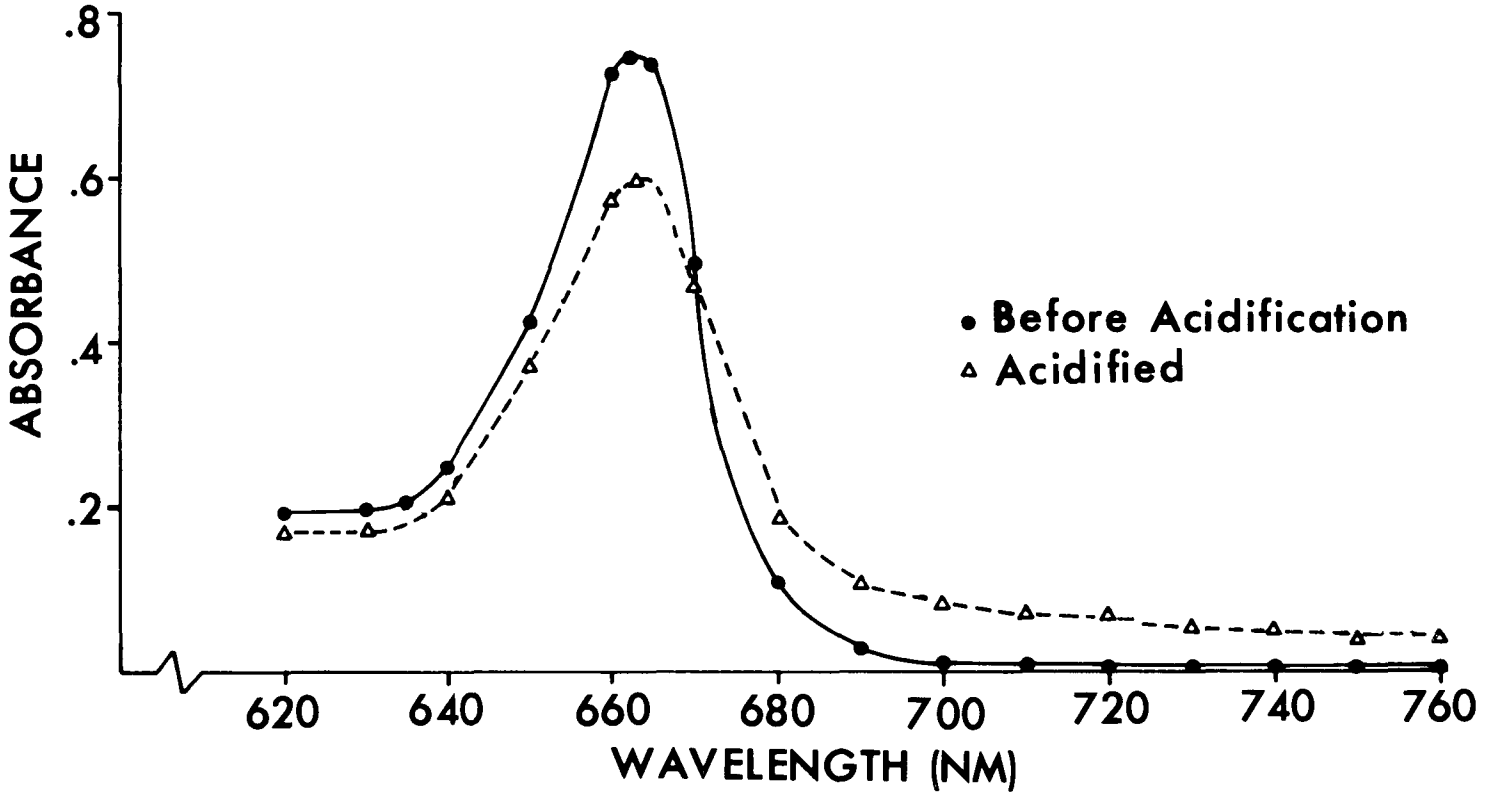

Figure 2. Visible spectra from unacidified and acidified standard chlorophyll sample.

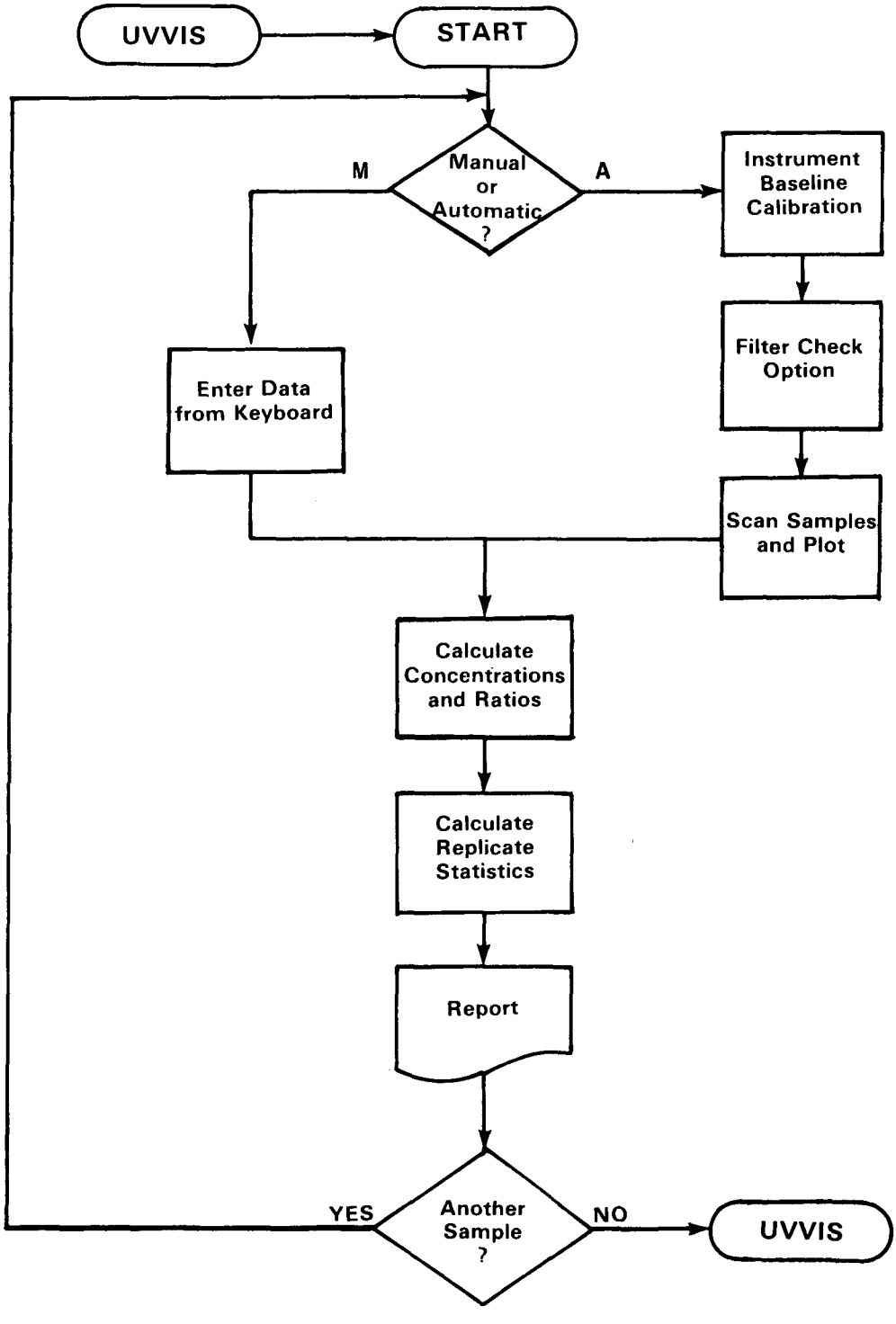

Figure 3. Flow chart of the chlorophyll analysis module.

Normally the errors introduced by this deviation are minor compared to other method errors; however, a peak search routine has been installed in the automated segment of the module in order to systematically verify this hypothesis. A peak search is made on every automated sample scan and the user is supplied with the "true" sample peak as seen by the automated spectrometer. At the user's discretion, this peak absorbance can be used in place of the peak defined by the particular computation method. For example, if a scan is made on a chlorophyll sample and the peak occurs at 662 nm, the user may elect to retain the absorbance at 662 nm in place of the method-defined peak found at 663, 664 or 665 nm. Presently this feature is considered a research tool and caution should be exercised in order to avoid inconsistencies inherent in this type of option.

The discussion above has dealt only with measurements obtained from an automated spectrometer; however, it is likely that chlorophyll samples will sometimes be measured using an unautomated instrument, and strip chart recordings will be generated from these scans. The chlorophyll analysis module is able to accept data from these charts through the manual mode. The analyst must type in the absorbances recorded at the appropriate wavelengths, and when data entry is complete, the computer will calculate and display the results in the same manner as described above for the automated run.

The chlorophyll analysis module is a fast, flexible, and accurate tool for the evaluation of chlorophyll concentrations in environmental samples. As computational methods are perfected and algae families are characterized in terms of chlorophyll a, b, and c, the chlorophyll analysis module will assist in quantifying ocean food supplies and monitoring the ecosystems which facilitate the growth of these algae.

SECTION III

COLOR ANALYSIS MODULE

The color analysis module of the UVVIS software package incorporates newly developed techniques for the evaluation of color levels in environmental samples. For this reason a more descriptive discussion will be presented on the color analysis module.

The American Public Health Association (APHA) color method has, in the past, been adequate for the evaluation of yellow color levels in river and stream waters. The method required the preparation of a number of platimum cobalt standard solutions with known APHA colorimetric units. An unknown sample was then visually located between two of these standards. In this way an approximate APHA number could be assigned to the unknown. This method was not only inexact but it was also unusable on industrial wastes which had colors significantly different from the yellow of the platinum cobalt standards. The American Dye Manufacturers Institute (ADMI), through its Ecology Analytical subcommittee, developed an alternative method for measuring color levels in water (6). Their goal was to devise a method that would meet four criteria:

1. The method should be applicable to any color (hue).

2. The method should be sensitive to small color differences.

3. The method results should be related to APHA colormetric units (platimum cobalt standards).

4. The method should require relatively inexpensive instrumentation.

The method devised by the subcommittee will be referred to as the ADMI method for the determination of color in water. The following is a brief summary of the evolution of this method.

The committee first assumed that the color of water has a negative impact on the environment only in an esthetic sense. For this reason, it was decided to determine color in water as a strictly visual perception.The International Commission on Illumination (CIE) system of specifying color based on tristimulus values X, Y, and Z has had wide acceptance in relating physical measurements to the stimulus perceived by the normal observer, and can be used to specify the color of environmental samples in terms of dominant wave length (hue), purity, and luminosity (7).

Tristimulus values are hue dependent, however, and cannot be used directly to determine the uniform color difference between the color of a solution and that of a colorless solution. By transforming the tristimulus values to Munsell coordinates (8), a uniform color difference equation can be used to produce a single number which represents the vector length between the color of the sample and colorless water.

One of the simpler equations used to evaluate this color difference is that known as the Adams-Nickerson equation (9) which combines the chromatic value transformation of the CIE chromaticity diagram proposed by Adams with the lightness or luminosity modification proposed by Nickerson. This single number color difference is known as Delta-E or the change in sensitivity from the colorless state.

All non-phosphorescent solutions regardless of hue can be labeled with this Delta-E (or DE) number. The DE number represents the uniform color difference from colorless. By measuring platinum-cobalt standard solutions and calculating DE by the Adams-Nickerson formula, a calibration curve relating DE to standard ADMI values can be used to obtain accurate ADMI values to describe color level in water of any hue. A plot of measured values of DE as a function of ADMI values for six APHA standards is shown in Figure 4. Any colored solution whose DE number has been calculated can be related to the standards curve, and an ADMI number can be assigned to the solution. Table 2 shows the method for preparation of the standards, the definitions of ADMI values, and the measured DE values used in Figure 4.

The ADMI method for the determination of color in water described above can be divided into five parts.

1. Measurement of the visible spectrum of the sample on suitable instrument.

2. Calculation of CIE tristimulus values X, Y, and Z.

3. Conversion of tristimulus X, Y, and Z to Munsell coordinates Vx, Vy, and Vz.

4. Calculation of Adams-Nickerson color difference (DE).

5. Conversion of DE to ADMI values using standards curve.

By Federal law section 304g, PL 92-500, the EPA was required to develop a procedure for the measurement of color in water. The ADMI method described above was adopted as a means to measure color levels in wastewater (10). However, the steps involved in the ADMI method have traditionally involved complicated and lengthy measurement and computation. To solve this problem the color analysis module was developed for the UVVIS system. The module was designed to make repetative measurements, hand calculations, and table referencing unnecessary.

RECORDS SHELF LIST

FROM: Bill Potter (Name) DATE: 1 Mar 99

Room 567 (Building/Room Number) EPA - NERL (Bureau or Office)

(Telephone Extension) MCEARD (Division)

CONTAINER NO.	DESCRIPTION & DATE (Please list each folder)	ESTIMATED DISPOSAL DATE/NARA SCHEDULE NUMBER
	EPA-440/9-75-010 Minimal Requirements For WQ/Assurance Program	
	EPA/600/F-98/007 Research - Ecological Exposure	
	EPA/600/R-92/005 Arsenic & Mercury Work Shop	
	_____ Field Screening Methods 1988	
	EPA/600/R-93/093 Environmental Monitoring Issues; Intergovernment Task Force - Ambient WQ. Monitoring	

EPA-660/3-74-024	Conceptual Model . Reticide
EPA/600/4-87/014	Natl Surface Water Survey
EPA/600/2-79-210i	Status Assessment of Toxic Chemicals
EPA-600/4-81-056	Total Organic Halide
WFPC - Dept Health HEW	Dissolved Oxygen
EPA Jo Vol 12- NO7 1986	Your Drinking Wata
EPA 16010 DXU 11/71	The Carbon Dioxide System
EPA 16070 DCD 09/70	Oxygen Uptake Demand
EPA 600/7-77-017	Nitrate Interference in Total Kjeldahl
EPA 600/4-77-035	Study - Chemical Oxygen Demand
EPA-600/2-74-079	Ion Selective Membrane -
EPA 600/4-78-015	Evaluation of Technicon Digestor...
FWPCA - Apr 1969	Study I - orthophosphate Methods
" Jun 1969	" - Mineral - methods
" 1970	Nutrient Analyses

TABLE 2. ADMI VS. DE FOR PLATINUM COBALT STANDARD SOLUTIONS

Milliliters of Standard Solution Diluted to 100 ml with distilled water[1]	Color in ADMI Units	Measured DE Values[2]	Standard Deviation
5.0	25	0.090	0.003
10.0	50	0.180	0.004
20.0	100	0.342	0.006
30.0	150	0.512	0.004
40.0	200	0.666	0.006
50.0	250	0.810	0.008

1 Preparation of Standard Solution:

Dissolve 1.246g of potassium chloroplatinate, K_2PtCl_4 (equivalent to 500 mg metalic platinum) and 1.00g crystallized cobaltous chloride, $COCl_2\ 6H_2O$ (equivalent to about 250 mg metalic Cobalt) in distilled water with 100 ml concentrated hydrochloric acid and dilute to 1000 ml with distilled water. This stock standard has a color of 500 ADMI units.

2 Represents ten non-consecutive replicates of each standard.

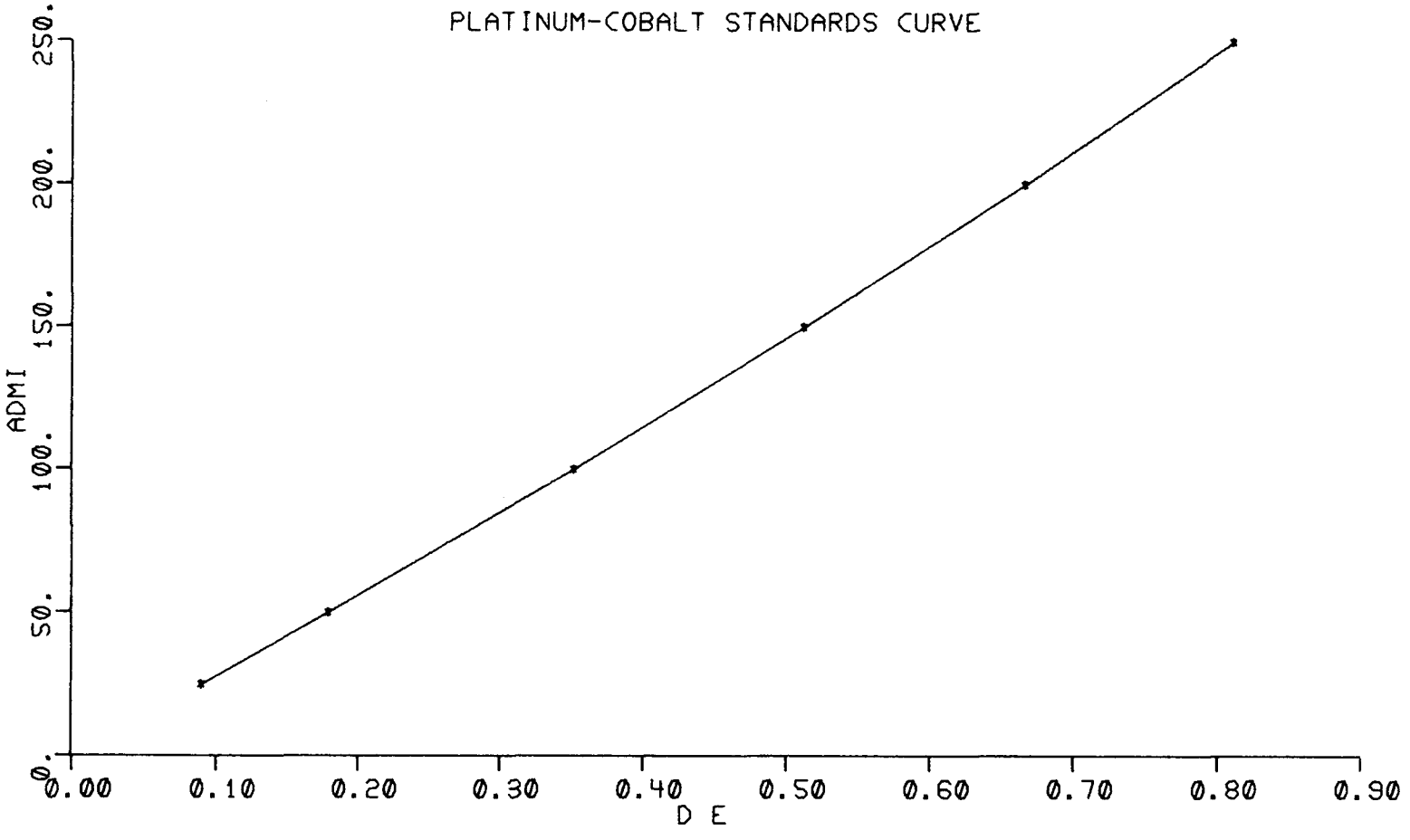

Figure 4. A plot of ADMI values for six platinum-cobalt standard solutions as a function of measured DE values.

The five steps of the ADMI method are stated in terms of this automated procedure as follows:

1. A dual-beam scanning spectrometer was selected and interfaced to a Data General NOVA 840 mini-computer. The BASIC computer language was chosen to allow for maximum computer-operator interaction.

2. The module was designed to make a spectrometric scan of a sample between 400 and 700 nanometers, acquire and store transmittance measurements through the range, and reduce these values to the tristimulus values X, Y, and Z (7).

3. A simple iterative algorithm was used to convert the tristimulus values X, Y, and Z to Munsell values Vx, Vy and Vz (8).

4. The DE value could then be calculated from the following Adams-Nickerson equation:

$$DE = \left\{(0.23 * \Delta Vy)^2 + [\Delta(Vx - Vy)]^2 + [0.4 * \Delta(Vy - Vz)]^2\right\}^{1/2}$$

   Where Vx, Vy, and Vz are the Munsell value equivalents of tristimulus values X, Y, and Z respectively, and where Delta-V is the difference between the Munsell value of a sample (Vxs, Vys, and Vzs) and the Munsell value of a colorless solution (Vxc, Vyc and Vzc)(9).

5. The ADMI value of the sample could then be determined from a calibration equation of ADMI as a function of DE for the standard solutions. In the computer program this was accomplished by fitting the standard points to a second degree equation using the least squares regression technique. In this way the relative ADMI value of any sample could then be obtained as a function of DE (Figure 4).

Because of the excellent reproducibility obtained in repetative measurements of the platinum cobalt standards, it was decided that the entire set of standards need not be run each time samples are analyzed. Instead the user is required to run at least one standard, commonly referred to as a check standard, to verify the validity of the calibration curve. If the outcome of this measurement is within statistical bounds, the user may begin measuring color levels in environmental samples. However, if the check standard values are repeatedly unacceptable, the user may update the standard equation.

Figure 5 shows an overall flow chart for the color analysis module.

The Color Analysis Module serves as a versatile research tool for the evaluation of the color characteristics of virtually any uniform-nonopaque substance or solution, while still fulfilling the legal requirements for determining the ADMI color levels in environmental samples.

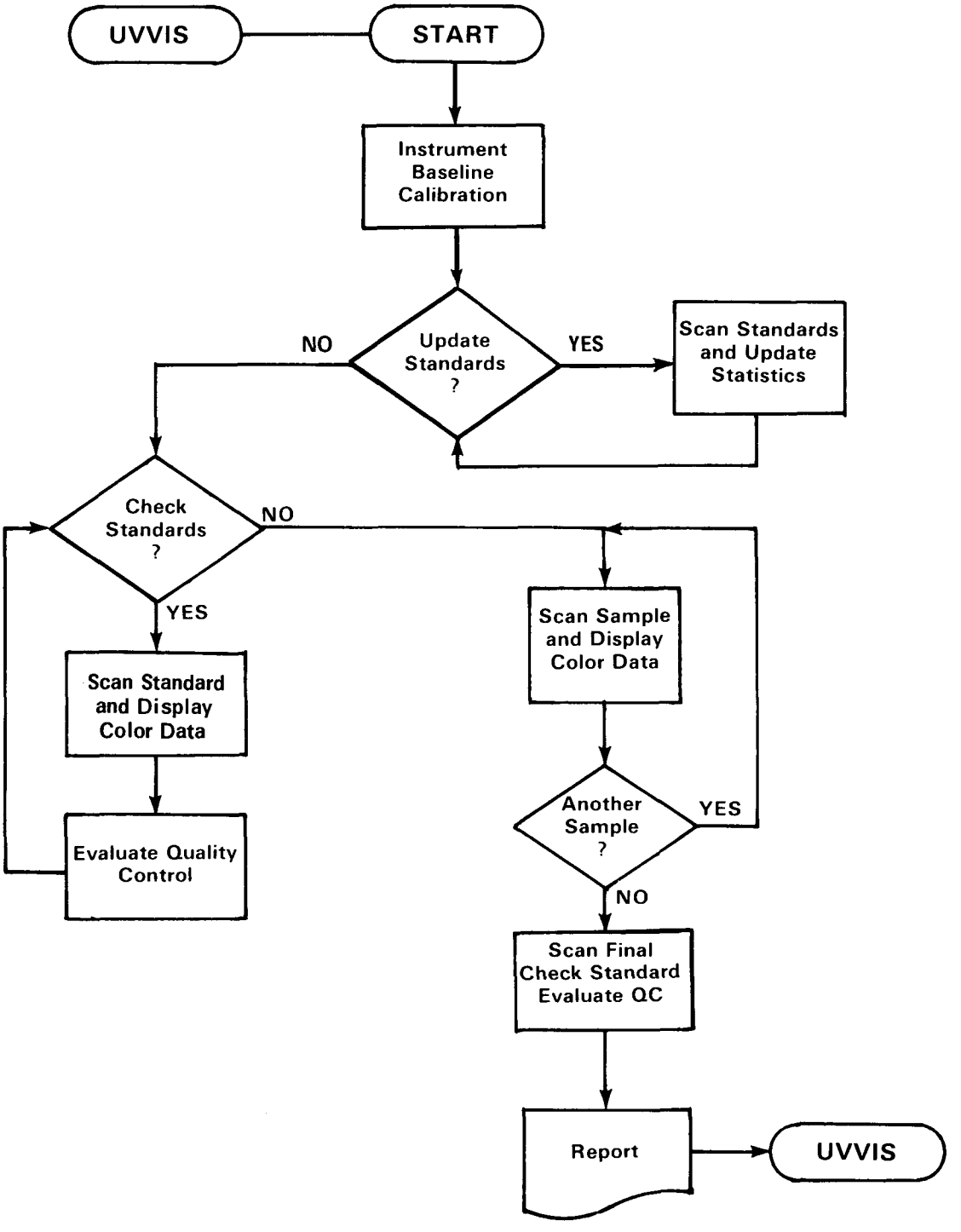

Figure 5. Flowchart of the Color Analysis Module.

SECTION IV

MULTI-OPTION ANALYSIS OF ENVIRONMENTAL POLLUTANTS

As was stated in the introduction of this report, there are many compounds, ions, and elements that exhibit spectrometric properties in compliance with Beer's law. In these cases, the concentration of an analyte is proportional to the absorbance of the sample measured at a specific wavelength. Table 3 shows the list of analytes, methods, units of measurement and wavelengths for several methods currently approved for EPA monitoring programs (10). The module may also be used to evaluate newly proposed methods for determining pollutant concentration levels in environmental samples.

The multi-option analysis module of the UVVIS system was developed as a general purpose approach to this type of spectrometric measurement. In all instances where this methodology is used, it is necessary to measure the absorbances (at the specified wavelength) of standards with known concentrations before an analysis of environmental samples with unknown concentrations can be made. This process entails the development of a standards calibration equation relating the absorbance of the standard to its concentration. This is done by calculating coefficients for the first and second order equations using the least squares regression technique. Once an accurate calibration curve has been developed, unknown sample absorbances are measured, and these absorbances are substituted into the standard calibration equation in order to determine the analyte concentration in the unknown sample.

The multi-option module normally facilitates data acquisition through an automated mode. Absorbance data may be input manually through keyboard entry in cases where standards and samples were measured using a non-automated instrument. This facility speeds up data reduction, and produces a formal report describing the outcome of the analysis.

In the on-line or automated mode, data is collected directly from the spectrometer. The user is responsible for processing and labeling standards, unknowns, and quality control samples including spiked samples, check standards, and duplicate unknowns. The user inputs various run parameters, sets the appropriate wavelength, and then loads standards with known concentrations. The computer records each standard absorbance. Standard calibration curves are created using the least squares regression technique. The program displays tables showing the results of this calibration. The multi-option module allows for a great deal of

flexibility in manipulating standards. After standard values have been input in the initialization segment of the run, standards can be loaded and readings can be taken in any order. Divergent points can be eliminated and calibration equations can be updated. Internally generated error commands, warnings, and suggestions aid the operator in developing a standards equation in compliance with analysis specifications. With this kind of flexibility, the effective concentration range for a given test can be determined easily, and the least squares fit parameters can be calculated to optimize measurement accuracy.

Samples of unknown concentration are then loaded into the spectrometer, and the absorbances measured. By utilizing the calibration equation, unknown concentrations are determined. Quality control samples can be measured during the samples run to document the accuracy and precision of the unknown measurement. As many as ten replicate measurements can be made on any standard or sample to evaluate precision and assure measurement reliability.

Interim reports are displayed during the course of a run, and consist of: equations obtained by least squares fit of standards data; standard calibration tables; concentrations of samples; concentrations and statistical data for spiked samples, check standards, and unknown duplicates; and other miscellaneous messages.

Hard copy reports may be obtained during the course of a run or at the end of a run on the line printer.

The multi-option analysis module performs each of the functions described above through the execution of user-supplied instructions. The module is therefore termed a command structured system. Since flexibility is the key to a command structured system, the internal processes do not exhibit a uniform flow. Because of this, Table 4 is presented to describe the numerous commands available in the multi-option module.

The multi-option analysis module is a very flexible and highly adaptable element of the UVVIS package and as such will serve the future as well as the present EPA analytical monitoring requirements.

TABLE 3. MULTI-OPTION ANALYSIS TECHNIQUES CURRENTLY AVAILABLE USING THE UVVIS SYSTEM (10)

Analyte	Method	Units	Wavelength
Kjeldahl Nitrogen	Nesslerization	Miligrams/liter	425 NM
Ammonia	Nesslerization	Miligram/liter	425 NM
Nitrate	Brucine	Miligrams/liter	410 NM
Nitrate-Nitrite	Cadmium Reduction	Miligrams/liter	540 NM
Phosphorus	Total Phosphorus	Miligrams/liter	650 NM
Phosphorus	Ortho-Phosphorus	Miligrams/liter	650 NM
Phosphorus	Hydrolyzable Phosphorus	Miligrams/liter	650 NM
Arsenic	Silver Diethyldithiocarbamate	Micrograms/liter	535 NM
Chlorine	DPD Colorimetric	Miligrams/liter	515 NM
Cyanide	Pyridine-Pyrazolone	Miligrams/liter	620 NM
Cyanide	Pyridine-Barbituric Acid	Miligrams/liter	578 NM
Sulfide	Methylene Blue Photometric	Miligrams/liter	625 NM
Fluride	SPADNS	Miligrams/liter	570 NM
Silica	Molybdosilicate	Miligrams/liter	410 NM
Phenol	4AAP Direct Photometric	Micrograms/liter	510 NM
Phenol	4AAP $CHCL_3$ Extraction	Micrograms/liter	460 NM
C O D	Colorimetric	Milligrams/liter	600 NM

TABLE 4. SAMPLE TYPES AND COMMAND SUMMARY FOR THE MULTI-OPTION ANALYSIS MODULE

Sample Type ($$)	Sample Description
S	Standard in Calibration Curve
U	Unknown Sample
RB	Reagent Blank (Quality Control)
CS	Check Standard (Quality Control)
DU	Unknown Duplicate (Quality Control)
SP	Spiked Sample (Quality Control)

Command	Description
$$x/y	Measure $$ number x for the y^{th} time.
C	Develop calibration equations for current standards.
B	Measure instrument blank.
$$x/A	Show concentration of $$ number x using a 1^{st} degree equation.
$$x/B	Show concentration of $$ number x using a 2^{nd} degree equation.
$$x/I	Show concentration of $$ number x by interpolating between two bracketting standards.
$$x/yD	Delete replicate y of $$ number x.
$$x/E	Erase all replicates of $$ number x.
$$x/L	Look at current replicate data on $$ number x.
R	Report on completed run results.

SECTION V

QUALITY CONTROL MODULE

Clearly from the previous sections, the automated ultraviolet-visible spectrometer can be used in a variety of ways. These include broad spectrum measurements as used in the chlorophyll application, static transmittance/absorbance measurements as used in the multi-option module, and tristimulus integrator measurements for evaluating the color characteristics of samples as used in the color analysis module.

In order to verify the precision and accuracy of the automated instrument, a quality control module has been developed for the UVVIS software package. The module allows for two types of filter tests using National Bureau of Standards (NBS) certified filters (11). These tests facilitate the evaluation of the automated spectrometer as a color tristimulus integrator system or as a transmittance/absorbance measuring device. Each type of test is made by scanning the appropriate NBS glass filter, acquiring data on the scan, reducing the data, and comparing the results with certified NBS standard values. An additional feature allows for the comparison of the test results with historical test results obtained on the same instrument.

The filter test to evaluate the performance of the automated spectrometer as a color tristimulus integrator system uses NBS filters 2101, 2102, 2103, 2104 and 2105 (12). Each filter is a 2-inch square of transparent colored glass. A chart of tristimulus values for CIE source C, representing average day light, is furnished with each set of glasses, and these values are certified by the National Bureau of Standards. Each filter exhibits unique color characteristics as shown in Figure 7. Table 5 shows the NBS certified tristimulus values for each filter, along with the mean and standard deviation acquired with eight non-consecutive replicates of each filter made on the prototype UVVIS system.

After measurements are made on a particular tristimulus filter from 400 to 700 nm, the computer automatically calculates the tristimulus and trichromatic values using the thirty ordinate CIE spectrometric method (7). No internal corrections are made for back-reflectance, slit-width or inertial errors, but the accuracy and precision are very good, especially for filter 2105 which exhibits color characteristics likely to be found in environmental samples.

The filter test to evaluate the performance of the automated spectrometer as a transmittance/absorbance measuring device uses NBS linearity filters 1-282, 2-282 and 3-282 (SRM 930b). Each of these neutral

glass filters is mounted in a standard 1 centimeter holder and exhibits transmittance of approximately 10, 20, and 30 percent over the scanning range. Each filter is individually calibrated and certified by the National Bureau of Standards for absorbance and transmittance values at wavelengths of 440, 465, 564.1, 590 and 635 nanometers. Table 6 shows the NBS certified transmittance values for each filter (in %T) along with the mean and standard deviaton acquired with eight nonconsecutive replicates of each filter made on the prototype UVVIS system.

Note that the standard deviation remains relatively constant as the transmittance (%T) increases. This implies that for environmental samples, which normally exhibit much higher transmittance values, the precision will improve proportionally. Another point worth noting is that the test on these filters is dynamic since the filter is scanned over the wavelength region. The accuracy improves under static conditions where the wavelength is set before a measurement is taken.

A typical quality control run using the NBS filters can be broken down into three parts: spectrometric baseline calibration, filter scans including calculations and interim reports on each filter, and the summary output report including precision and accuracy evaluations on all filter scans. Test filters are run in any order, and a single filter can be run up to 10 times if desired. Test results on each filter will be displayed after each filter has been scanned, and, at the operator's discretion, a more detailed report can be generated on the lineprinter, including a spectral plot of the filter. Once the desired filters have been run, a summary report is output to the lineprinter detailing the accuracy of the tests relative to NBS standards and the precision of the test relative to historical statistics maintained in the system.

In general, the quality control module of the UVVIS software package can be used to rigorously test the precision and accuracy of the automated spectrometer under extreme conditions. This points the way to the use of the system as a tristimulus integrator and transmittance/absorbance measuring device for environmental samples which exhibit more normal color characteristics.

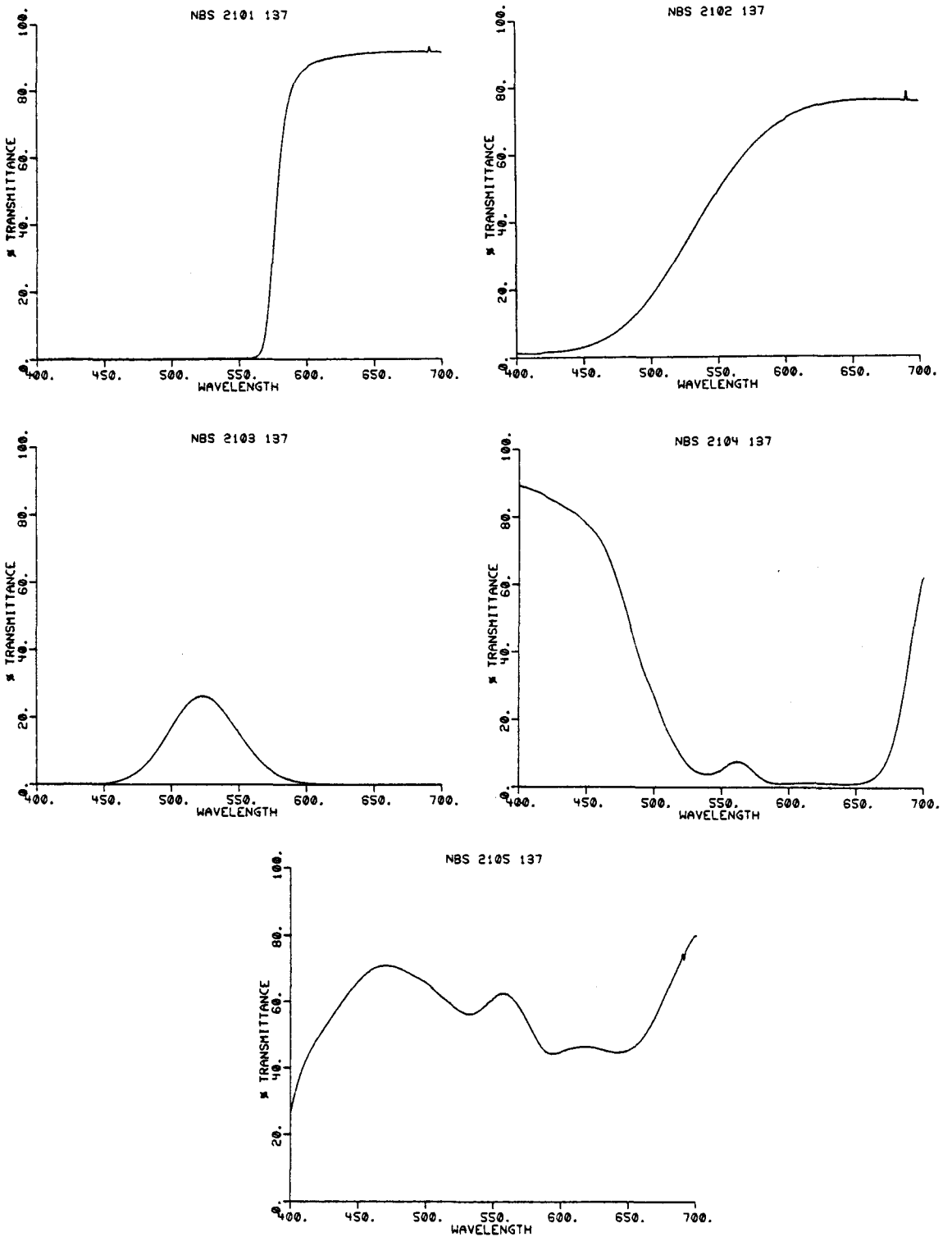

Figure 7. Color characteristics for five National Bureau of Standards Tristimulus Filters.

TABLE 5 - CERTIFIED AND MEASURED TRISTIMULUS VALUES FOR FIVE NATIONAL BUREAU OF STANDARDS FILTERS

Filter	X	Y	Z
2101			
NBS value	45.0	25.3	0.0
Measured mean	46.83	26.73	0.20
SD	0.57	0.48	0.13
2102			
NBS value	51.5	48.9	5.6
Measured mean	52.16	49.50	5.98
SD	0.21	0.27	0.14
2103			
NBS value	3.6	11.3	2.6
Measured mean	3.38	10.90	2.84
SD	0.17	0.19	0.13
2104			
NBS value	17.2	9.1	84.3
Measured mean	17.08	9.05	83.34
SD	0.18	0.21	0.34
2105			
NBS value	51.8	56.1	75.4
Measured mean	51.75	56.00	75.43
SD	0.05	0.11	0.28

TABLE 6. NBS CERTIFIED TRANSMITTANCE VALUES FOR THREE LINEARITY FILTERS

Filter	%T at Wavelength (NM)				
	440.0	465.0	546.1	590.0	635.0
1-282					
NBS Value	9.15	10.86	9.78	8.68	9.70
Measured Mean	9.26	10.85	9.89	8.81	9.82
SD	0.15	0.14	0.18	0.17	0.16
2-282					
NBS Value	18.90	21.26	19.78	18.22	19.67
Measured Mean	18.83	21.01	19.73	18.24	19.69
SD	0.13	0.12	0.16	0.17	0.16
3-282					
NBS Value	29.16	32.27	30.73	27.71	27.83
Measured Mean	29.33	32.25	30.93	27.97	28.07
SD	0.12	0.11	0.16	0.17	0.17

SECTION VI

HARDWARE INTERFACING AND SYSTEM CALLS IN THE UVVIS SYSTEM

The major hardware components of the automated ultrviolet-visible spectrometric system include the following items: the Data General Nova 840 mini-computer, fixed and moving head disks, medium or high speed line-printer, hard copy or CRT-display computer terminal, digital and analog interfaces, spectrometer remote interface box, and the scanning ultraviolet-visible spectrometer.(Figure 1) The computer terminal serves as the user-computer interface which allows the analyst to access the UVVIS software package for spectrometric analysis. After the analyst chooses a particular option, the CPU loads the appropriate module from disk to core memory. The chosen BASIC program then questions and directs the operator through well defined steps in order to complete the analysis. In cases when data are acquired from the on-line spectrometer, a BASIC CALL is used to access assembler language routines which control the start/stop scanning mechanism, and which acquire wavelength and transmittance signals from the spectrometer. Scanning is controlled through the digital interface, and data acquisition is controlled in the analog interface. Once the analog signals representing wavelength and transmittance are acquired by the analog interface, they are converted to relative digital counts in the analog to digital converter. These digital signals are then manipulated by system software to give values of wavelength and transmittance for use in software data reduction routines. As data is reduced, it is stored in disk data files and is retrieved as output for lineprinter reports during the course of the analysis.

Two BASIC data acquisition CALLs are used in the UVVIS system (13). CALL 6 is used in static measurement applications such as the multi-option module. Static measurements do not require a spectrometric scan. Instead, the operator sets the wavelength on the spectrometer and the computer repeatedly measures the transmittance signal of the sample at predefined time intervals (2/15 sec). These signals are then averaged and presented as a single signal to the system software. CALL 6 parameters are shown as follows:

```
CALL 6, C, N, A
```

where C is the single A/D channel number for the spectrometric transmittance signal, N is the number of points to be averaged and A is the returned average of the converted digital transmittance signals.

CALL 7 is used in dynamic measurement applications such as the chlorophyll computation module and the color analysis module. In the

dyanmic mode, the operator sets the wavelength at which the scan is to begin and the computer starts the scan, collects the wavelength and transmittance signals at specified intervals, converts the signals to digital values and stores them in a sequential software array. CALL 7 parameters are shown as follows:

CALL 7, C, D1, D2, N, V(1)

where C is the composite A/D channel number for scan control, wavelength, and transmittance channels of the spectrometer, D1 is the delay time (in 1/60th second intervals) between when the CALL is made and when scanning and data acquisition begin (this parameter may be 0), D2 is the delay time (in 1/60th second intervals) between data point pairs, N is the total number of points to be taken, and V(1) is the first element of the array used to store the converted digital signals.

CALL 6 is a rudimentary single channel CALL and, as such, requires no further explanation. CALL 7, on the other hand, involves a much more complicated interplay of hardware components which require a more detailed description. To do this, the hardware system used to acquire wavelength and transmittance signals must be examined in more detail. Then we will show how these signals are passed to the computer, converted to digital values, and stored in the software array.

Remember that CALL 7 is used in the scan mode. This implies that at certain discrete intervals, a pair of signals representing transmittance and wavelength is acquired at the spectrometer by the computer and translated to digital equivalents in the A/D converter. The wavelength signal is supplied by a ten turn (.005%) potentiometer which is permanently attached to the shaft of the scanning motor. Compensation for potentiometer non-linearity is made through a piecewise linear transformation within the systems software. Transmittance signals are taken directly from the spectrometer output electronics. The transmittance voltage may range from about 1 volt for opaque solids to about 9 volts for air. Interim voltage levels are nearly proportional to the transmittance of substances within this range. Certain instrument idiosyncrasies which interfere with transmittance signal linearity have been compensated for in the system software. The wavelength and transmittance signals are continuously available as long as the instrument is turned on.

When CALL 7 is invoked and scanning begins, the computer begins to acquire signal pairs - one voltage level representing wavelength and one voltage level representing transmittance at that wavelength. The transfer of these voltages to the A/D converter occurs simultaneously. They are converted to 14 bit digital equivalents and stored in the software array. Points continue to be taken in discrete intervals until all of the signal pairs required by the CALL (N/2) have been acquired. System software then utilizes internally stored reference values to reduce the digital array values to the actual wavelength and transmittance values. These wavelength/transmittance pairs represent the spectral response of the sample throughout the scanning range.

SECTION VII

CONCLUSION

This report was intended to succinctly describe the UVVIS system without being overly simplistic. No progress will ever be made in lab automation if system developers sacrifice user awareness for the sake of expediency. On the other hand an attempt has been made to be descriptive without burdening the reader with minute technical details. Let there be no doubt that any automated system abounds with technical complexities at all levels. With the right balance of order and detail, a foundation can be built through which the user can understand even the most sundry elements of a system. New patterns of communication arising from a systematic approach to lab automation will benefit both the user community and the systems developer. On the one hand, the designers will be pressed by the user to explain rather than complicate. Conversely, the users will be asked to organize and solidify their thinking rather than to postulate nebulous needs.

The UVVIS system has been shown to be a potentially powerful tool in the lab automation arsenal, yet its primary value may reside in its form rather than in its content. Because of advances in lab methodology and because of an expansion in lab instrument complexity, it is necessary to build automated instrument systems with qualities of multi-functionality.

The UVVIS system is a simple example of this approach. The main elements of the system are viewed as modules, and these modules are broken down into easily understandable components. In the UVVIS system the software modules are also reduced to program segments which perform individual tasks. More complicated systems require more levels of task differentiation. It is the duty of systems designers and the user community to work together to structure understandable segments within each system level. Certainly the current gap which exists between the user community and the systems designers will not close overnight, but a common adherence to a general system design methodology may do much to reduce misunderstanding and encourage productive communication.

SECTION VIII

REFERENCES

1. W.L. Budde, B.P. Almich, and J.M. Teuschler, The Status of the EPA Laboratory Automation Project, EPA report 600/4-77-025, April 1977.

2. C.I. Weber (Editor), Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents, EPA report 670/4-73-001, July 1973.

3. C.I. Weber, et. al., "The Status of Methods for the Analysis of Chlorophyll in Periphyton and Plankton", U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio (in preparation).

4. S. W. Jeffrey and G. F. Humphrey, Biochem. Physiol. Plazen (BPP), 167, pp. 191-194 (1975).

5. T.R. Parsons and J.D.H. Strickland, J. Mar. Res. 21, pp. 155-163 (1963).

6. W. Allen, et. al., Proceedings of the 28th Industrial Waste Conference, Purdue University, 28, pp. 661-675 (1973).

7. "Standard Methods for the Examination of Water and Waste Water", 14th Edition, APHA, Washington, D.C. (1976).

8. W.C. Rheinbolt, J. P. Menard, J. Opt. Soc. Amer. 50 80Z (1960).

9. K. McLaren, J. Soc. Dyers Colour. 86, 354 (1970).

10. Methods for Chemical Analysis of Water and Wastes, EPA Report 625/6-74-003, 1974.

11. Nat. Bur. Stand. (U.S.), Spec. Publ. 260-1975-76, catalog, p. 55 (June 1975).

12. H. J. Keegan, J. C. Schleter, D. P. Judd, J. Res. Nat. Bur. Stand., A., 66A 203 (1962).

13. L. Taber, et. al., Real Time Code for Laboratory Automation, Lawrence Livermore Laboratories, UCRL-52, 392 (1978).

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/4-78-064		

4. TITLE AND SUBTITLE	5. REPORT DATE
Automation of an Ultraviolet-Visible Spectrometer	December 1978
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Dennis P. Ryan	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Southwestern Ohio Regional Computer Center University of Cincinnati Cincinnati, Ohio 45220	1BD621
	11. CONTRACT/GRANT NO.
	GS-055-10458
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Monitoring and Support Lab. - Cinn, OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268	Extramural
	14. SPONSORING AGENCY CODE
	EPA/600/06
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

This report is an overview of the functional description and major features of an automated ultraviolet-visible spectrometer system intended for environmental measurements application. As such, it defines functional specifications and requirements which are divided into the chlorophyll, color, multi-option, and quality control modules. The general system design methodology is discussed with regard to the EPA laboratory automation project. The interfacing hardware requirements are included in general terms only.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Computer programming Environmental tests Spectrophotometers Algorithms Ultraviolet spectrophotometers		09/B 14/B

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	Unclassified	36
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Environmental Research Information Center
Cincinnati, Ohio 45268

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE, $300
AN EQUAL OPPORTUNITY EMPLOYER

POSTAGE AND FEES PAID
U S ENVIRONMENTAL PROTECTION AGENCY
EPA-335

Special Fourth-Class Rate
Book

If your address is incorrect, please change on the above label tear off; and return to the above address.
If you do not desire to continue receiving these technical reports, CHECK HERE ☐; tear off label, and return it to the above address,

EPA-600/4-78-064