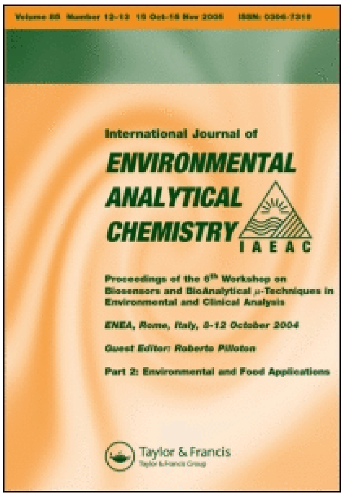

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Continuous Flow Analysis of Phosphate in Natural Waters Using Hydrazine as a Reductant

Jia-Zhong Zhang[a,b]; Charles J. Fischer[b]; Peter B. Ortner[b]
[a] Cooperative Institute of Marine and Atmospheric Studies, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL, USA [b] Ocean Chemistry Division, Atlantic Oceanographic and Meteorological Laboratory, National Oceanic and Atmospheric Administration, Miami, FL, USA

To cite this Article Zhang, Jia-Zhong , Fischer, Charles J. and Ortner, Peter B.(2001) 'Continuous Flow Analysis of Phosphate in Natural Waters Using Hydrazine as a Reductant', International Journal of Environmental Analytical Chemistry, 80: 1, 61 — 73

To link to this Article: DOI: 10.1080/03067310108044386

URL: http://dx.doi.org/10.1080/03067310108044386

Intern. J. Environ. Anal. Chem., Vol. 80(1), pp. 61-73
Reprints available directly from the publisher
Photocopying permitted by license only

CONTINUOUS FLOW ANALYSIS OF PHOSPHATE IN NATURAL WATERS USING HYDRAZINE AS A REDUCTANT

JIA-ZHONG ZHANG[ab]*, CHARLES J. FISCHER[b] and PETER B. ORTNER[b]

[a]Cooperative Institute of Marine and Atmospheric Studies, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA and [b]Ocean Chemistry Division, Atlantic Oceanographic and Meteorological Laboratory, National Oceanic and Atmospheric Administration, Miami, FL 33149, USA

(Received 4 September 2000; In final form 2 November 2000)

The use of hydrazine to reduce 12-molybdophosphoric acid to phosphomolybdenum blue in continuous flow analysis of phosphate in natural water samples is characterized. Using hydrazine in gas-segmented continuous flow phosphate analysis minimizes coating and silicate interference in comparison with using ascorbic acid. The addition of Sb to the molybdate reagent increases sensitivity at temperatures greater than 50°C but causes severe additional coating. The degree of coating was found to be a function of pH. Minimal coatings were achieved at a final solution pH of 0.5. Silicate interference was found to increase dramatically with color development temperature. At room temperature no detectable silicate interference was found. We recommend hydrazine in preference to ascorbic acid for gas-segmented continuous flow phosphate analysis with optimal reaction conditions of room temperature color development and a final solution pH of 0.5.

Keywords: Phosphate determination; hydrazine; silicate interference; coating; continuous flow analysis

INTRODUCTION

As an essential micronutrient required for plant growth in terrestrial and aquatic environment, phosphate is one of the most frequently analyzed compounds in soils, sediments and natural waters[1-5]. The most common analytical method for determining phosphate concentration is spectrophotometry. The technique relies upon the reaction of phosphate with molybdate in acidic solution to form 12-molybdophosphoric acid and subsequent reduction to phosphomolybdenum blue complex[6–18]. Several different reductants have been used to reduce the

---

* Corresponding author. Fax: +1-305-361-4392. Email: zhang@aoml.noaa.gov

12-molybdophosphoric acid. Stannous chloride was used as a reductant in the early 1930s[19,20]. The instability and substantial temperature, salinity dependencies of the resultant blue complex stimulated a search for more suitable reducing agents[21]. P-methyl aminophenol sulfate (metol) in conjunction with sulfite was proposed by Burton and Riley in 1956. However, their method required a boiling temperature to develop phosphomolybdenum blue color[21]. Ascorbic acid was first used as the reductant by Greenfield and Kalber in seawater analyses in 1954[22] and has been remained the most widely used reductant for phosphate analysis in seawater[18], freshwater and waste water[23] and ground water[24].

Today automated gas-segmented continuous flow analyzers are commonly used for phosphate determination to routinely analyze large numbers of samples. Coating and silicate interference have become primary concerns to those using continuous flow methods[25,29]. Coating results from the colloidal nature of the phosphomolybdenum blue, a heteropoly acid resulting from the color development reaction. During manual phosphate analyses, Burton and Riley reported that if the same flasks were used several times for color development, high and erratic values resulted. Moreover this adverse effect became more pronounced at high phosphate concentration[21]. In continuous flow phosphate analysis, phosphomolybdenum blue is readily adsorbed onto flowcell surfaces and subsequently release into the wash solution, resulting in carry-over to subsequent samples. The coating effect is evident in the printer record of the output signal since the ideal symmetrical sample peak is distorted to a highly asymmetric one with excessive tailing at the end. Coating significantly degrades both precision and sample throughput in automated analysis[25]. It is essential therefore to use a phosphate method that minimizes coating.

In addition to coating, any spectrophotometric method of phosphate determination relying upon the formation of phosphomolybdenum blue complex is subject to silicate interference in that both phosphate and silicate form similar blue complexes with molybdate[26–30]. Since silicate is often present at concentrations one to three orders of magnitude higher than phosphate[31–33] this interference is particularly severe in natural water samples. It is essential therefore to use a phosphate method that not only minimizes coating but also silicate interference.

Hydrazine sulfate was used as a reductant by King et al., Boltz and Lueck in the 1950's[13,34]. Bernhard and Wilhelms incorporated it into the Technicon Autoanalyzer method for continuous flow phosphate analysis in 1967[35]. Since Murphy and Riley's ascorbic acid method became popular in 1960's, however, few laboratories have used hydrazine in phosphate analysis. Although some oceanographers preferred to use hydrazine in phosphate analysis because they felt it reduced dye coating on the flow cell window[36], there have been no systematic studies directly comparing it with ascorbic acid nor defining how reaction conditions affect the degree of coating and silicate interference. Following are the results of the first systematic comparison of hydrazine and ascorbic acid performance in regard to both carryover and silicate interference in phosphate analysis under different reaction conditions.

EXPERIMENTAL

The analytical method we use is essentially similar to that of Bernhard and Wilhelms[35]. 12-molybdophosphoris acid is formed from the reaction of phosphate with molybdate in an acidic solution. The 12-molybdophosphoris acid is then reduced by hydrazine to form a phosphomolybdenum blue complex. The absorbance of the phosphomolybdenum blue complex is then measured at 830 nm on a Hewlett Packard 8453 spectrophotometer. For continuous flow analysis we use an Alpkem Flow Solution auto-analyzer. This consists of an autosampler, a peristaltic pump, an absorbance detector and a manifold containing mixing coils and a heater. The model 510 detector is a monochrometer capable of measuring absorbance from 0.0005 to 2 Absorbance Units Full Scale (AUFS) at working wavelengths from 190–800 nm. The flow cell in the detector has a pathlength of 5.5 mm and a volume of 15 μl. A flow diagram for phosphate analysis using hydrazine is shown in Figure 1. Sample and wash time are 90 and 30 seconds, respectively. Alpkem Softpac Plus software is used in the data processing.

The absorbance of the phosphomolybdenum blue complex was measured at the highest wavelength available with this detector -i.e., 800 nm. Since phosphomolybdenum blue complex in the absence of Sb has a maximum absorbance at 830 nm[9,12], measurements at 800 nm had to be corrected to 830 nm by measuring absorbance on a Hewlett Packard 8453 spectrophotometer at both 800 and 830 nm. For experiments in which Sb was added to the molybdate reagent, the absorbances of the phosphomolybdenum blue complex with Sb was measured at 710 nm well within the limits of our autoanalyzer's detector.

To study the effect of reaction temperature upon sensitivity, coating and silicate interference, reaction temperature was varied from 25 –70°C and controlled to ± 0.1°C. Effects of pH on the sensitivity, coating and silicate interference were studied by varying pH from 0.29 to 1.25 while the ratio of hydrogen ion to molybdenum (H^+/Mo) was held constant at about 70[25].

The extent of coating at various reaction conditions was estimated by calculating carry-over coefficients based upon readings of a high standard followed by two identical low standards (HLL scheme) as provided for within the Softpac Plus[37]. The apparent phosphate concentrations as a result of silicate interfer-

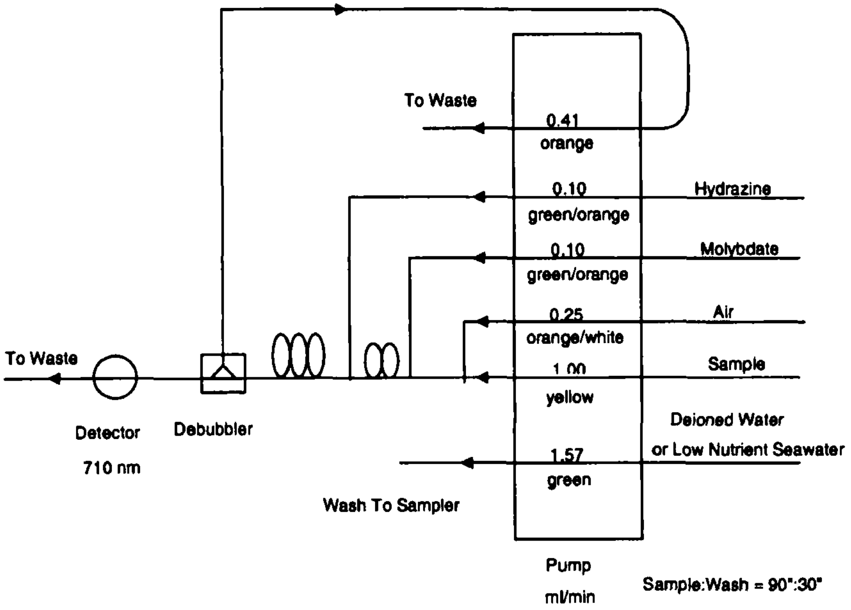

FIGURE 1 Flow diagram and manifold configuration for automated gas-segmented continuous flow phosphate analysis using hydrazine as a reductant

ence were calculated from absorbances measured in the samples containing silicate alone using a calibration curve generated from phosphate standards. The concentrations of phosphate standards (0.05–1 μM) used in each experiment were adjusted according to the magnitudes of silicate interference signals obtained under given temperature and pH conditions.

All reagents used were of analytical grade. Sodium dihydrogen phosphate was used to prepare the stock solution (2 mM) for the phosphate standards. Sodium hexaflourosilicate was used to prepare the silicate standards (2 mM). To ensure complete dissolution, 0.3761 g of sodium hexafluorosilicate was stirred in ~ 800 ml of water for 24 hours. The solution was transferred to a volumetric flask and then quantitatively diluted to the 1–1 mark. Working standards of phosphate (50 nM – 2 μM) and silicate (20–100 μM) were prepared daily by appropriate dilution. Working ammonium molybdate solutions were prepared daily by adding 2 ml of surfactant Dowfax to 100 ml of stock ammonium molybdate solution and mixing gently. Working hydrazine sulfate solution was prepared daily by dissolving 1.0 g hydrazine sulfate, $(NH_2)_2{\cdot}H_2SO_4$, (1% w/v, 0.062 M) in 100 ml of distilled water. To avoid contamination from glassware silicate, plastic containers were used for handling all standards, reagents and samples[38].

RESULTS AND DISCUSSION

The effect of Sb on phosphomolybdenum blue complex

The effect of Sb on phosphomolybdenum blue complex is best demonstrated in the absorbance spectra. When sample phosphate reacts with molybdate, they form a 12-molybdophosphoric acid that is subsequently reduced by a reducing agent, such as hydrazine or ascorbic acid, to phosphomolybdenum blue. Phosphomolybdenum blue has a single absorption maximum at 836 nm with a molar absorptivity about 26,000 $M^{-1}cm^{-1}$. Different reductants seem to produce similar spectrum (Figure 2), suggesting an identical or similar compound is formed during reduction irrespective of the reductant employed. When Sb is added to the molybdate reagent, however, the resulting complex has absorption maxima at 710 and 880 nm with molar absorptivities of 17,000 and 20,000 $M^{-1}cm^{-1}$, respectively (Figure 2). Again, similar spectra obtained from hydrazine and ascorbic acid suggest an identical or similar compound is obtained regardless of the reductant employed. Our results support earlier studies that Sb is readily incorporated into a Mo-Sb-P heteropoly acid compositionally distinguishable from Mo-P heteropoly acid. The stoichiometry of this reduced heteropoly acid was suggested to be $PSb_2Mo_{10}O_{40}$[12].

Formation of heteropoly acid was found to be sufficient fast even at room temperature, suitable for continuous flow analysis. In fact a steady state was approached within five minutes and absorbances were found to increase very slowly thereafter over prolonged reaction times (Figure 3). Since the mixing and reaction times in automated continuous flow analyses are usually less than 10 minutes, fast reaction kinetic are essential to permit sufficient color development. Markedly higher absorbance was obtained using hydrazine rather than ascorbic acid, suggesting hydrazine a stronger reducing agent that causes more substantial reduction of 12-molybdophosphoric acid and hence more intense color (Figure 3).

Effects of Sb, pH and temperature on overall sensitivity

Given the low concentrations of phosphate typical of natural waters, high sensitivity is essential. Overall methodological sensitivity was examined as a function of reagent, temperature and pH to define optimal reaction conditions.

Absorbances obtained from sample phosphate normalized to 1 μM concentration at different temperature and pH are given in Table I. No Sb was added. At any given pH absorbances increased slightly with temperature over a range of 30 to at least 70°C. Within a pH range of 0.30 to 1.25, absorbances changed little with pH. In contrast, with the addition of Sb, absorbances of phosphate vary with

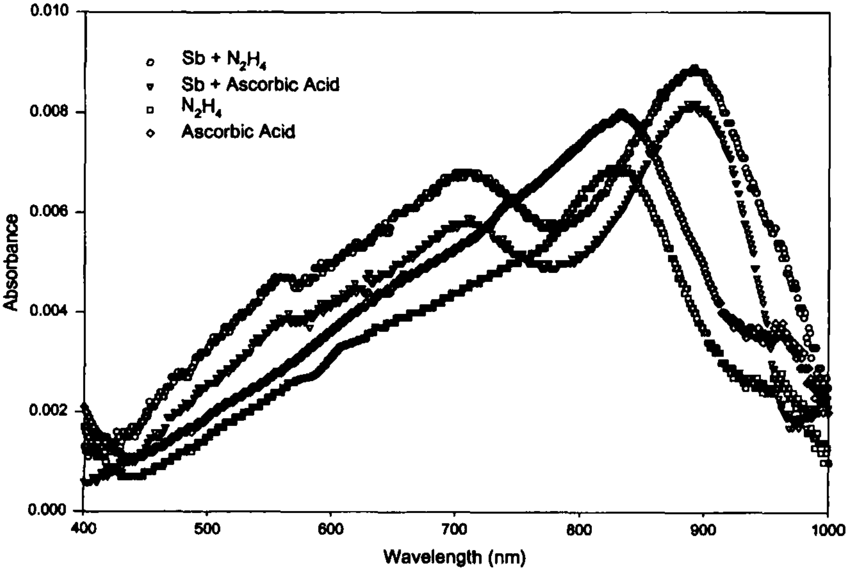

FIGURE 2 The absorption spectra of phosphomolybdum blue heteropoly acids formed with various reagents: a. Mo + Sb, reduced with hydrazine; b. Mo + Sb, reduced with ascorbic acid; c. Mo, reduced with hydrazine; d. Mo, reduced with ascorbic acid

pH and reach maxima at a pH of 0.51–0.78 (see Table II). Moreover with added Sb a strong and positive temperature dependence was observed at all pH conditions. The effect of Sb on the sensitivity of phosphate analysis using hydrazine as a reductant as function of temperature is shown in Figure 4. Higher sensitivities can be achieved using Sb at high reaction temperatures but as the subsequent experiments will illustrate, the disadvantages of using Sb may outweigh this advantage in potential sensitivity.

TABLE I Absorbances of phosphomolybdenum blue from the reaction of sample phosphate (normalized 1 μM) with molybdate reagent with no addition of Sb and subsequent reduction by hydrazine at different temperatures and pH of final mixture

pH	*Temperature (°C)*						
	30	*40*	*50*	*60*	*70*	*80*	*90*
0.30	0.0056	0.0061	0.0070	0.0084	0.0089	0.0086	
0.51	0.0064	0.0071	0.0082	0.0089	0.0091		
0.78	0.0058	0.0064	0.0070	0.0084	0.0083	0.0076	
1.00	0.0060	0.0066	0.0074	0.0081	0.0080	0.0076	0.0069
1.25	0.0055	0.0061	0.0068	0.0079	0.0082	0.0077	

TABLE II Absorbances of phosphomolybdenum blue from the reaction of sample phosphate (normalized 1 μM) with molybdate reagent with addition of Sb and subsequent reduction by hydrazine at different temperatures and pH of final mixture

pH	*Temperature (°C)*						
	30	*40*	*50*	*60*	*70*	*80*	*90*
0.30		0.0005	0.0006	0.0034	0.0084		
0.51	0.0069	0.0073	0.0082	0.0109	0.0275	0.0608	0.0907
0.78	0.0038	0.0049	0.0148	0.0295	0.0447		
1.00	0.0062	0.0067	0.0072	0.0082	0.0142	0.0225	

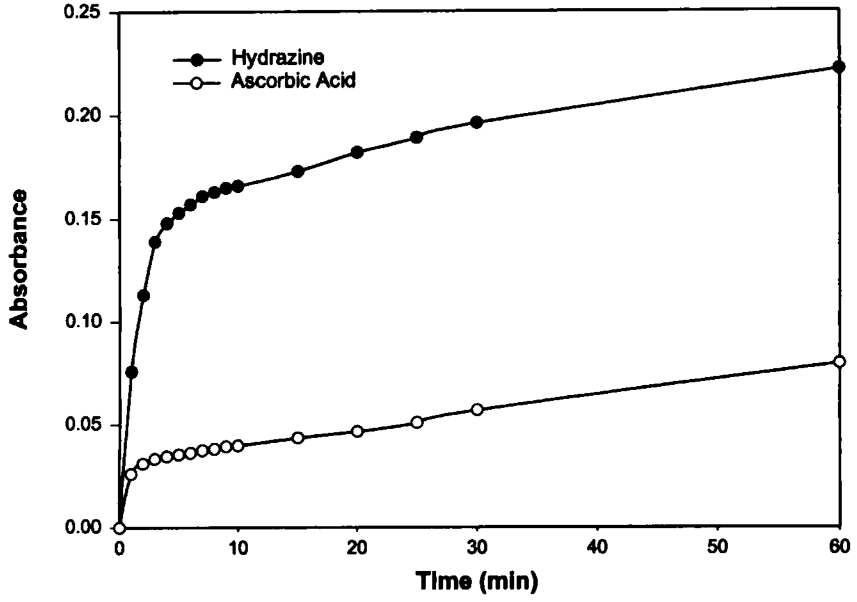

FIGURE 3 The kinetic of formation of phosphomolybdum blue heteropoly acids with different reducing agents: a. hydrazine; b. ascorbic acid

Effects of Sb, pH and temperature on coating

Addition of Sb to molybdate reagent was found to cause severe coating at all pH ranges tested (from 0.30 to 1.0, see Table III). The worst case of coating was found at low pH (0.3) with double-digit carryover coefficients at all temperatures tested (maximum 25% at 40°C). Carryover coefficients generally decreased with increasing temperature as found in an earlier study in which ascorbic acid was used as a

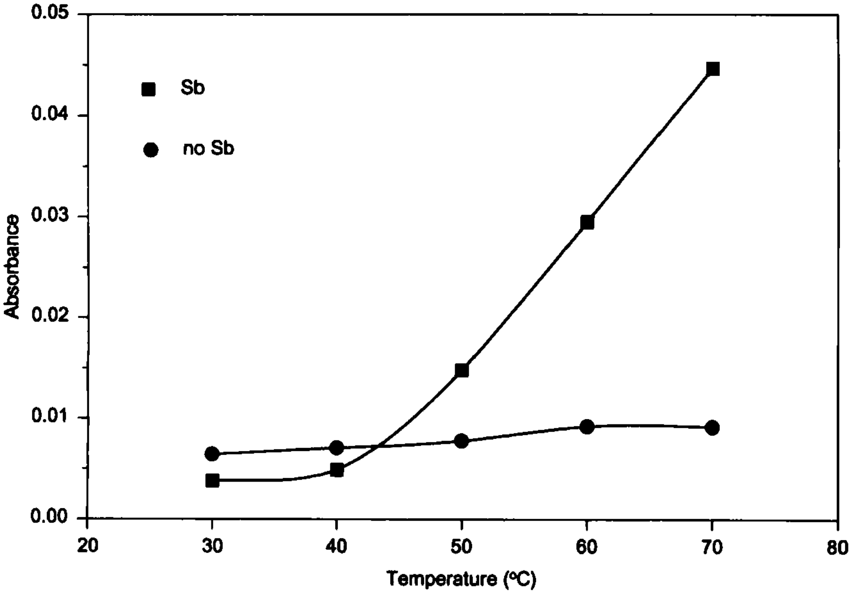

FIGURE 4 The effect of Sb on the sensitivity of phosphate analysis using hydrazine as a reductant as a function of temperature

reductant[25]. As discussed therein, this decrease is probably due to enhanced dissolution of colloidal phosphomolybdenum blue at elevated temperatures.

TABLE III Carry-over coefficients (%) in gas-segmented continuous flow phosphate analysis as a measure of coating at various temperatures and pH of final mixture with added Sb in the molybdate reagent. The carry-over coefficients are calculated based upon readings of a high standard followed by two identical low standards (HLL scheme) as provided for within the Softpac Plus[37]

pH	*Temperature (°C)*						
	30	*40*	*50*	*60*	*70*	*80*	*90*
0.30		25.0	22.3	17.9	11.2		
0.51	6.5	6.1	4.4	1.9	1.7	0.91	0.68
0.78	7.5	4.3	3.8	3.1	2.2		
1.00	10.5	7.7	5.3	2.3	1.7	1.5	

Without Sb in the molybdate reagent, coating was much reduced. At pH 0.30, the carry-over coefficient decreased from 5.11 to 1.16% as temperatures were elevated from 30 to 80°C (see Table IV). Minimal coating was seen at pH 0.51, where the carry-over coefficient was only 0.49% at 30°C and decreased to 0.12% at 70°C. These results suggest that the colloidal nature of phosphomolybdenum blue complex is altered by the presence of Sb in the complex. If the complex contains Sb it more readily forms colloids which cause severe coating problems. Without Sb the phosphomolybdenum blue forms a more soluble complex with correspondingly less coating. Coating problems associated with Sb are sufficiently severe at all pH and temperature conditions tested that Sb is not recommended despite its effect upon color development and consequent sensitivity.

TABLE IV Carry-over coefficients (%) in gas-segmented continuous flow phosphate analysis as a measure of coating at various temperatures and pH of final mixture with no added Sb in the molybdate reagent. The carry-over coefficients are calculated based upon readings of a high standard followed by two identical low standards (HLL scheme) as provided for within the Softpac Plus[37]

pH	*Temperature (°C)*						
	30	*40*	*50*	*60*	*70*	*80*	*90*
0.30	5.11	4.87	4.82	3.09	1.77	1.16	
0.51	0.49	0.42	0.38	0.21	0.12		
0.78	1.26	1.12	0.95	0.81	0.78	0.72	
1.00	1.42	0.51	0.63	0.73	0.28	0.22	0.16
1.25	1.58	1.25	0.95	0.81	0.78	0.72	

Reaction mixture acidity also seemingly affects phosphomolybdenum blue complex solubility as shown in Table IV. At pH 0.5 negligible coating effects were observed in comparison to both higher and lower pH values tested regardless of reaction temperature. Therefore, when hydrazine is used as a reductant at pH of 0.5 would appear to be optimal to minimize coating. Further study on the characterization of heteropoly acid and its solubility is needed to elucidate the effect of pH on coating.

Interference of silicate in the samples

Because the addition of Sb to the molybdate reagent causes severe coating problems in continuous flow analysis, all experiments on silicate interference were conducted without added Sb. The potential interference of silicate on phosphate analysis is quantified as in our previous study[25]. In summary, absorbances were measured in samples containing only silicate using the phosphate analytical method. In the absence of phosphate in the samples, absorbances measured by the detector can only result from the sequential reaction of sample silicate with

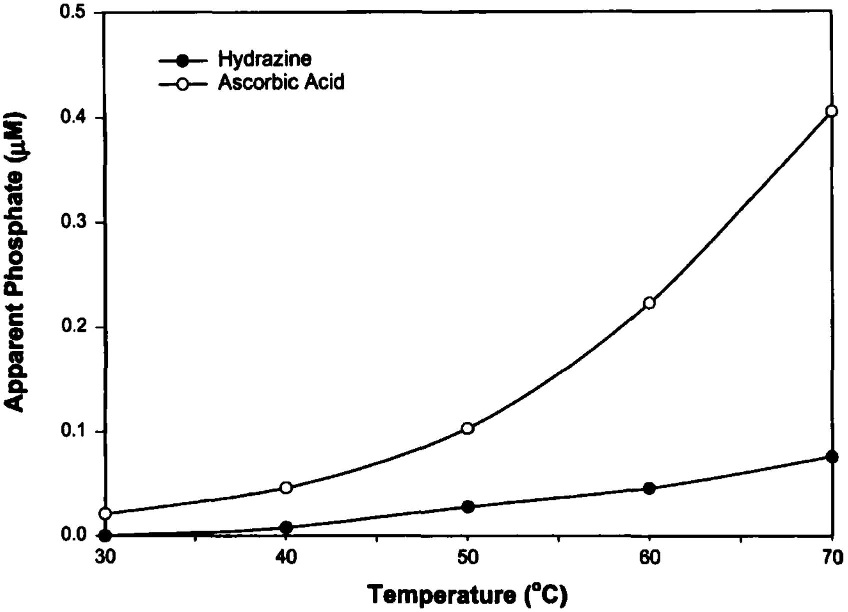

FIGURE 5 Comparison of hydrazine method and ascorbic acid method on the silicate interference (100 μM silicate) as a function of temperature

molybdate reagent and the subsequent reduction by hydrazine. These absorbances were then converted to apparent phosphate concentrations using a calibration curve constructed for phosphate standards measured under the same reaction conditions. Silicate concentrations tested ranged from 0–100 μM, which represent a range commonly found in natural waters.

The extent of apparent phosphate as a function of silicate concentrations over a temperature range from 30 to 70°C is shown in Table V. Apparent phosphate showed a minimal at pH 0.30 and 1.25 with slightly higher silicate interference at pH 0.51 and 0.78. The apparent phosphate concentrations increase with increasing reaction temperature but much less than that when ascorbic acid is used as the reductant (Figure 5). A 100 μM sample silicate results only 0.1 μM apparent phosphate at temperature higher than 70°C at pH 0.5 to 1.0. Note that virtually interference-free reactions can be achieved at low temperatures (≤ 30°C). Overall silicate interference is much less with hydrazine than ascorbic acid at all temperatures. By running the reaction at room temperature, the hydrazine method require no heater and provides a virtually silicate interference-free method for the determination of phosphate in natural waters.

TABLE V Apparent phosphate concentrations (in µM) measured in samples containing silicate alone at various silicate concentrations, temperatures and pH of final mixture. Molybdate reagent without Sb and hydrazine were used in phosphate analysis

Silicate (µM)	*Temperature (°C)*						
	30	*40*	*50*	*60*	*70*	*80*	*90*
				pH = 0.30			
0	0.000	0.000	0.000	0.000	0.000	0.000	
20	0.000	0.000	0.000	0.004	0.010	0.013	
40	0.000	0.000	0.002	0.008	0.015	0.019	
60	0.000	0.000	0.006	0.016	0.023	0.028	
80	0.000	0.000	0.008	0.020	0.030	0.040	
100	0.000	0.000	0.009	0.023	0.037	0.048	
				pH = 0.51			
0	0.000	0.000	0.000	0.000	0.000		
20	0.000	0.000	0.007	0.016	0.025		
40	0.000	0.000	0.012	0.024	0.040		
60	0.000	0.009	0.018	0.034	0.060		
80	0.000	0.014	0.024	0.048	0.080		
100	0.000	0.017	0.028	0.056	0.094		
				pH = 0.78			
0	0.000	0.000	0.000	0.000	0.000	0.000	
20	0.000	0.002	0.007	0.015	0.018	0.022	
40	0.000	0.005	0.012	0.032	0.035	0.044	
60	0.000	0.007	0.015	0.047	0.058	0.063	
80	0.000	0.009	0.019	0.050	0.068	0.087	
100	0.000	0.012	0.022	0.062	0.082	0.104	
				pH = 1.00			
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20	0.000	0.000	0.011	0.015	0.023	0.028	0.034
40	0.000	0.000	0.016	0.026	0.038	0.049	0.056
60	0.000	0.004	0.020	0.033	0.053	0.067	0.076
80	0.000	0.006	0.024	0.042	0.067	0.085	0.095
100	0.000	0.008	0.028	0.046	0.076	0.098	0.108
				pH = 1.25			
0	0.000	0.000	0.000	0.000	0.000	0.000	
20	0.000	0.000	0.000	0.000	0.000	0.005	
40	0.000	0.000	0.000	0.000	0.000	0.012	
60	0.000	0.000	0.000	0.000	0.005	0.022	
80	0.000	0.000	0.000	0.005	0.012	0.028	
100	0.000	0.000	0.000	0.011	0.025	0.037	

In summary, the hydrazine method can be used for automated continuous flow phosphate analysis and offers the considerable advantage of negligible silicate interference and minimal coating. Precision of hydrazine method is similar to ascorbic acid method with a relative standard deviation of 0.5% for replicate samples (n=8) containing 1 μM phosphate and the method detection limit is 0.005 μM phosphate. The suggested reaction condition for this method would be a final solution pH 0.5 and room temperature color development. The following recipe, along with the flow diagram in Figure 1, is recommended for automated gas-segmented continuous flow phosphate analysis in natural waters containing appreciable dissolved silicate:

1. *Stock Ammonium Molybdate Solution*: Dissolve 7.1 g of ammonium molybdate in 593 ml of 5 N H_2SO_4 solution, and then bring up to 1L with reagent water and mix. Store in a dark bottle at room temperature. If concentrated sulfuric acid is used, add 83 ml concentrated sulfuric acid slowly into 800 ml reagent water while mixing. Let the solution to cool to room temperature before adding ammonium molybdate. Dilute the solution to 1L with reagent water and mix.
2. *Working Hydrazine Sulfate Solution*: Dissolve 1.0 g hydrazine sulfate, $(NH_2)_2{\cdot}H_2SO_4$, (1% w/v) in 100 ml of reagent water. Prepare it daily.
3. *Working Ammonium Molybdate Solution*: Add 2 ml of Dowfax in 100 ml of stock ammonium molybdate solution, and mix gently.

Acknowledgements

This work was supported by the NOAA's South Florida Ecosystem Restoration Prediction and Modeling Program with Coastal Ocean Program funds and the NOAA's Global Carbon Cycle program with Global Climate Change Program funds. This research was carried out, in part, under the auspices of the Cooperative Institute of Marine and Atmospheric Studies (CIMAS), a joint institute of the University of Miami and the National Oceanic and Atmospheric Administration, cooperative agreement #NA67RJ0149.

References

[1] J.H. Ryther and W.M. Dunstan, *Science*, **171**,1008–1013 (1971).
[2] S.V. Smith and M.J. Atkinson, *Nature*, **307**, 626–627 (1984).
[3] S.V. Smith, *Limnol. Oceanogr.*, **29**, 1149–1160 (1984).
[4] D.W. Schindler, *Science*, **195**, 260–262 (1977).
[5] M.D. Krom, N. Kress, S. Brenner and L.I. Gordon, *Limnol. Oceanogr.*, **36**, 424–432 (1991).
[6] D.F. Boltz, in: M. Hammann (Ed.), *Analytical Chemistry of Phosphorus Compounds*, Wiley, New York, 1972, pp. 9–65.
[7] J.E. Harwood, and W.H.J. Hattingh In E.J. Griffith, A.M. Beeton, J.M. Spencer and D.J. Michell (Eds.), *Environmental Phosphorus Handbook*, Wiley & Sons, New York, 1973, pp. 289–302.

[8] J. Murphy and J.P. Riley, *J. Mar. Biol. Ass. U.K.*, **37**, 9–14 (1958).
[9] J. Murphy and J.P. Riley, *Anal. Chem. Acta*, **27**, 31–36 (1962).
[10] K.M. Chan and J.P. Riley, *Deep-Sea Res.*, **13**, 467–471 (1966).
[11] S.R. Crouch and H.V. Malmstadt, *Anal. Chem.*, **39**, 1084–1089 (1967).
[12] J.E. Going and S.J. Eisenreich, *Anal. Chim. Acta*, **70**, 95–106 (1974).
[13] D.F. Boltz, C.H. Lueck, R.J. Jakubiec, in: D.F. Boltz, J.A. Howell (Eds.), *Colorimetric Determination of nonmetals, Chemical Analysis, Vol. 8*, 2nd edn., Wiley, New York, 1978, pp. 337–369.
[14] O. Broberg and K. Pettersson, *Hydrobiologia*, **170**, 45–59 (1988).
[15] S.-C. Pai and C.-C. Yang, *Anal. Chim. Acta*, **229**, 115–120 (1990).
[16] L. Drummond and W. Maher, *Anal. Chim. Acta*, **302**, 69–74 (1995).
[17] A. Sjosten and S. Blomqvist, *Water Res.*, **31**, 1818–1823 (1997).
[18] H.P. Hansen and F. Koroleff, in K.Grasshoff, K. Kremling and M. Ehrhardt, (Eds.), *Methods of Seawater Analysis*, 3rd edn., Weinheim, Wiley-VCH, 1999, pp. 159–228.
[19] W.R.G. Atkins, *J. Mar. Biol. Ass. U.K.*, **13**, 119–150 (1923).
[20] H.W. Harvey, *J. Mar. Biol. Ass. U.K.*, **27**, 337–359 (1948).
[21] D.J. Burton and J.P. Riley, *Mikrochimica Acta*, **9**, 1350–1365 (1956).
[22] L.J. Greenfield and F.A. Kalber, *Bull. Mar. Sci. Gulf Caribb.*, **4**, 323–335 (1954).
[23] *Standard methods for the examination of water and wastewater.* 1995. APHA. Washington, D.C. 1193 pp.
[24] R.C. Antweiler, C.J. Patton and H.E. Taylor, US Geological Survey, 1996, Open-File Report 93–638.
[25] J.-Z. Zhang, C.J. Fischer and P.B. Ortner, *Talanta*, **49**, 293–304 (1999).
[26] J.D. Burton, *Water Res.*, **7**, 291–307 (1973).
[27] A. Henriksen, *Analyst*, **91**, 290–291 (1966).
[28] R.E. Stauffer, *Anal. Chem.*, **55**, 1205–1210 (1983).
[29] S. Blomqvist, K. Hjellstrom and A. Sjosten, *Intern. J. Environ. Anal. Chem.*, **54**, 31–43 (1993).
[30] J.D. Ingle, Jr. and S.R. Crouch, *Anal. Chem.*, **43**, 7–10 (1971).
[31] G.E. Hutchinson, *Treatise on Limnology, Vol. 1*, 1957, Wiley, New York.
[32] C.P. Spencer, in J.P. Riley, and G. Skirrow (Eds.) *Chemical Oceanography*, Vol 2, 2nd edn. Academic Press, London, 1975, pp245–300.
[33] M.S.Quinby-Hunt, R.D. McLaughlin and A.T. Quintanilha (Eds.) *Instrumentation for Environmental Monitoring: Water*, Vol. 2, Wiley-Interscience, 1983, pp. 185–228.
[34] J.E. King, T.S. Austin, and M.S. Doty, *Spc. Sci. Rep., U.S. Fish Wildl. Serv., Fish.* **No.201**, Washinton, D.C., 1957, 155 pp.
[35] H. Bernhard, and A. Wilhelms Technicon Symp., 1967, Vol. I, 386.
[36] Gordon, L.I., Joe C. Jennings, Jr., Andrew A. Ross, and James M. Krest. OSU Coll. of Oc. Descr. Chem. Oc. Grp. Tech. Rpt. 93-1(1993).
[37] J.-Z. Zhang, *J. Automatic Chem.*, **19**, 205–212 (1997).
[38] J.-Z. Zhang, C. Fischer and P.B. Ortner, *Water Res.*, **33**, 2879–2883 (1999).