AUTOMATED, COLORIMETRIC METHODS FOR DETERMINATION OF NITRATE PLUS NITRITE, NITRITE, AMMONIUM AND ORTHOPHOSPHATE IONS IN NATURAL WATER SAMPLES

By Ronald C. Antweiler, Charles J. Patton and Howard E. Taylor

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CONVERSION FACTORS AND ABBREVIATIONS

Multiply	By	To obtain
	Volume	
milliliter (mL)	0.03382	ounces, fluid
liter (L)	0.2642	gallon
	Mass	
microgram (µg)	0.00000003527	ounce, avoirdupois
milligram (mg)	0.00003527	ounce, avoirdupois

The following abbreviations also are used in this report:

Concentration unit	Abbreviation
milligrams per liter	mg/L
micrograms per liter	mg/L

Chemicals	Abbreviation
Deionized water	DI water
Sodium lauryl sulfate	SLS
Sulfanilamide	SAN
N-(1-naphthyl)ethylenediamine dihyrochloride	NED

Miscellaneous	Abbreviation
millimoles per liter	mM
milliliters per minute	mL/min
packed bed cadmium reactor	PBCR
Standard reference water sample	SWRS
pounds per square inch	psi
High density polyethylene	HDPE
weight-to-weight	w/w
weight-to-volume	w/v
volume-to-volume	v/v

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ABSTRACT

The apparatus and methods used for the automatic, colorimetric determinations of dissolved nutrients (nitrate plus nitrite, nitrite, ammonium and orthophosphate) in natural waters are described. These techniques allow for the determination of nitrate plus nitrite for the concentration range 0.02 to 8 mg/L (milligrams per liter) as N (nitrogen); for nitrite, the range is 0.002 to 1.0 mg/L as N; for ammonium, the range is 0.006 to 2.0 mg/L as N; and for orthophosphate, the range is 0.002 to 1.0 mg/L as P (phosphorus). Data are presented that demonstrate the accuracy, precision and quality control of the methods.

INTRODUCTION

This report describes methods used to determine dissolved nutrients (nitrate plus nitrite, nitrite, orthophosphate and ammonium) in natural waters by air-segmented continuous-flow absorption spectrophotometry. Concentration ranges for the methods described in this report are tabulated below.

Analyte	Concentration ranges (mg/L as N or P)	
Nitrate + nitrite	0.02 - 2.00;	0.08 - 8.00
Nitrite	0.002 - 0.200;	0.01 - 1.00
Orthophosphate	0.002 - 0.200;	0.01 - 1.00
Ammonium	0.006 - 0.400;	0.02 - 2.00

Samples with dissolved nutrient concentrations that exceed these ranges must be diluted prior to analytical determinations. These working concentration ranges could be modified; 10- to 500- fold dilution of samples could be achieved using dilution loops or dialyzers, separately or in combination (Coverly, 1985; Patton and Wade, 1990); analytical sensitivity could be increased by increasing the gain of the photometer up to the limiting signal-to-background ratio, increasing the path length of photometer flow cells, increasing the flow rate of the sample pump tube or decreasing the flow rate of the diluent pump tube.

APPARATUS

An air-segmented continuous-flow analyzer (Alpkem RFA-300) was used to implement the automated, colorimetric methods described in this report. System components included a 301 sampler, a 302 peristaltic pump, a 313 analytical cartridge base, a 314 power module, three 305A photometers, and a PC-based data acquisition and processing system. Alternative procedures for these methods could have been implemented using flow-injection analyzers or other types of air-segmented continuous-flow analyzers.

Operation Principles

The configuration of a generalized bench-top air-segmented continuous-flow analyzer is shown in figure 1. An automatic sampler alternately moves the sample withdrawal tube between a reservoir containing analyte-free wash solution and disposable cups containing samples. The sample withdrawal tube connects to one of the peristaltic pump channels, which in turn connects directly to the analytical cartridge (the part of the analyzer between the pump and the photometer in figure 1). Samples and the wash solution are pumped into the analytical cartridge as discrete plug flows that are separated by one or more air bubbles that form at the tip of the sample withdrawal tube

each time it is exposed to the atmosphere. The introduction of several intersample air bubbles at the beginning of each sample and wash cycle -- "pecked sampling" of Diebler and Pelavin (1972) and Patton and Wade (1990) -- substantially decreases interaction among these plugs that would otherwise occur as they flow between the sampler and the analytical cartridge. As each plug enters the analytical cartridge, it is divided into many smaller, nominally identical segments with bubbles of a suitable gas (usually air). Alternately, sample and wash plugs may be pumped into an air-segmented diluent or reagent stream. Gas bubbles are not pumped into the analytical cartridge continuously, but instead are admitted in short bursts in phase with pump pulsations -- the "air-bar" technique. This practice minimizes proportioning errors -- differences in segment-to-segment sample to reagent volume ratios -- that would otherwise occur (Snyder and others, 1976; Patton and Wade, 1990). In this manner, a small portion of each plug is proportioned into many analytical stream segments, the actual number of which is simply the product of the sample or wash time and the segmentation frequency. Chemical reactions are effected in appropriate sections of the analytical cartridge as the segmented stream flows in box-car fashion toward a recording detector. For more complete details of the theory and practice of continuous-flow analysis, see Patton and Wade (1990).

Sample Preparation

Samples must be filtered -- 0.45 µm nominal pore size or smaller -- prior to colorimetric determinations. Samples should be filtered in the field as soon after collection as practical.

Calculations

Instrument calibration requires the preparation of a set of solutions (calibrants) in which the analyte concentration is known. These calibrants are used to establish a calibration function that is estimated from a least-squares fit of nominal calibrant concentrations (x) and absorbance peak heights (y). A second-order polynomial ($y = a + bx + cx^2$) function often provides better concentration estimates at calibration range extremes than the more conventional linear model ($y = a + bx$). There is no loss of accuracy when a second-order fit is used even if the calibration function is strictly linear, because in this case the value estimated for the quadratic parameter will approach zero.

Before the calibration function can be estimated, the baseline absorbance component of measured peak heights including drift (continuous increase or decrease in the baseline absorbance during the course of a run), if present, must be removed. Baseline absorbance in continuous-flow analysis is analogous to reagent blank absorbance in batch analysis. Correction for baseline absorbance is an automatic function of most data acquisition and processing software sold by vendors of continuous-flow analyzers. These correction algorithms, however, are based on linear interpolation between initial and intermediate or final baseline absorbance values and do not, therefore, accurately correct for abrupt, step-changes in baseline absorbance that usually indicate partial flow cell blockage. It is prudent, therefore, to reestablish baseline absorbance at intervals of 20 samples or so.
After peaks are baseline corrected, regression parameters (a, b, and c terms) for the calibration function are estimated using a second-order polynomial least-squares algorithm. The quadratic equation is used to convert peak heights into concentrations for second-order calibration functions.

Most software packages provide a data base into which appropriate dilution factors can be entered. Usually these factors can be entered before or after a run is performed. Ibf dilution factors are entered, reported values will be compensated automatically for the extent of dilution. The dilution factor is the number by which measured concentrations must be multiplied to reflect the concentration of analyte in the sample prior to dilution. For example, if sample and diluent were combined in volume ratios of 1 + 1, 1 + 4, and 1 + 9, dilution factors would be 2, 5, and 10, respectively.

METHOD DESCRIPTIONS

Nitrate plus Nitrite

Cadmium metal, in the form of a miniature packed bed reactor (PBCR, figure 2) incorporated into the analytical cartridge of the continuous-flow analyzer, is used to chemically reduce nitrate to nitrite. Because reduction products and kinetics of this reaction are highly pH dependent (Nydahl, 1976), the analytical stream must be well buffered as it flows through the cadmium reactor to insure uniformity of results for a wide range of sample matrices. For the flow rate and reactor geometry used in this method, the optimum pH during the reduction step is about 7.5 (Patton, 1982). Furthermore, a reagent that forms strong complexes with Cd (II) in mildly alkaline media is also essential to long-term reactor stability during the reduction step, because these ions -- produced not only by reduction of nitrate, but also by quantitative reduction of oxygen dissolved in both samples and reagents (~ 0.5 mM) -- would otherwise form insoluble hydroxides and quickly foul the active surfaces of the cadmium reactor (Nydahl, 1974, 1976; Patton, 1982). In this regard, imidazole (figure 2) is an excellent reagent for buffering the analytical stream (pK_b = 7.09) and complexing Cd (II) ions ($\beta_4 \sim 7.5$). It is therefore a better reagent than ammonium chloride, the complexing reagent used in most other published procedures, which has no appreciable buffer capacity at this pH. The high and constant reduction efficiency of the packed bed reactor is further insured by adding a trace of copper (II) -- a potent surface activator for cadmium metal (Sherwood and Johnson, 1981; Patton, 1982) -- to the imidazole reagent.

Nitrite ions originally present in samples along with those produced by the near-quantitative reduction of nitrate by the packed bed cadmium reactor (PBCR) are determined colorimetrically by diazotization with sulfanilamide and subsequent coupling with N-(1-naphthyl) ethylenediamine (figure 2) to form an intensely-colored azo dye (Greiss reaction). The dye formed has a molar absorptivity of $\sim 4 \times 10^4$ at 543 nanometers, which is its absorbance maximum (Bendschneider and Robinson, 1952). The Greiss reaction has been widely applied as documented in an exhaustive review article (Fox, 1985). Details of its kinetics and mechanistic aspects are also available (Fox, 1979; Pai and others, 1990a). Previous, commonly cited methods based on this general analytical scheme include those of Brewer and Riley (1965), Morris and Riley (1963), Strickland and Parsons (1972), Wood and others (1967), and the U.S. Environmental Protection Agency (1983).

Concentration Ranges

The concentration range of this method can be adjusted by changing the volume of sample pumped into the analytical cartridge, changing the photometer gain, or flow cell path length of the photometer, or all three. For example, a working concentration range of 0.02 - 2.00 mg/L results when a sample pump tube with a nominal flow rate of 0.166 mL/min is used, whereas a working concentration range of 0.08 - 8.00 mg/L results when the sample pump tube flow rate is reduced to 0.074 mL/min. The analytical cartridge diagram is shown in figure 2. Flow cell path length and photometer gain settings are listed in table 1.

Interferences

Concentrations of potentially interfering substances are generally negligible in unpolluted surface and ground waters. Specific details of inorganic and organic compounds that interfere with the Greiss reaction are presented by Norwitz and Keliher (1985, 1986); more general information is presented by the American Society for Testing and Materials (1991).

Sulfides, often present in anoxic waters, rapidly deactivate cadmium reactors by forming an insoluble layer of cadmium sulfide on the active metal surface (Strickland and Parsons, 1972).

Because the volume ratio of imidazole reagent to sample is ~10:1 and its buffer capacity is high, moderately acidic (pH > 1) samples can be determined without prior neutralization using this method.

Nitrite

Nitrite is determined identically to nitrate plus nitrite, except that the packed bed cadmium reactor is removed from the analytical cartridge and deionized water containing surfactant is substituted for the imidazole buffer reagent. Thus, only nitrite ions are determined upon addition of the Greiss reagents. See the nitrate plus nitrite method summary and the references cited for additional details.

Concentration Ranges

The concentration range of this method can be adjusted by changing the volume of sample pumped into the analytical cartridge, changing the photometer gain, or flow cell path length of the photometer, or all three. For example, working concentration ranges of 0.002 - 0.200 mg/L and 0.01 - 1.00 mg/L result when sample pump tubes with nominal flow rates of 0.284 mL/min and 0.166 mL/min, respectively, are installed. Photometer gain settings and flow cell path lengths are listed in table 1.

Interferences

Concentrations of potentially interfering substances are generally negligible in unpolluted surface and ground waters. Specific details of inorganic and organic compounds that interfere with the Greiss reaction are presented by Norwitz and Keliher (1985, 1986); more general information is presented by the American Society for Testing and Materials (1991).

Ammonium

Ammonium ion reacts with hypochlorous acid and salicylate ions in the presence of nitroferricyanide ions -- actually their hydrolysis product, pentacyanoaquoferroate -- to form the salicylic acid analog of indophenol blue (Reardon and others, 1966; Patton and Crouch, 1977; Harfmann and Crouch, 1989; Alpkem Corp., 1989) The optimum pH for formation of this chromophore is about 13.4. The absorption maximum at this pH is about 660 nanometers (Pym and Milham, 1976; Krom, 1980, Kempers and Kok, 1989). Tartrate ions are added to the highly alkaline reaction medium to prevent precipitation of Ca (II) and Mg (II) ions that would otherwise occur.

Concentration Ranges

The concentration range of this method can be adjusted by changing the volume of sample pumped into the analytical cartridge, changing the photometer gain, or flow cell path length of the photometer, or all three. For example, a working concentration range of 0.006 - 0.400 mg/L results when a sample pump tube with a nominal flow rate of 0.482 mL/min is used, whereas a working concentration range of 0.02 - 2.00 mg/L results when the sample pump tube flow rate is reduced to 0.226 mL/min. The analytical cartridge diagram is shown in figure 3 and the table 1 for flow cell path lengths and photometer gain settings are shown in table 1.

Interferences

Interference by calcium and magnesium hydroxides are eliminated by adding potassium sodium tartrate to the working buffer (Alpkem Corp., 1989). Formation of the chromophore in this method is suppressed by a number of agents including primary and secondary amines, sulfides, thiols, and ascorbic acid (Ngo and others, 1982). Bromide ions can also interfere by reacting with hypochlorite to form hypobromite. The hypobromite thus formed removes ammonium ion from the reaction mixture by oxidizing it to nitrogen gas (C.J. Patton, U.S. Geological Survey, unpublished results, 1992).

Samples are easily contaminated by ammonium ion in the laboratory atmosphere. Blanks and samples with ammonium ion concentrations less than 0.01 mg/L are especially problematic and require extra care during all steps of analysis: sample cups should be rinsed with 5 percent volume-to-volume hydrochloric acid immediately prior to use; working reagents should be prepared and filtered daily. Ammonia contamination from the laboratory atmosphere can be reduced by filling sample cups in an argon-rich atmosphere and by maintaining a positive pressure of argon above the filled sample cups on the automatic sampler during analysis. A sample tray cover was

designed to make this possible. In addition, a sponge ring saturated with deionized water and mounted on the sampler tray could prevent evaporative concentration of the samples.

Transmission tubing connecting the analytical cartridge to the photometer flow cell should be thermally insulated for maximum baseline stability (Alpkem Corp., 1987).

Orthophosphate

Orthophosphate ions are determined at a rate of 90 tests per hour by an automated version of the phosphoantimonylmolybdenum blue procedure of Murphy and Riley (1962). The absorbance maximum for this method is 880 nanometers (Whitledge and others, 1981).

When making concentration range changes, it is important that $[H^+]$ and $[H^+]:[Mo^{6+}]$, at the flow cell, are ~ 0.40 and ~ 74, respectively (Pai and others, 1990b) in order to maximize the absorbance.

Concentration Ranges

The concentration range of this method can be adjusted by changing the volume of sample pumped into the analytical cartridge, changing the photometer gain, or flow cell path length of the photometer, or all three. For example, a working concentration range of 0.002 - 0.200 mg/L results when a sample pump tube with a nominal flow rate of 0.482 mL/min is used, whereas a working concentration range of 0.01 - 1.00 mg/L results when the sample pump tube flow rate is reduced to 0.226 mL/min. The analytical cartridge diagram is shown in figure 4. Flow cell path lengths and photometer gain settings are listed in table 1.

Transmission tubing connecting the analytical cartridge to the photometer flow cell, and the flow cell itself, should be thermally insulated for maximum baseline stability (Alpkem Corp., 1987).

Interferences

Potential interferents in natural water, Fe^{+3}, Cu^{+2} and SiO_2, do not affect this method at concentration levels less than 40 mg/L, 10 mg/L and 10 mg/L, respectively (Alpkem Corp., 1991). If orthophosphate is to be determined in acidified samples, the pH should be adjusted to the range of 3 to 5 prior to colorimetric analysis (Pai and others, 1990b).

Even for very low concentration level samples (< 5 µg/L), contamination is seldom a problem provided that sample containers and analytical glassware are acid rinsed prior to use. Working reagents should be prepared fresh daily from stock solutions, and both samples and reagents should be filtered prior to analysis.

ANALYSIS PROTOCOL

For a typical nutrient analysis, two runs are performed: nitrate plus nitrite, orthophosphate and ammonium are determined simultaneously in the first; nitrite alone is determined in the second. A set of six mixed calibrants containing appropriate concentrations of nitrate, ammonium ion and orthophosphate ions are included in the first run to establish the calibration function. For the second run, six nitrite working calibrants are used. The first 18 positions in each run are reserved for calibration and quality-control purposes. Positions 1-10 are used to determine calibration curves. Positions 11-13 are used to estimate sample interaction or carryover; the high calibrant (S1) is followed by two of the low calibrants (S6). Comparison of these two S6 peaks gives an indication of how much carryover occurred from S1 (see Quality Control). Positions 14 and 15 are used to determine the reduction efficiency of the cadmium column (PBCR). A nitrate sample is run next to a nitrite sample having the same concentration; if the nitrate is being properly reduced to nitrite by the PBCR, these two peaks will have the same height (see Quality Control). Positions 16-18 are used to gauge accuracy, by running standard reference samples. For the second run (nitrite only), the final 5 of these 18 positions are reserved for standard reference samples (there is no purpose to testing the efficiency of the nitrate reduction). Samples are always run in duplicate, and after every sixth sample, two standard reference samples and two working calibrants are run to insure accuracy. Finally, the last eight positions (usually positions 83-90) are reserved for the working calibrants to redetermine a new

calibration curve. This protocol helps to guarantee that the analyses reflect the "true" concentrations of the analytes.

Absorbance data are transmitted from the photometers to a computer by an Alpkem 406 signal converter, and processed within the computer by the software package SOFTPAC-PLUS. The operating parameters for this software package are listed in table 2. The software converts the raw data into peak heights, which are converted into concentrations from the calibrants by linear or quadratic regression within a spreadsheet program.

QUALITY CONTROL

This section covers all matters concerning the quality of the data: the detection limits and precisions, the accuracy, the precision, and the instrument and cadmium column efficiencies.

Detection Limits and Precisions

The detection limit (*DL*) is the smallest concentration that the methodology can discern from "noise". It is defined by Skogerboe and Grant (1970): one measures the peak height of the blank several (*n*) times, calculates a standard deviation (SD_b), determines the slope of the calibration curve (peak height versus concentration, (*dh/dc*) and divides SD_b by *dh/dc*:

$$DL = SD_b \cdot t_{(n-1,0.95)}/(dh/dc),$$

where $t_{(n-1,0.95)}$ is the student t statistic at the 95 percent confidence level for $n-1$ degrees of freedom. The detection limits using the larger sample line tubing are given in table 3: 0.02 mg/L for nitrate plus nitrite; 1.6 µg/L for nitrite; 0.006 mg/L for ammonium; and 1.9 µg/L for orthophosphate.

The precision of the data is the same statistic as the detection limit except that SD_b is replaced by SD_s, where SD_s is the standard deviation of the peak heights of the standard (or sample) *s*, i.e.,

$$P_s = SD_s \cdot t_{(n-1,0.95)}/(dh/dc),$$

where P_s is the precision of the standard s. Alternatively,

$$P_s = sd_s \cdot t_{(n-1,0.95)},$$

where sd_s is the standard deviation of the calculated concentrations of standard *s*. Table 4 lists the precisions (both absolute and relative) for a variety of standards covering the working concentration ranges. Figure 5 shows relative precision against concentration for each of the four analytes. The precisions for nitrate plus nitrite, nitrite and orthophosphate are less than 5 percent when the concentration is sufficiently greater than the detection limit; for ammonium, the precision is between 5 and 10 percent.

Accuracy

To ascertain the accuracy of the methodology, several standard reference materials were routinely used: U.S. Geological Survey Standard Reference Water Samples (SRWS) N-30, N-32 and P-11. Also, NBS-1 and NBS-2 were used; they are standards made from National Bureau of Standards reference nitrate and phosphate. Each of these standards was non-preserved. The analyses are consistently close to the reported value (table 5).

Instrument and Cadmium Column Efficiencies

As stated above, to determine the amount of sample interaction -- carryover from one sample to another -- a triplet of calibrants was consistently used. The high standard (S1) was run followed by two low standards (S6). Any significant carryover was displayed as a difference in the peak heights of the two S6 standards. As summarized in table 6, carryover is negligible for nitrate plus nitrite, nitrite alone and ammonium; there is a slight amount of

carryover for orthophosphate. Practically, this means that low concentration samples that are run directly after high concentration samples may give erroneously high values for orthophosphate. This problem can be adequately solved by always running samples in duplicate. In those cases where the two peaks do not match, the sample should be re-run.

Reported in table 7 is the reduction efficiency of the PBCR. A nitrate standard of a given concentration was analyzed followed by a nitrite standard of the same concentration. If the cadmium column was successfully reducing all the nitrate to nitrite, these two peaks should have the same height. The data in table 7 indicate that nitrate was being quantitatively reduced to nitrite in the PBCR.

SUMMARY

This report describes apparatus and methods used for automatic, colorimetric determinations of dissolved nutrients (nitrate plus nitrite, nitrite, ammonium and orthophosphate). Data characterizing the performance of these methods for a number of natural water samples and synthetic reference materials are also provided. In general, for filtered samples the methods described here are successful for the determination of nitrate plus nitrite for the concentration range 0.02-8 mg N/L; for nitrite, the range is 0.002-1.0 mg N/L; for ammonium, the range is 0.006-2 mg N/L; and for orthophosphate it is 0.002-1 mg P/L.

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Table 1. Instrument operating parameters and calibrant concentrations of the determinations of nitrate plus nitrite, nitrite, ammonium and orthophosphate.

[psi, pounds per square inch; µL/min, microliters per minute; mg/L, milligrams per liter; N, nitrogen; P, phosphorus; °C, degrees Celsius]

Parameter	Nitrate plus nitrite	Nitrite	Ammonium	Ortho-phosphate
INSTRUMENT OPERATING PARAMETERS				
Sample time in seconds	25	25	25	25
Wash reservoir time, in seconds	20	20	20	20
Argon pressure, in psi	2	2	2	2
Number of calibrants	6	6	6	6
Sample pump tube flow rate, in µL/min.	74	166	482	482
Cell length, in millimeters	10	10	30	30
Absorbance, in nanometers	540	540	660	880
Bubble rate, in bubbles per minute	45	45	45	45
Temperature of bath, in °C	37	37	37	37
Photometer gain (STD CAL)	78	160	520	420
CALIBRANT CONCENTRATIONS				
Calibration range, mg/L as N or P	0.1-5.0	0.004-0.2	0.005-0.4	0.002-0.2
Calibrant 1, mg/L as N or P	5.0	0.200	0.400	0.200
Calibrant 2, mg/L as N or P	3.0	0.120	0.250	0.120
Calibrant 3, mg/L as N or P	2.0	0.080	0.100	0.080
Calibrant 4, mg/L as N or P	1.0	0.050	0.050	0.040
Calibrant 5, mg/L as N or P	0.4	0.020	0.020	0.010
Calibrant 6, mg/L as N or P	0.1	0.004	0.005	0.002

Table 2. Software operating parameters

	Channel number			
Parameter	1	2	3	4
Channel name	NO3+NO2	PO4	NH4	NO2
Start ignore time (seconds)	0	0	0	0
Initial baseline lead time (seconds)	60	60	60	60
Final baseline lag time (seconds)	60	60	60	60
Corrections code	Yes	Yes	Yes	Yes
Cycle time (seconds)	45	45	45	45
Collection rate (points per second)	1	1	1	1
Invert raw data	No	No	No	No
Plot curve	No	No	No	No
Auto/Interactive	I	I	I	I
Decimal places	3	0	3	0
1st sample number	1	1	1	1
Peak height/Area	Height	Height	Height	Height
Threshold (points)	10	10	10	10
Ascending slope (points)	1	1	1	1
Apex (points)	10	10	10	10
Descending slope (points)	1	1	1	1
Plateau (points)	2	1	3	2
Integration points	7	5	7	7

Table 3. Detection limits for the determinations of nitrate plus nitrite, nitrite, ammonium and orthophosphate.

Species	Number of samples	Detection limit, in milligrams nitrogen or phosphorus per liter
Nitrate plus nitrite	17	0.02
Nitrite	29	0.0016
Ammonium	37	0.006
Orthophosphate	45	0.0019

Table 4. Precisions and relative precisions for the determinations of nitrate plus nitrite, nitrite, ammonium and orthophosphate.

Standard[1]	Concentration, in milligrams nitrogen or phosphorus per liter	Number of samples	Precision, in milligrams nitrogen or phosphorus per liter	Relative precision, in percent
		NITRATE PLUS NITRITE		
S1	7.98	16	0.153	1.9
S1	4.99	20	0.078	1.6
NBS-2	4.51	16	0.137	3.0
S2	3.01	21	0.049	1.6
S1	2.00	7	0.083	4.2
S2	1.61	12	0.054	3.4
S3	1.18	18	0.065	5.5
NBS-1	0.90	8	0.034	3.8
S4	0.81	6	0.029	3.6
N-30	0.42	40	0.050	11.9
S5	0.40	35	0.035	8.9
N-34	0.20	30	0.036	17.9
N-32	0.14	30	0.044	30.7
S6	0.10	40	0.033	32.4
		NITRITE		
S1	0.200	25	0.0046	2.3
N-30	0.188	21	0.0046	2.5
N-34	0.167	26	0.0030	1.8
S2	0.120	23	0.0031	2.6
S3	0.080	28	0.0017	2.1
N-32	0.055	18	0.0027	4.9
S4	0.050	25	0.0024	4.7
N-33	0.046	22	0.0045	9.8
N-31	0.036	17	0.0020	5.6
S5	0.020	25	0.0029	14.1
S6	0.004	35	0.0016	42.4
		AMMONIUM		
S1	0.398	39	0.013	3.1
S2	0.249	50	0.010	4.1
N-30	0.199	28	0.009	4.8
N-34	0.153	30	0.010	6.7
S3	0.098	45	0.009	8.9
NBS-1	0.078	8	0.004	4.8
NBS-2	0.077	16	0.006	7.3
S4	0.051	37	0.008	15.3
N-32	0.033	28	0.006	17.5
S5	0.020	33	0.006	28.3
S6	0.004	45	0.006	144.7

Table 4. Precisions and relative precisions for the determinations of nitrate plus nitrite, nitrite, ammonium and orthophosphate – cont.

Standard[1]	Concentration, in milligrams nitrogen or phosphorus per liter	Number of samples	Precision, in milligrams nitrogen or phosphorus per liter	Relative precision, in percent
		ORTHOPHOSPHATE		
N-30	0.297	12	0.0095	3.2
N-34	0.210	30	0.0090	4.3
S1	0.199	42	0.0088	4.4
NBS-2	0.164	16	0.0095	5.8
S2	0.121	49	0.0067	5.6
N-32	0.097	30	0.0046	4.8
S3	0.080	48	0.0059	7.4
NBS-1	0.064	8	0.0039	6.1
S4	0.040	37	0.0028	7.0
S5	0.010	37	0.0016	15.6
S6	0.002	37	0.0014	88.2

[1]S1-S6 are calibrants used to standardize the instrument. N-30, N-31, N-32, N-33 and N-34 are U.S. Geological Survey standard reference water samples. NBS-1 and NBS-2 are standards made up from National Bureau of Standards reference materials.

Table 5. Reported and measured concentrations for U.S. Geological Survey Standard Reference water samples.

[Conc., concentration; SD, standard deviation. Both in milligrams nitrogen or phosphorus per liter]

Standard[1]	Reported			Measured			Number of Samples
	Conc.		SD	Conc.		SD	
			NITRATE PLUS NITRITE				
N-30	0.442	±	0.012	0.421	±	0.030	40
N-32	0.148	±	0.024	0.143	±	0.026	30
N-34	0.26			0.199	±	0.021	30
NBS-1	0.905			0.896	±	0.018	8
NBS-2	4.52			4.51	±	0.08	16
P-11	0.28			0.274	±	0.010	8
NITRITE							
N-34	0.030			0.046	±	0.003	22
			AMMONIUM				
N-30	0.210	±	0.005	0.199	±	0.006	28
N-32	0.040	±	0.021	0.033	±	0.003	28
N-34	0.17			0.153	±	0.006	30
			ORTHOPHOSPHATE				
N-30	0.260	±	0.006	0.297	±	0.005	12
N-32	0.091	±	0.010	0.097	±	0.003	30
N-34	0.190			0.210	±	0.005	30
NBS-1	0.065			0.064	±	0.002	8
NBS-2	0.160			0.164	±	0.005	16

[1]N-30, N-32, N-34 and P-11 are U.S. Geological Survey non-preserved Standard Reference water samples; NBS-1 and NBS-2 are prepared from National Bureau of Standards stock solutions.

Table 6. Sample interaction (amount of carryover) in the determination of nitrate plus nitrite, nitrite, ammonium and orthophosphate.

[S1 and S6, high and low calibration standards; Pct. I = (1st S6 - 2nd S6)/S1 * 100, which represents the amount of "contamination" carried over from the previous sample; N, nitrogen; P, phosphorus]

Species	Standard	Number of samples	Average Concentration in milligrams N or P per liter	Pct. I
Nitrate plus nitrite	S1	12	4.99	
	1st S6	12	0.143	0.8
	2nd S6	12	0.104	
Nitrite	S1	9	0.200	
	1st S6	9	0.0040	0.2
	2nd S6	9	0.0036	
Ammonium	S1	12	0.398	
	1st S6	12	0.005	0.3
	2nd S6	12	0.003	
Orthophosphate	S1	11	0.199	
	1st S6	11	0.0035	1.0
	2nd S6	11	0.0014	

Table 7. Cadmium column efficiency

Species	Number of Samples	Concentration, in milligrams nitrogen per liter	
		Standard	Analytical result
Nitrate	9	1.6	1.60
Nitrite	9	1.6	1.65
Nitrate	12	5.0	5.04
Nitrite	12	5.0	4.91
Nitrate	12	3.0	3.02
Nitrite	12	3.0	2.98

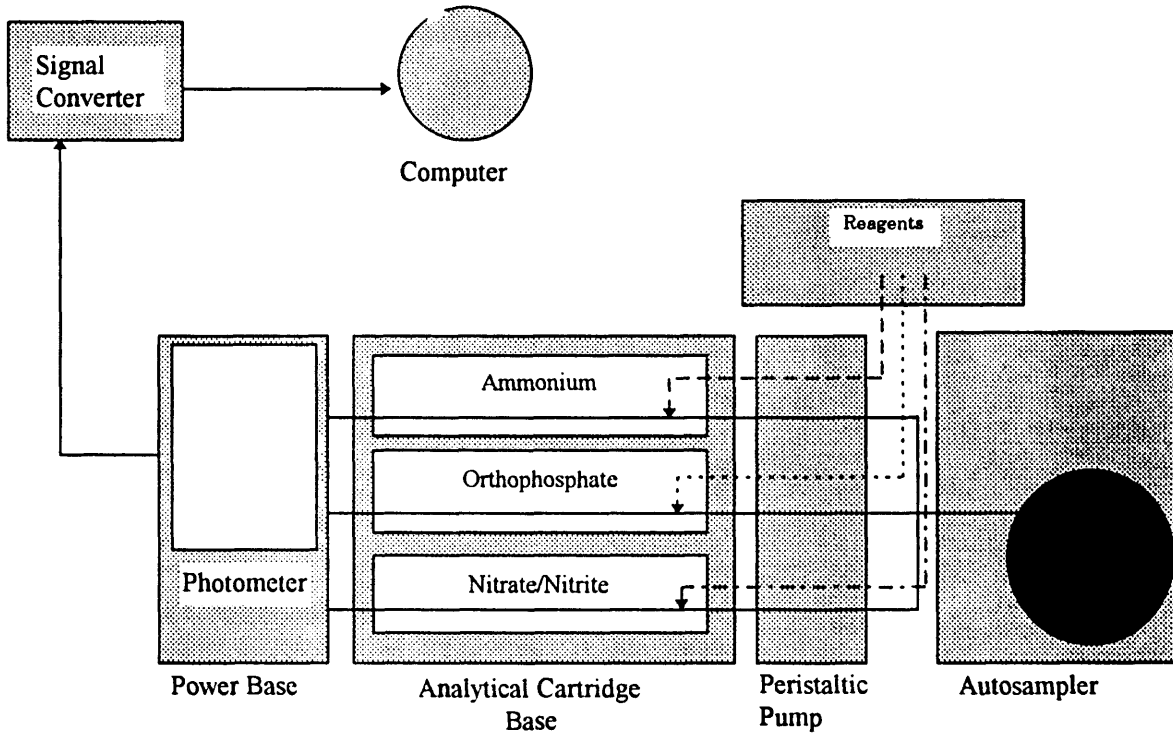

Figure 1. Schematic of automated nutrient analysis equipment.

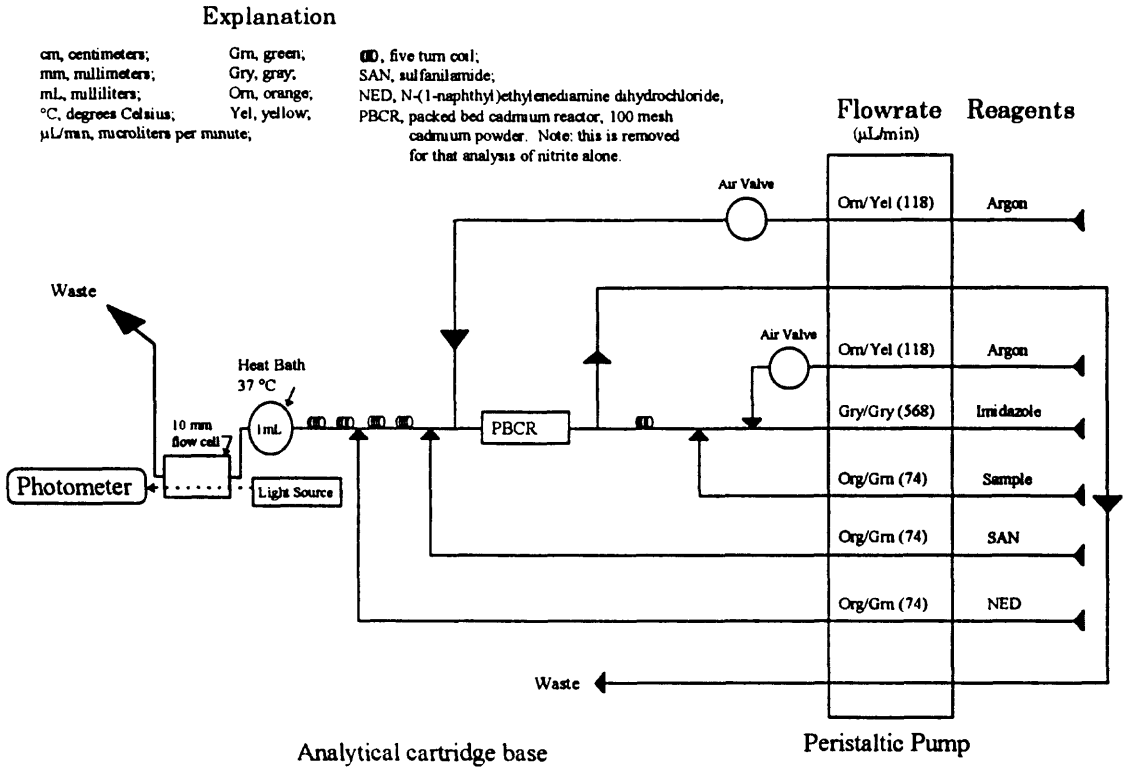

Figure 2. The flow regime used for the analysis of nitrate and nitrite.

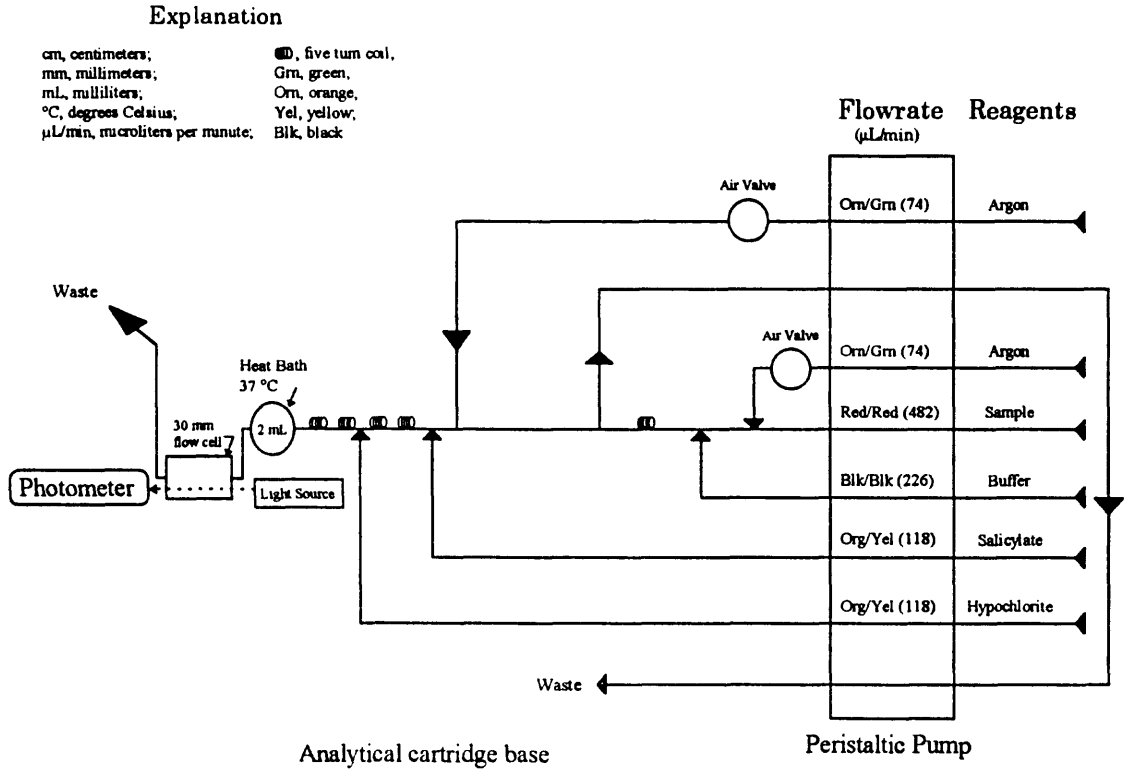

Figure 3. The flow regime used for the analysis of ammonium

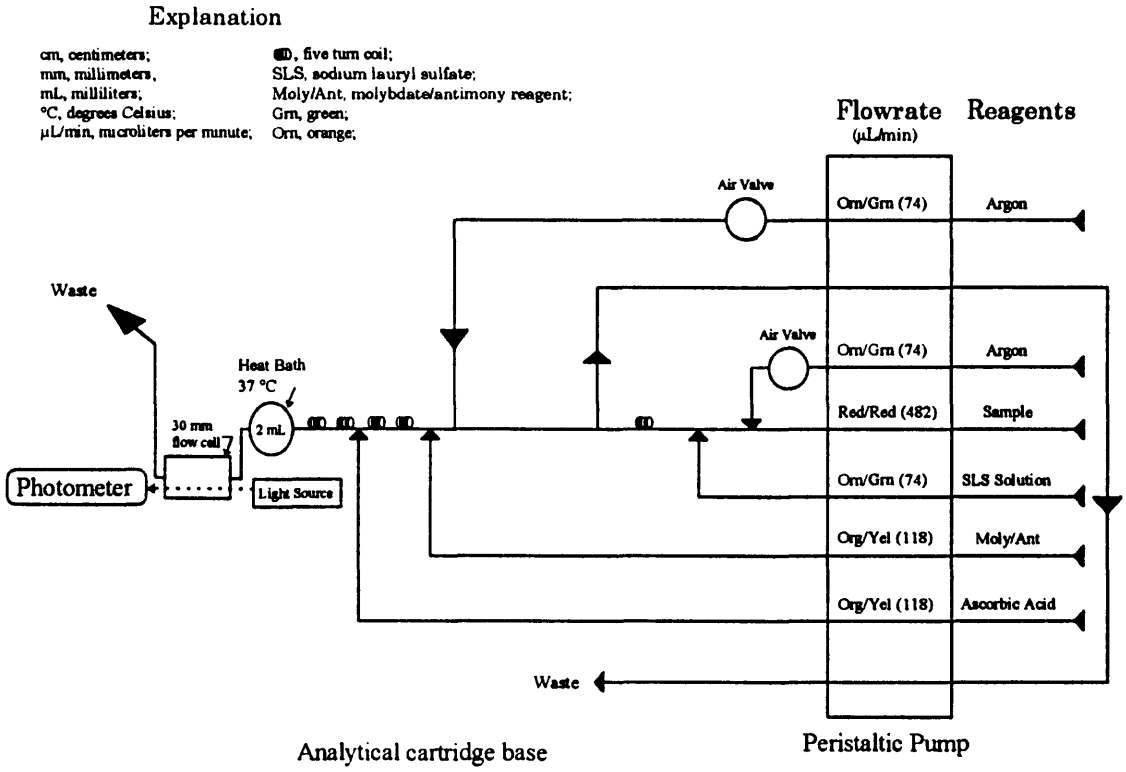

Figure 4. The flow regime used for the analysis of orthophosphate.

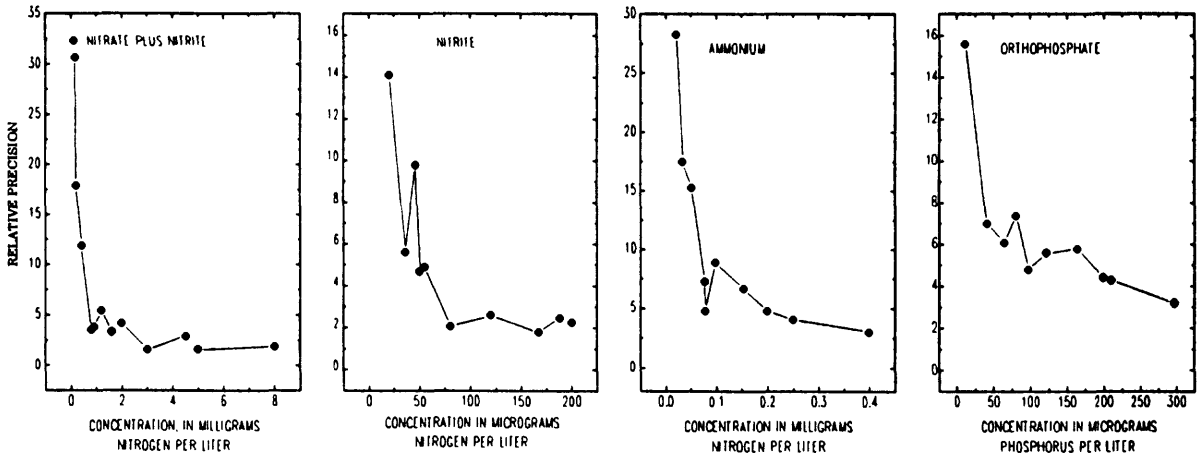

Figure 5. Relative precisions of nitrate plus nitrite, nitrite, ammonium and orthophosphate analyses as a function of concentration.

Appendix I : Operating instructions for the ALPKEM system.

1. Empty the waste bottles into the large waste container labeled "Nutrient Waste."
2. If the cadmium column is spent, prepare a new one (see below).
3. Prepare the working reagents from the stock solutions (see below). Also, rinse the deionized (DI) water reservoir and fill it.
4. Inspect the tubing (and replace if necessary) as it passes through the micropump. Clean it and the platens on the micropump with a tissue sprayed with silicone.
5. Turn on the argon : pressure should be 1-2 pounds per square inch.
6. Turn on the master power switch on the 314 power module. Turn on the autosampler, micropump and temperature baths for the three cartridges. Turn on the computer.
7. Lower and secure the platens; the micropump should begin pumping when this is accomplished.
8. After waiting about 5 minutes, visually trace the forward progress of bubbles through the system. They should be uniform in size, shape and number (for each cartridge) as they pass through the flow cells; their shape should be ovoid and their number should be such that only one bubble enters the flow cell at a time. If these conditions are not met, troubleshooting is necessary.
9. Wipe off the reagent straws one at a time and place each one into its appropriate reagent.
10. Turn on the light switch on the photometers.
11. After waiting 5 minutes, inspect the bubble patterns again.
12. Insert the cadmium column in the nitrate/nitrite cartridge: it is critical that this step come after the addition of imidazole buffer to the system.
13. On the computer, access SOFTPAC PLUS by typing "alpkem" at the system prompt. Once the software is accessed, press the F5 key to access the Display menu, turn on channels 1, 2 and 3, press ALT-1 to turn on the computer access of signal, and then press F3. The monitor should display the signals from the three channels.
14. After waiting another 5 minutes, adjust the gain control on the sample and reference channel of each photometer to about 5.00 volts. Turn the output switch to "Absorbance" and adjust the reference channel gain slightly until a steady reading of 0.10 to 0.20 volts is obtained on the photometer display.
15. Rinse each sample cup with a 5 percent HCl solution prior to pouring a sample into it.
16. Put the autosampler tray into the argon bath, pour the samples into the acid-rinsed cups, and put the filled sample cups into the argon-bathed tray. All the samples must be poured prior to beginning analysis, and every slot on the tray must be filled with a sample cup, even if there is no sample in it. Follow the sample order protocol (below) as the samples are poured. This step is important because the data acquisition and processing within the software requires a certain order.
17. Examine the monitor to ascertain that stable baselines have been obtained during the time the samples have been dispensed. If a stable baseline is present for each channel, press ESC and ALT-1 to turn off the display. Press ESC again, then press F4 to access the SAMPLE TABLE menu. Add the sample IDs to this table. When this is complete, press ALT-N to name the sample table, then press ALT-S to save it. When the file is named and saved, press ESC to return to the main menu and press F5 to enter the Display menu.
18. Take the filled sample tray from the argon bath and put it on the autosampler. Attach the tray cover with the argon lines to the tray. At this point, the tray should again be immersed in argon.
19. On the computer, turn on the data acquisition by pressing ALT-1. As soon as data is being accumulated, press the START button on the autosampler.
20. As data are received, assure that S1 (the high calibrant) is on scale (on the photometers, the reading should be between 4.5 and 4.9 volts). Adjust if necessary by turning the standard calibration knob on the photometers. Also, assure that the cadmium column is reducing the nitrate by comparing S2 and the 3 mg N/L NO_2 standards; if these peaks are not equivalent in height, a new cadmium column may need to be prepared.
21. When the run is complete (after about an hour), wait about 5 minutes to obtain a post-analysis stable baseline. Press the ESC button on the computer and ALT-1 to stop the data acquisition.
22. Remove the cadmium column: it should be stored in imidazole buffer.
23. Remove the reagent straws from their respective reagents. If nitrite is to be determined, leave the straws in the SAN and NED bottles (but remove the imidazole straw).
24. If nitrite is not to be determined, go to instruction 36.
25. Remove the orange/green tube from the nitrate/nitrite sample line and insert the orange/white tube in its place.

26. From the 406 signal converter, remove the channel 1 cable and insert it in channel 4.
27. Turn the standard calibration knob on the nitrate/nitrite photometer from 080 to 840.
28. On the computer, press ESC to return to the main menu, F4 to get to the Sample Table. Then press ALT-F4 to access channel 4. Input the sample IDs into this table. When this is accomplished, press ALT-N to name the file, ALT-S to save it, and ESC to return to the main menu. Press F5 to get to the Display menu, and by pressing the spacebar, turn off the displays for channels 1, 2 and 3 and turn on the display for channel 4.
29. Remove the autosampler tray from the autosampler, and pour nitrite calibrants in place of the mixed calibrants used earlier. When this is done, replace the tray onto the autosampler.
30. Examine the nitrate/nitrite bubble pattern, and adjust the gain controls as in instruction 14.
31. On the computer, press ALT-4 to turn on the data acquisition of channel 4. When this is done, press F3 to monitor the signal.
32. Press the RESET button on the autosampler, and then the START button. Be sure the sample tray is lined up properly.
33. Similar to instruction 20, adjust the standard calibration knob if necessary to receive a voltage of 4.5 to 4.9 for the high calibrant.
34. When the nitrite run is complete, wait about 5 minutes to obtain a post-stable baseline. Press the ESC button on the computer and ALT-4 to stop the data acquisition.
35. Turn the standard calibration knob on the photometer from 840 to 080. Remove the channel 4 cable on the 406 signal converter and insert it into channel 1. Replace the orange/white tube with the orange/green tube.
36. Remove the reagent straws from SAN and NED, and let the system pump DI water for 5-10 minutes.
37. Turn off the photometer light and the temperature baths. Then turn off the autosampler, micropump and master power switches. Release the platens on the micropumps.
38. Turn off the argon.

Appendix II. Data Processing within SOFTPAC-PLUS.

1. Once the data acquisition is complete, return to the main menu by pressing ESC twice. Press ALT-F1 to access the nitrate plus nitrite, orthophosphate and ammonium channels.
2. Press F4 to get to the Sample table and ALT-F2 to get to the orthophosphate channel. Press ALT-C and copy everything from channel 1 to channel 2. Press ALT-F3 to get to the ammonium channel and press ALT-C to copy everything from channel 1 to channel 3. Press ALT-F1 to return to the nitrate/nitrite channel, and press ESC to return to the main menu.
3. Press F8 to access the calculations menu, and F8 again the begin the calculations. Hit RETURN thrice to display the nitrate plus nitrite data. Examine these data assure that the software has properly marked all the right peaks (as opposed to glitches).
4. If it is necessary to edit the computer choices, press F4 or F3 to move to the errant peak and F5 to edit it. Within the edit menu, press F4 to enlarge the picture and the arrow keys to properly locate the peak. When this is done, press F2 to mark the new choice and F10 to escape the edit menu. Do this procedure for the errant peaks.
5. When the proper peaks are chosen, press F9 twice to display the calculated concentrations and peak heights. The computer automatically will access the orthophosphate data next, followed by the ammonium data. If necessary, the data can be edited as in instruction 4.
6. When the software finally returns to the Calculations menu press ALT-F4 to access the nitrite data, and then process these data as in instructions 3, 4 and 5.
7. To export the data as an ASCII file, press F6 to enter the Spreadsheet menu, ALT-F to access the Files menu, ALT-E (for export). At the new screen, press ALT-F and input the name of the ASCII file to be created. Then, when the software prompts for a template file name, type Q. Press ESC twice to return to the Spreadsheet menu. Pressing ALT-F and ALT-L allows another file to be loaded and exported. Pressing ALT-Q escapes from the Spreadsheet menu.

Appendix III. Preparation of a packed bed cadmium reactor (PBCR).

The following are brief instructions. For more details, one should consult Patton (1982).

1. a. Cadmium powder should be wet-sieved 100 mesh and subsequently copperized using cupric sulfate and imidazole. The cupric sulfate solution should be removed by subsequent washing with imidazole. The cadmium powder can be stored indefinitely in imidazole.
2. b. One should have a porous frit material to hold the cadmium powder in the PBCR. The authors used Interflo Technologies High Density Polyethylene (HDPE) porous plastic with a nominal pore size of 40 μm. and had constructed a stainless steel punch of outer diameter 1/16 inch to punch out the HDPE frit plugs.
3. c. A 3-cm length of 1/16 inch ID Teflon tubing, a funnel, a spatula, a paper clip and a syringe are needed.
4. Attach a 3-cm section of Teflon tubing to the end of the funnel. Put a porous plug in the funnel and plunge it into the end of the tubing with the paper clip.
5. Remove the tubing and connect the other end to the funnel. To the end containing the plug, attach a syringe filled with imidazole. Force the imidazole into the funnel until it is about one-half full.
6. Scoop a small portion of the copperized cadmium into the funnel. Careful tapping the side of the funnel causes the powder to trickle into the Teflon tube. Add powder to the funnel only sufficient to fill the Teflon tube. When the tube is full, draw in imidazole with the syringe which helps pack the cadmium.
7. Take another plug, drop it into the funnel, and with the paper clip, force it into the Teflon tube. Remove the tube from the funnel, attach 0.015 inch ID polyethylene (PE) tubing to this end, and fill the system with the imidazole.
8. Remove the syringe and connect the 0.015 inch ID PE tubing to form an enclosed loop. The PBCR is now complete and ready to use.
9. The cadmium powder may need to be re-copperized. To do this, pour the powder and imidazole into a beaker, and add an equal quantity of cupric sulfate solution. Swirl this for a minute or two, and decant off the liquid. Rinse three times with imidazole. Store the powder in the imidazole.

Appendix IV. The working calibrants and sample order protocol.

The Working Calibrants

Primary standard solutions are made from reagent grade desiccated KNO_3, KNO_2, KH_2PO_4 and NH_4Cl. Adding 1.4434 g KNO_3 to 1 L deionized (DI) water makes a 200 mg N/L standard for nitrate; 0.6075 g KNO_2 in 1 L DI water makes a 100 mg N/L standard for nitrite; 0.4394 g KH_2PO_4 in 1 L DI water makes a 100 mg P/L standard for orthophosphate; and 0.7637 g NH_4Cl in 1 L DI water makes a 200 mg N/L standard for ammonium. These salts *must* be dried for several hours at 110 - 120 degrees C, cooled and stored in a desiccator prior to weighing and dissolution.

The working calibrants S1 - S6 are made from these stock solutions (through suitable intermediate dilution if necessary). A typical set of mixed working calibrants are shown in the table below (here, "mixed" means that the nitrate, orthophosphate and ammonium standards are combined).

	Concentrations		
Calibrant	Nitrate (mg N/L)	Orthophosphate (µg P/L)	Ammonium (mg N/L)
S1	5.0	200	0.400
S2	3.0	120	0.250
S3	2.0	80	0.100
S4	1.0	40	0.050
S5	0.4	10	0.020
S6	0.1	2	0.005

For nitrite, a working set of calibrants is given below:

Calibrant	Nitrite (µg N/L)
S1	200
S2	120
S3	80
S4	50
S5	20
S6	4

A set of working calibrants should be prepared fresh every 2 weeks (at least), and should be stored in the dark in a refrigerator.

A set order of samples and standards was used in the analyses, both for consistency and rigor. This protocol is as follows:

Position	Standard/Sample	Position	Standard/Sample	Position	Standard/Sample
1	S1	10	DI water	...	...
2	DI water	11	S1	83	S1
3	DI water	12	S6	84	S2
4	S1	13	S6	85	A nitrite standard whose concentration is the same as S2
5	S2	14	S2	86	S3
6	S3	15	A nitrite standard whose concentration is the same as S2	87	S4
7	S4	16	Standard Reference Sample	88	S5
8	S5	17	Standard Reference Sample	89	S6
9	S6	18	Standard Reference Sample	90	DI water

In addition, every sample is run in duplicate, and after every fifth or sixth sample (depending on the number of samples) four cups are used for standards and standard reference samples.

Appendix V : Preparation of the reagents used in the nutrient analyses.

Nitrate/Nitrite

1. Stock imidazole buffer, 0.1 M

Imidazole ($C_3H_4N_2$)	6.81 g
Hydrochloric Acid (HCl)	as required (~ 4 mL)
DI water	dilute to 1L

Insert a pH electrode and a magnetic stirring bar into about 900 mL of DI water. Dissolve 6.81 g of imidazole in the DI water. Adjust the pH of the solution to pH 7.5 with the hydrochloric acid. Dilute to 1L.

2. Cupric sulfate, 2 percent (w/v)

Cupric sulfate (CuSO4.5H2O)	20 g
DI water	dilute to 1L

3. Working imidazole buffer

Stock imidazole buffer	100 mL
Cupric sulfate, 2 percent (w/v)	50 µL
Brij-35 surfactant (30 percent w/v)	0.1 mL (2 drops)

4. Stock sulfanilamide (SAN)

Sulfanilamide (H2NC6H4SO2NH2)	10.0 g
Hydrochloric acid (HCl)	100 mL
DI water	dilute to 1L

5. Working SAN

Stock SAN	50 mL
Brij-35 surfactant (30 percent)	2 drops

6. N-(1-naphthyl)ethylenediamine Dihydrochloride (NED)

NED (C10H7NHCH2CH2NH2.2HCl)	1.0 g
DI water	dilute to 1L

Orthophosphate

1. Sodium lauryl sulfate (SLS), 15 percent w/w

SLS (CH3(CH2)11OSO3Na)	150 g
DI water	dilute to 1L

2. Stock antimony potassium tartrate

Antimony potassium tartrate (KSbOC4H4O6.½H2O)	3.0 g
DI water	dilute to 1L

3. Stock molybdate/antimony reagent

Sulfuric acid (H2SO4)	70 mL
Ammonium molybdate (NH4)6MoO24.4H2O	6.0 g
Stock antimony potassium tartrate	50 mL
DI water	dilute to 1L

4. Working molybdate/antimony reagent, 100 mL

Stock molybdate/antimony	100 mL

SLS, 15 percent	2 mL

5. Stock ascorbic acid reagent, 400 mL

Ascorbic acid (C6H8O6)	6.0 g
Acetone (CH3COCH3)	200 mL
DI water	200 mL

This reagent should be stored chilled in the dark.

6. Working ascorbic acid reagent, 60 mL

Stock ascorbic acid	10 mL
DI water	50 mL

This reagent should be prepared fresh daily.

7. Working SLS, 110 mL

Stock SLS	10 mL
DI water	100 mL

Ammonium

1. Stock 5 N sodium hydroxide

Sodium hydroxide (NaOH)	200 g
DI water	dilute to 1L

2. Stock potassium sodium tartrate

Potassium sodium tartrate (KNaC4H4O6.4H2O)	200 g
DI water	dilute to 1L

3. Stock buffer sodium phosphate dibasic

Sodium phosphate dibasic (Na2HPO4)	134 g
Stock 5 N sodium hydroxide	100 mL
DI water	dilute to 1L

4. Working buffer

Stock buffer	40 mL
Stock potassium sodium tartrate	50 mL
Stock 10 N sodium hydroxide	24 mL
Brij-35 surfactant	3 drops
DI water	dilute to 200 mL

5. Sodium salicylate/sodium nitroferricyanide

Sodium salicylate (NaC7H5O3)	75 g
Sodium nitroferricyanide (Na2Fe(CN)5NO.2H2O)	0.15 g
DI water	dilute to 500 mL

This solution should be stored in a dark bottle.

6. Sodium hypochlorite

Sodium hypochlorite solution (NaOCl), 5.25 percent solution (Clorox bleach)	3 mL
DI water	47 mL

This solution should be prepared fresh daily.