Method 365.5

Determination of Orthophosphate in Estuarine and Coastal Waters by Automated Colorimetric Analysis

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Revision 1.4
September 1997

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1.0 Scope and Application

1.1 This method provides a procedure for the determination of low-level orthophosphate concentrations normally found in estuarine and/or coastal waters. It is based upon the method of Murphy and Riley[1] adapted for automated segmented flow analysis[2] in which the two reagent solutions are added separately for greater reagent stability and facility of sample separation.

Analyte	Chemical Abstracts Service Registry Numbers (CASRN)
Phosphate	14265-44-2

1.2 A statistically determined method detection limit (MDL) of 0.0007 mg P/L has been determined by one laboratory in 3 parts per thousand (ppt) saline water.[3] The method is linear to 0.39 mg P/L using a Technicon AutoAnalyzer II system (Bran & Luebbe, Buffalo Grove, IL).

1.3 Approximately 40 samples per hour can be analyzed.

1.4 This method should be used by analysts experienced in the use of automated colorimetric analyses, and familiar with matrix interferences and procedures for their correction. A minimum of 6-months experience under experienced supervision is recommended.

2.0 Summary of Method

2.1 An automated colorimetric method for the analysis of low-level orthophosphate concentrations is described. Ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of phosphate to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color produced is proportional to the phosphate concentration present in the sample. Positive bias caused by differences in the refractive index of seawater and reagent water is corrected for prior to data reporting.

3.0 Definitions

3.1 Calibration Standard (CAL) -- A solution prepared from the stock standard solution that is used to calibrate the instrument response with respect to analyte concentration. One of the standards in the standard curve.

3.2 Dissolved Analyte (DA) -- The concentration of analyte in an aqueous sample that will pass through a 0.45-μm membrane filter assembly prior to sample acidification or other processing.

3.3 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether method performance is within acceptable control limits. This is basically a standard prepared in reagent water that is analyzed as a sample.

3.4 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or apparatus.

3.6 Linear Dynamic Range (LDR) -- The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.7 Method Detection Limit (MDL) -- The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

3.8 Reagent Water (RW) -- Type 1 reagent grade water equal to or exceeding standards established by American Society of Testing Materials (ASTM). Reverse osmosis systems or distilling units that produce 18 megohm water are two examples of acceptable water sources.

3.9 Refractive Index (RI) -- The ratio of the velocity of light in a vacuum to that in a given medium. The relative refractive index is the ratio of the velocity of light in two different media, such as sea or estuarine water versus reagent water. The correction for this difference is referred to as the refractive index correction in this method.

3.10 Stock Standard Solution (SSS) -- A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

4.0 Interferences

4.1 Interferences caused by copper, arsenate and silicate are minimal relative to the orthophosphate determination because of the extremely low concentrations normally found in estuarine or coastal waters. High iron concentrations can cause precipitation of and subsequent loss of phosphate from the dissolved phase. Hydrogen sulfide effects, such as occur in samples collected from deep anoxic basins, can be treated by simple dilution of the sample since high sulfide concentrations are most often associated with high phosphate values.[4]

4.2 Sample turbidity is removed by filtration prior to analysis.

4.3 Refractive Index interferences are corrected for estuarine/coastal samples (Section 12.2).

5.0 Safety

5.1 Water samples collected from the estuarine and/or ocean environment are generally not hazardous. However, the individual who collects samples should use proper technique.

5.2 Good laboratory technique should be used when preparing reagents. A lab coat, safety goggles, and gloves should be worn when preparing the sulfuric acid reagent.

6.0 Equipment and Supplies

6.1 Continuous Flow Automated Analytical System Consisting of:

6.1.1 Sampler.

6.1.2 Manifold or Analytical Cartridge equipped with 37°C heating bath.

6.1.3 Proportioning pump.

6.1.4 Colorimeter equipped with 1.5 X 50 mm tubular flow cell and a 880 nm filter.

6.1.5 Phototube that can be used for 600-900 nm range.

6.1.6 Strip chart recorder or computer based data system.

6.2 Phosphate-Free Glassware and Polyethylene Bottles

6.2.1 All labware used in the determination must be low in residual phosphate to avoid sample or reagent contamination. Washing with 10% HCI (v/v) and thoroughly rinsing with distilled, deionized water was found to be effective.

6.2.2 Membrane or glass fiber filters, 0.45 μm nominal pore size.

7.0 Reagents and Standards

7.1 Stock Reagent Solutions

7.1.1 Ammonium Molybdate Solution (40 g/L) -- Dissolve 20.0 g of ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}\bullet 4H_2O$, CASRN 12027-67-7) in approximately 400 mL of reagent water and dilute to 500 mL. Store in a plastic bottle out of direct sunlight. This reagent is stable for approximately three months.

7.1.2 Antimony Potassium Tartrate Solution (3.0 g/L) -- Dissolve 0.3 g of antimony potassium tartrate [$(K(SbO)C_4H_4O_6\bullet 1/2H_2O$, CASRN 11071-15-1] in approximately 90 mL of reagent water and dilute to 100 mL. This reagent is stable for approximately three months.

7.1.3 Ascorbic Acid Solution (18.0 g/L) -- Dissolve 18.0 g of ascorbic acid ($C_6H_6O_6$, CASRN 50-81-7) in approximately 800 mL of reagent water and dilute to 1 L. Dispense approximately 75 mL into clean polyethylene bottles and freeze. The stability of the frozen ascorbic acid is approximately three months. Thaw overnight in the refrigerator before use. The stability of the thawed, refrigerated reagent is less than 10 days.

7.1.4 Sodium Lauryl Sulfate Solution (30.0 g/L) -- Sodium dodecyl sulfate ($CH_3(CH_2)_{11}OSO_3Na$, CASRN 151-21-3). Dissolve 3.0 g of sodium lauryl sulfate (SLS) in approximately 80 mL of reagent water and dilute to 100 mL. This solution is the wetting agent and its stability is approximately three weeks.

7.1.5 Sulfuric Acid Solution (4.9 N) -- Slowly add 136 mL of concentrated sulfuric acid (H_2SO_4, CASRN 7664-93-9) to approximately 800 mL of reagent water. After the solution is cooled, dilute to 1 L with reagent water.

7.1.6 Stock Phosphorus Solution -- Dissolve 0.439 g of pre-dried (105°C for 1 hr) monobasic potassium phosphate (KH_2PO_4, CASRN 7778-77-0) in reagent water and

dilute to 1000 mL. (1.0 mL = 0.100 mg P.) The stability of this stock standard is approximately three months when kept refrigerated.

7.1.7 Low Nutrient Seawater -- Obtain natural low nutrient seawater (36 ppt salinity; <0.0003 mg P/L) or dissolve 31 g analytical reagent grade sodium chloride, (NaCl, CASRN 7647-14-5); 10 g analytical grade magnesium sulfate, ($MgSO_4$) CASRN 10034-99-8); and 0.05 g analytical reagent grade sodium bicarbonate, ($NaHCO_3$, CASRN 144-55-8), in 1 L of reagent water.

7.2 Working Reagents

7.2.1 Reagent A -- Mix the following reagents in the following proportions for 142 mL of Reagent A: 100 mL of 4.9 N H_2SO_4 (Section 7.1.5), 30 mL of ammonium molybdate solution (Section 7.1.1), 10 mL of antimony potassium tartrate solution (Section 7.1.2), and 2.0 mL of SLS solution (Section 7.1.4). Prepare fresh daily.

7.2.2 Reagent B -- Add approximately 0.5 mL of the SLS solution (Section 7.1.4) to the 75 mL of ascorbic acid solution (Section 7.1.3). Stability is approximately 10 days when kept refrigerated.

7.2.3 Refractive Reagent A -- Add 50 mL of 4.9 N H_2SO_4 (Section 7.1.5) to 20 mL of reagent water. Add 1 mL of SLS (Section 7.1.4) to this solution. Prepare fresh every few days.

7.2.4 Secondary Phosphorus Solution -- Take 1.0 mL of Stock Phosphorus Solution (Section 7.1.6) and dilute to 100 mL with reagent water. (1.0 mL = 0.0010 mg P.) Refrigerate and prepare fresh every 10 days.

7.2.5 Prepare a series of standards by diluting suitable volumes of standard solutions (Section 7.2.4) to 100 mL with reagent water. Prepare these standards daily. When working with samples of known salinity, it is recommended that the standard curve concentrations be prepared in low-level natural seawater (Section 7.1.7) diluted to match the salinity of the samples. Doing so obviates the need to perform the refractive index correction outlined in Section 12.2. When analyzing samples of varying salinities, it is recommended that the standard curve be prepared in reagent water and refractive index corrections be made to the sample concentrations (Section 12.2). The following dilutions are suggested.

mL of Secondary Phosphorus Solution (7.2.4)	Conc. mg P/L
0.1	0.0010
0.2	0.0020
0.5	0.0050
1.0	0.0100
2.0	0.0200
4.0	0.0400
5.0	0.0500

8.0 Sample Collection, Preservation and Storage

8.1 Sample Collection -- Samples collected for nutrient analyses from estuarine and coastal waters are normally collected using one of two methods: hydrocast or submersible pump systems. Filtration of the sample through a 0.45-μm membrane or glass fiber filter immediately after collection is required.

8.1.1 A hydrocast uses a series of sampling bottles (Niskin, Nansen, Go-Flo or equivalent) that are attached at fixed intervals to a hydro wire. These bottles are sent through the water column open and are closed either electronically or via a mechanical "messenger" when the bottles have reached the desired depth.

8.1.2 When a submersible pump system is used, a weighted hose is sent to the desired depth in the water column and water is pumped from that depth to the deck of the ship for processing.

8.1.3 Another method used to collect surface samples involves the use of a plastic bucket or large plastic bottle. While not the most ideal method, it is commonly used in citizen monitoring programs.

8.2 Sample Preservation -- After collection and filtration, samples should be analyzed as quickly as possible. If the samples are to be analyzed within 24 hr of collection, then refrigeration at 4°C is acceptable.

8.3 Sample Storage -- Long-term storage of frozen samples should be in clearly labeled polyethylene bottles or polystyrene cups compatible with the analytical system's automatic sampler (Section 6.1.1). If samples cannot be analyzed within 24 hr, then freezing at -20°C for a maximum period of two months is acceptable.[5-8]

9.0 Quality Control

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, the continued analysis of LRBs, laboratory duplicates, and LFBs as a continuing check on performance.

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (MDLs and linear dynamic range) and laboratory performance (analysis of QC samples) prior to analyses of samples using this method.

9.2.2 MDLs should be established using a low-level estuarine water sample fortified to approximately five

times the estimated detection limit.[3] To determine MDL values, analyze seven replicate aliquots of water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t)(S)$$

where, S = the standard deviation of the replicate analyses.

t = the Student's t value for n-1 degrees of freedom at the 99% confidence limit. t = 3.143 for six degrees of freedom.

MDLs should be determined every six months or whenever a significant change in background or instrument response occurs or when a new matrix is encountered.

9.2.3 Linear Dynamic Range (LDR) -- The LDR should be determined by analyzing a minimum of five calibration standards ranging in concentration from 0.001 mg P/L to 0.20, mg P/L across all sensitivity settings of the autoanalyzer. Normalize responses by dividing the response by the sensitivity setting multiplier. Perform the linear regression of normalized response vs. concentration and obtain the constants *m* and *b,* where *m* is the slope and *b* is the y-intercept. Incrementally analyze standards of higher concentration until the measured absorbance response, R, of a standard no longer yields a calculated concentration C_C, that is ± 10% of the known concentration, C, where $C_C = (R - b)/m$. That concentration defines the upper limit of the LDR for your instrument. Should samples be encountered that have a concentration that is ≥90% of the upper limit of the LDR, then these samples must be diluted and reanalyzed.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory Reagent Blank (LRB) -- A laboratory should analyze at least one LRB (Section 3.5) with each set of samples. LRB data are used to assess contamination from the laboratory environment. Should an analyte value in the LRB exceed the MDL, then laboratory or reagent contamination should be suspected. When LRB values constitute 10% or more of the analyte level determined for a sample, fresh samples or field duplicates of the samples must be prepared and analyzed again after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory Fortified Blank (LFB) -- A laboratory should analyze at least one LFB (Section 3.3) with each batch of samples. Calculate accuracy as percent recovery. If the recovery of the analyte falls outside the required control limits of 90-110%, the analyte is judged out of control and the source of the problem should be identified and resolved before continuing the analyses.

9.3.3 The laboratory must use LFB data to assess laboratory performance against the required control limits of 90-110% (Section 9.3.2). When sufficient internal performance data become available (usually a minimum of 20 to 30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{Upper Control Limit} = x + 3S$$

$$\text{Lower Control Limit} = x - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each 5 to 10 new recovery measurements, new control limits can be calculated using only the most recent 20 to 30 data points. Also, the standard deviation (S) data should be used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.4 Assessing Analyte Recovery - Laboratory Fortified Sample Matrix

9.4.1 A laboratory should add a known amount of analyte to a minimum of 5% of the routine samples or one sample per sample set, whichever is greater. The analyte concentration should be two to four times the ambient concentration and should be at least four times the MDL.

9.4.2 Calculate the percent recovery of the analyte, corrected for background concentrations measured in the unfortified sample, and compare these values with the values obtained from the LFBs.

Percent recoveries may be calculated using the following equation:

$$R = \frac{(C_s - C)}{S} x\ 100$$

where, R = percent recovery

C_S = measured fortified sample concentration (background + concentrated addition in mg P/L)

C = sample background concentration (mg P/L)

S = concentration in mg P/L added to the environmental sample.

9.4.3 If the recovery of the analyte falls outside the designated range of 90-110% recovery, but the laboratory performance for that analyte is in control, the fortified

sample should be prepared again and analyzed. If the result is the same after reanalysis, the recovery problem encountered with the fortified sample is judged to be matrix related, not system related.

10.0 Calibration and Standardization

10.1 Calibration (Refer to Sections 11.5 and 12.0).

10.2 Standardization (Refer to Section 12.2).

11.0 Procedure

11.1 If samples are frozen, thaw the samples to room temperature.

11.2 Set up manifold as shown in Figure 1. The tubing, flow rates, sample:wash ratio, sample rate, etc., are based on a Technicon AutoAnalyzer II system. Specifications for similar segmented flow analyzers vary, so slight adjustments may be necessary.

11.3 Allow both colorimeter and recorder to warm up for 30 min. Obtain a steady baseline with reagent water pumping through the system, add reagents to the sample stream and after the reagent water baseline has equilibrated, note that rise (reagent water baseline), and adjust baseline.

For analysis of samples with a narrow salinity range, it is advisable to use low nutrient seawater matched to sample salinity as wash water in the sampler in place of reagent water. For samples with a large salinity range, it is suggested that reagent wash water and procedure (Section 12.2) be employed.

11.4 A good sampling rate is approximately 40 samples/hr with a 9:1, sample:wash ratio.

11.5 Place standards (Section 7.2.5) in sampler in order of decreasing concentration. Complete filling the sampler tray with samples, LRBs, LFBs, and LFMs.

11.6 Commence analysis.

11.7 Obtain a second set of peak heights for all samples and standards with Refractive Reagent A (Section 7.2.3) being pumped through the system in place of Reagent A (Section 7.2.1). This "apparent" concentration due to coloration of the water should be subtracted from concentrations obtained with Reagent A pumping through the system.

12.0 Data Analysis and Calculations

12.1 Concentrations of orthophosphate are calculated from the linear regression obtained from the standard curve in which the concentrations of the calibration standards are entered as the independent variable and the corresponding peak height is the dependent variable.

12.2 Refractive Index Correction for Estuarine/ Coastal Systems

12.2.1 Obtain a second set of peak heights for all samples and standards with Refractive Reagent A (Section 7.2.3) being pumped through the system in place of Reagent A (Section 7.2.1). Reagent B (Section 7.2.2) remains the same and is also pumped through the system. Peak heights for the refractive index correction must be obtained at the same Standard Calibration Setting and on the same colorimeter as the corresponding samples and standards.[9]

12.2.2 Subtract the refractive index peak heights from the heights obtained for the orthophosphate determination. Calculate the regression equation using the corrected standard peak heights. Calculate the concentration of samples from the regression equation using the corrected sample peak heights.

12.2.3 When a large data set has been amassed in which each sample's salinity is known, a regression for the refractive index correction on a particular colorimeter can be calculated. For each sample, the apparent orthophosphate concentration due to refractive index is calculated from its peak height obtained with Refractive Reagent A (Section 7.2.3) and Reagent B (Section 7.2.2) and the regression of orthophosphate standards obtained with orthophosphate Reagent A (Section 7.2.1) and Reagent B (Section 7.2.2) for each sample. Its salinity is entered as the independent variable and its apparent orthophosphate concentration due to its refractive index in that colorimeter is entered as the dependent variable. The resulting regression equation allows the operator to subtract an apparent orthophosphate concentration when the salinity is known, as long as other matrix effects are not present. Thus, the operator would not be required to obtain the refractive index peak heights for all samples after a large data set has been found to yield consistent apparent orthophosphate concentrations due to salinity. An example follows:

Salinity (ppt)	Apparent orthophosphate conc. due to refractive index (mg P/L)
1	0.0002
5	0.0006
10	0.0009
20	0.0017

12.2.4 An example of a typical equation is:

mg P/L apparent PO_4^{3-} = 0.000087 X Salinity (ppt) where, 0.000087 is the slope of the line.

where, 0.000087 is the slope of the line.

12.3 Results should be reported in mg PO_4^{3-}- P/L or µg PO_4^{3-}- P/L.

mg PO_4^{3-} - P/L = ppm (parts per million)

µg PO_4^{3-} - P/L = ppb (parts per billion)

13.0 Method Performance

13.1 Single Analyst Precision -- A single laboratory analyzed three samples collected from Chesapeake Bay, Maryland, and East Bay, Florida. Seven replicates of each sample were processed and analyzed randomly throughout a group of 75 samples with salinities ranging from 3 to 36 ppt. The results were as follows:

Sample	Salinity (ppt)	Concentration (mg P/L)	Percent Relative Standard Deviation
1	36	0.0040	6.5
2	18	0.0024	10
3	3	0.0007	24

13.2 Multilaboratory Testing

13.2.1 This method was tested by nine laboratories using reagent water, high salinity seawater from the Sargasso Sea (36 ppt) and three different salinity waters from Chesapeake Bay, Maryland (8.3 ppt, 12.6 ppt, and 19.5 ppt). The reagent water and the Sargasso Seawater were fortified at four Youden pair concentrations ranging from 0.0012 mg P/L to 0.1000 mg P/L.[10] The Chesapeake Bay waters were fortified at three Youden pair concentrations ranging from 0.0050 mg P/L to 0.0959 mg P/L with the highest salinity waters containing the lowest Youden pair and the lowest salinity waters containing the highest Youden pair. Analysis of variance (ANOVA) at the 95% confidence level found no statistical differences between water types indicating that the refractive index correction for different salinity waters is an acceptable procedure. Table 1 contains the linear equations that describe the single-analyst standard deviation, overall standard deviation, and mean recovery of orthophosphate from each water type.

13.2.2 Pooled Method Detection Limit (p-MDL) -- The p-MDL is derived from the pooled precision obtained by single laboratories for the lowest analyte concentration level used in the multilaboratory study. The p-MDLs using reagent water and Sargasso Sea water were 0.00128 and 0.00093 mg P/L, respectively.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better. Laboratory Chemical Management for Waste Reduction,* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036, (202)872-4477.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel,* available from the American Chemical Society at the address listed in Section 14.2.

16.0 References

1. Murphy, J. and J.P. Riley. 1962. A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. *Analytica Chim. Acta 27,* 31-36.0.

2. Technicon Industrial Systems. 1973. Orthophosphate in Water and Seawater. Industrial Method 155-71 W. Technicon Industrial Systems, Tarrytown, NY 10591.

3. 40 CFR, 136 Appendix B. Definition and Procedure for the Determination of the Method Detection Limit. Revision 1.11.

4. Grasshoff, K., M. Ehrhardt, and K. Kremling. 1983. *Methods of Seawater Analysis.* Verlag Chemie, Federal Republic of Germany, 419 pages.

5. Klingamann, E.D. and D.W. Nelson. 1976. Evaluation of Methods for Preserving the Levels of Soluble Inorganic Phosphorus and Nitrogen in Unfiltered Water Samples. *J. Environ. Qual.,* 5:1, 42-46.

6. MacDonald, R.W. and F.A. McLaughlin. 1982. The Effect of Storage by Freezing on Dissolved Inorganic Phosphate, Nitrate, and Reactive Silicate for Samples from Coastal and Estuarine Waters. *Water Research,* 16:95-104.

7. Thayer, G.W. 1979. Comparison of Two Storage Methods for the Analysis of Nitrogen and Phosphorus Fractions in Estuarine Water. *Ches. Sci.,* 11:3, 155-158.

8. Salley, B.A., J.G. Bradshaw, and B.J. Neilson. 1986. Results of Comparative Studies of Preservation Techniques for Nutrient Analysis on Water Samples. VIMS, Gloucester Point, VA 23062. 32 pp.

9. Froelich, P.N. and M.E.Q. Pilson. 1978. Systematic Absorbance Errors with Technicon AutoAnalyzer II Colorimeters. *Water Research* 12: 599-603.

10. Edgell, K.W., E.J. Erb, and J.E. Longbottom, "Determination of Orthophosphate in Estuarine and Coastal Waters by Automated Colorimetric Analysis: Collaborative Study," submitted in November 1992 for publication in *Marine Chemistry.*

17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Single-Analyst Precision, Overall Precision and Recovery from Multilaboratory Study

Reagent Water	
(0.0012 - 0.100 mg P/L)	
Mean Recovery	X = 0.972C - 0.000018
Overall Standard Deviation	S_R = 0.033X + 0.000505
Single-Analyst Standard Deviation	S_r = 0.002X + 0.000448
Sargasso Sea Water	
(0.0012 - 0.100 mg P/L)	
Mean Recovery	X = 0.971C - 0.000002
Overall Standard Deviation	S_R = 0.021X + 0.000550
Single-Analyst Standard Deviation	S_r = 0.010X + 0.000249
Chesapeake Bay Water	
(0.005 - 0.100 mg P/L)	
Mean Recovery	
X = 1.019C - 0.000871	
Overall Standard Deviation	S_R = 0.066X + 0.000068
Single-Analyst Standard Deviation	S_r = 0.030X + 0.000165

C True value of spike concentration, mg P/L
X Mean concentration found, mg P/L, exclusive of outliers.
S_R Overall standard deviation, mg P/L, exclusive of outliers.
S_r Single-analyst standard deviation, mg P/L, exclusive of outliers.

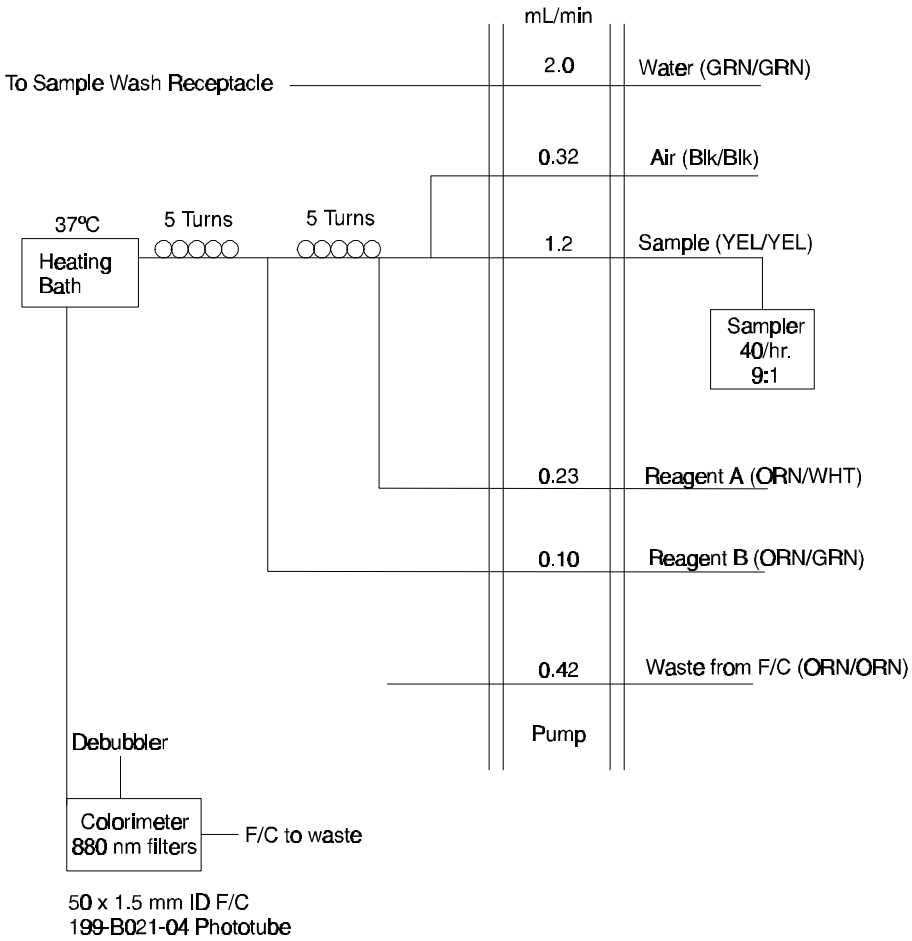

Figure 1. Manifold Configuration for Orthophosphate.