Evaluation of a number of methods for the determination of trace amounts of phosphates with flow injection analysis (FIA)

JF van Staden* and J van der Merwe,
Department of Chemistry, University of Pretoria, Pretoria 0002, South Africa

Abstract

Four different FIA/spectrophotometric analytical systems, namely tin(II) chloride ($SnCl_2$), ascorbic acid, malachite green and a rhodamine B methods, were optimised and evaluated in order to find the lowest detectable limit for each for the determination of trace amounts of phosphate on a routine basis. The $SnCl_2$ analytical system gave the best overall results. A low detection limit (2 µg/ℓ) combined with a relatively large linear working range (1 to 400 µg/ℓ) made this method superior to the others. The relatively unstable nature of $SnCl_2$ solutions together with blockages that occurred due to high concentration ranges are, however, factors to be considered when employing the method in routine laboratories.

Introduction

The nutritional needs of plants depend mainly on various concentrations of phosphates. A large number of commercially useful compounds contain phosphates in varying forms. Phosphates are added to boiler feed water to prevent scaling and are an essential constituent of fertilisers, of the body fluids, of soil and plants, and are also found in many industrial products from steels to detergents. The eutrophication of many natural waters has been attributed to the presence of appreciable concentrations of phosphate, so that many spectrophotometric determinations are performed to detect contamination or to control treatment of industrial effluent. Soluble orthophosphate and polyphosphates, in addition to organic-bound phosphates, may be found in water samples.

A variety of flow injection methods have been developed for the determination of phosphate in water. Of these methods, flow injection spectrophotometry is the best known and is currently most often used in various routine laboratories (Ruzicka and Hansen, 1975; 1988; Valcarcel and Luque de Castro, 1987; Ueno and Kina, 1983; Karlberg and Pacey, 1989; and Moeller, 1988). Flow injection voltammetry with voltammetric detection and reversed flow injection analysis also formed the basis of a procedure designed by Fogg and Bsebsu (1981; 1982; 1984).

The spectrophotometric determination of phosphate is based on the complexation of orthophosphate with acidic molybdate. The two main spectrophotometric methods available for phosphate determination are either the yellow coloured vanadomolybdate procedure for relatively high phosphate concentrations (Basson et al., 1981) or the molybdenum blue procedure for relatively low phosphate concentrations (Pauer et al., 1988). The molybdenum blue method, based on the reaction between orthophosphate and molybdate in an acidic medium to form a molybdophosphoric acid, is the most commonly used. Selective reduction of the molybdophosphoric acid produces a substance with a blue colour. Various reducing agents have been reported for the reduction of the phosphomolybdate complex to molybdenum blue, including hydrazine sulphate and sodium sulphite (Boltz, 1958); hydroquinone, iron(II) sulphate and tin(II) chloride (Jackson, 1962); and ascorbic acid (Lacy, 1965).

In a developing country with limited water resources it is necessary to develop simple, economic methods to analyse pollutants on the µg level. The growth of living organisms in water can begin when orthophosphate concentrations reach a concentration of 6.5 x 10^{-7} mol/ℓ (20 µg/ℓ P). It is, therefore, of utmost importance to be able to determine phosphate concentrations at this level. In the molybdenum blue procedure for relatively low phosphate concentrations the phosphomolybdate complex is reduced by either $SnCl_2$ or ascorbic acid to form a product which is detected. It is also possible to determine phosphate by complexing the phosphomolybdate-complex with malachite green or rhodamine B. The main objective of this study was to develop a method by which these low phosphate concentrations could be determined on a routine basis. Four flow injection analysis (FIA)-spectrophotometric methods for phosphate determination were optimised using univariate and simplex optimisation (Deming and Morgan, 1973; 1987; Moore, 1991; Moore and Böhmer, 1991; Massart et al., 1978; Malinowski and Howery, 1980; Massart et al., 1988; Meier and Zünd, 1993).

Experimental

Four different FIA methods were optimised and evaluated; $SnCl_2$, ascorbic acid, malachite green and a rhodamine B method.

Instrumentation

Flow system

Schematic diagrams of the different flow systems used, are outlined in Figs. 1, 2, 3 and 4. The manifolds consisted of Tygon tubing (inside dia. of 0.50 mm) cut into the required lengths and wound around glass tubes with an outside dia. of 10 mm. The following equipment also formed part of the FIA systems: Cenco and Gilson minipuls peristaltic pumps (operating at 10 r/min) were used to supply the different reagent streams and VICI 10 port multi-functional valves were used for injection of the samples. The valves, peristaltic pumps and the uv/vis detector were coupled to a computer. The whole FIA system in each procedure was controlled from the computer with a *FlowTEK* program (Marshall and Van Staden, 1992).

*To whom all correspondence should be addressed.
☎(012) 420-2515; fax: (012) 43-2863; e-mail: jvstaden@scientia.up.ac.za
Received 17 July 1996; accepted in revised form 28 October 1996.

Detector

A Unicam 8625 uv/vis spectrophotometer equipped with a 10-mm Hellma type flow-through cell (volume: 80 µℓ) was used as the detector in all four methods. The output signal of the detector was fed to the *FlowTEK* (Marshall and Van Staden, 1992) program for data processing.

Reagents and solutions

All reagents were prepared from AR grade chemicals unless specified otherwise. Aqueous solutions were prepared with doubly distilled, deionised water. Filtrations were done through an 0.45 µm filter paper. All solutions were degassed before use.

Phosphate stock solution

Weigh accurately 0.4390 g of sodium dihydrogen phosphate, dried at 105°C for 2 h, and dissolve in 500 mℓ water. Dilute quantitatively to 1 ℓ with doubly distilled, deionised water. The stock solution contains 100 mg/ℓ PO_4^{3-}-P. Prepare working standard phosphate solutions by suitable dilution of the stock solution. Both the stock solution and phosphate standards must be preserved using 20 mg/ℓ mercury(II) chloride.

$SnCl_2$ method

Ammonium molybdate solution

Dissolve 1.62 g of ammonium heptamolybdate-tetrahydrate and 0.0212 g sodium laurel sulphate in water and dilute to 500 mℓ with water.

$SnCl_2$ solution

Dissolve 0.10 g of $SnCl_2$ and 1.0 g of hydrazinium sulphate in 400 mℓ water (Purged with N_2-gas and degassed). Add 10 mℓ of a 98% sulphuric acid solution and dilute to 500 mℓ with water (Purged with N_2-gas and degassed). Transfer the solution to an amber bottle and cover the bottle with plastic film to protect from O_2. As the $SnCl_2$ is subject to oxidation by O_2 in acid solution, the $SnCl_2$ solutions should be freshly prepared daily.

Ascorbic acid method

Ammonium molybdate solution

Dissolve 5.0 g of ammonium heptamolybdate-tetrahydrate and 0.30 g of $K(SbO)C_6H_4O_6$ in water and add 22 mℓ of a 98% sulphuric acid solution. Dilute quantitatively to 500 mℓ with water.

Ascorbic acid solution

Dissolve 25 g of ascorbic acid and dilute to 250 mℓ with water.

Malachite green method

Malachite green and ammonium molybdate solution

Dissolve 0.070 g of malachite green and 8.6 g of ammonium heptamolybdate-tetrahydrate in water. Add 35 mℓ of a 98% sulphuric acid solution and mix well. Add 120 mℓ of a 98% ethanol solution. Dilute to 500 mℓ with water. Filter the solution through a 0.45 µm filter paper.

Carrier stream

Dilute 15 mℓ of a 98% sulphuric acid solution to 2 ℓ with water.

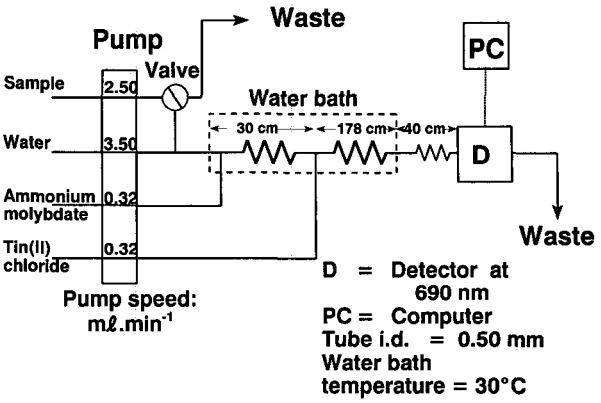

Figure 1
Schematic flow diagram of the FIA/spectrophotometric analytical system employing the $SnCl_2$ method. Valve loop size = 390 µℓ. Tube i.d. = 0.50 mm. Tube lengths are given in cm.

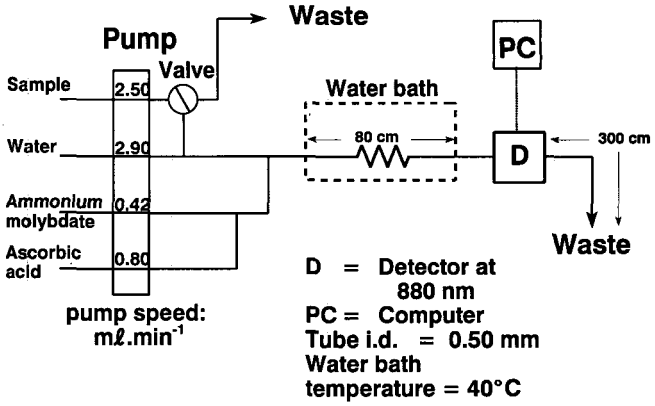

Figure 2
Schematic flow diagram of the FIA/spectrophotometric analytical system employing the ascorbic acid method. Valve loop size = 465 µℓ. Tube i.d. = 0.50 mm. Tube lengths are given in cm.

Rhodamine B method

Rhodamine B and ammonium molybdate solution

Dissolve 0.010 g of rhodamine B and 25 g of ammonium heptamolybdate-tetrahydrate in water. Add 40 mℓ of a 98% sulphuric acid solution. Dilute to 1 ℓ with water. Filter the solution through an 0.45 µm paper filter.

0.1 % PVA solution

Dissolve 1.0 g of polyvinyl alcohol in 1 ℓ water.

Results and discussion

Optimisation of the four methods

Sensitivity

The main objective of the study was to find the lowest detectable limit for each of the four flow injection-spectrophotometric methods and to evaluate the methods for the determination of trace amounts of phosphate on a routine basis. To obtain the

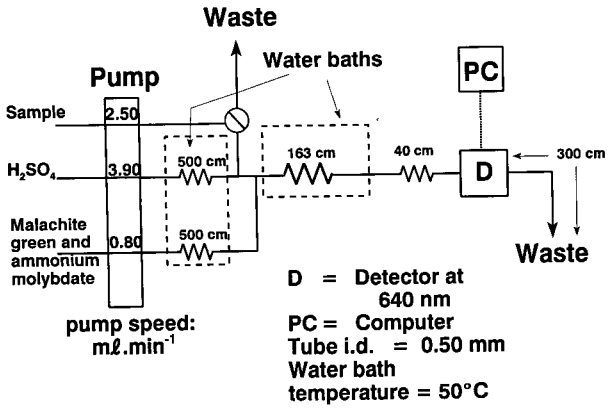

Figure 3

Schematic flow diagram of the FIA/spectrophotometric analytical system employing the malachite green method. Valve loop size = 393 µℓ. Tube i.d. = 0.50 mm. Tube lengths are given in cm.

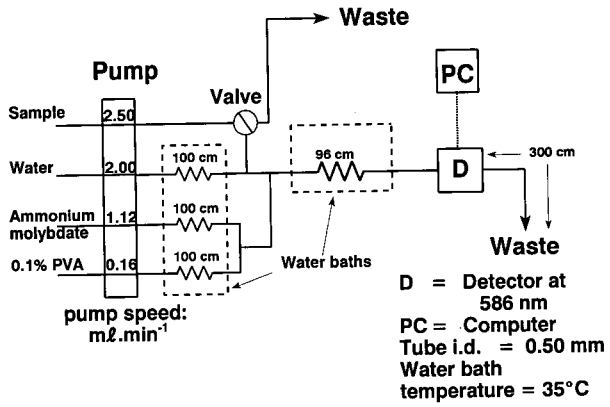

Figure 4

Schematic flow diagram of the FIA/spectrophotometric analytical system employing the rhodamine B method. Valve loop size = 140 µℓ. Tube i.d. = 0.50 mm. Tube lengths are given in cm.

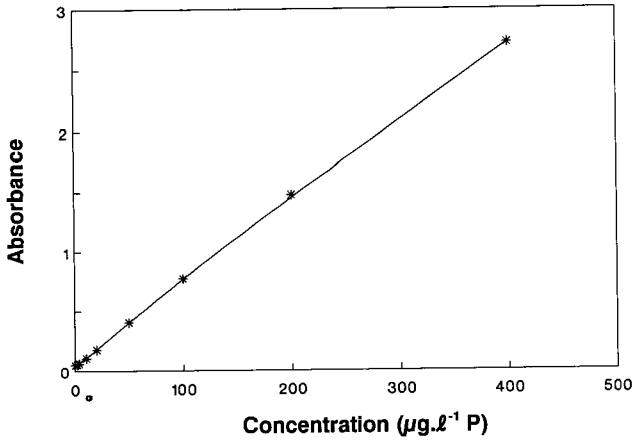

Figure 5

Calibration curve obtained for the determination of trace amounts of phosphate using the tin(II) chloride method.

optimum sensitivity, the effects of physical and chemical parameters such as sample volume, line length, coil diameter, flow rates, reagent concentrations and temperature were studied. Of the various methods of optimisation (Deming and Morgan, 1973; 1987; Moore, 1991; Moore and Böhmer, 1991; Massart et al., 1978; Malinowski and Howery, 1980; Massart et al., 1988; Meier and Zünd, 1993) i.e. the univariate, multivariate or simplex optimisation, the univariate search (where each variable was optimised, while the others were kept constant) was first used to give a preliminary optimised condition. The parameters were then further optimised using simplex optimisation (Deming and Morgan, 1973; 1987; Moore, 1991; Moore and Böhmer, 1991; Massart et al., 1978). Certain factors, however, like the availability of transmission tubing in specific fixed inner diameters and pump tubing at fixed flow rates restricted optimisation in this regard to a certain extent. In the **optimised $SnCl_2$ method**, outlined in Fig. 1, 390 µℓ of an aqueous sample was injected into a water carrier stream (flow rate 3.5 mℓ/min), merged with an ammonium molybdate solution added at a flow rate of 0.32 mℓ/min and mixed in a mixing coil (30 cm x 0.5 mm i.d.) at a constant temperature of 30°C. The complex formed was then reduced to molybdenum blue at a constant temperature of 30°C in a 178 cm x 0.5 mm i.d. mixing coil after addition of a $SnCl_2$ solution at a flow rate of 0.32 mℓ/min. The product was further mixed in a mixing coil of 40 cm x 0.5 mm i.d. before detected at 390 nm in a 80 µℓ Hellma type flow cell. In the **optimised ascorbic acid method**, illustrated in Fig. 2, 465 µℓ of an aqueous sample was injected into a water carrier stream (flow rate 2.90mℓ/min) and merged with a premixed ammonium molybdate (flow rate 0.42 mℓ/min) and an ascorbic acid solution (flow rate 0.80 mℓ/min). The resulting stream was homogenised and processed in a mixing coil (80 cm x 0.5 mm i.d.) at a constant temperature of 40°C. The product was detected at 880 nm in a 80 µℓ Hellma type flow cell. In the **optimised malachite green method**, shown in Fig. 3, 393 µℓ of an aqueous sample was injected into an acidified carrier stream (flow rate 3.90 mℓ/min), merged with a malachite green and ammonium molybdate solution added at a flow rate of 0.80 mℓ/min and mixed in a 163 cm x 0.5 mm i.d. mixing coil at a constant temperature of 50°C. The product was channelled via a 400 mm x 0.5 mm i.d. coil to a detector where the product was measured at 640 nm in a 80 µℓ Hellma type flow cell. In the **optimised rhodamine B method**, given in Fig. 4, 140 µℓ on an aqueous sample was injected into a water carrier stream (flow rate 2.00 mℓ/min) and merged with a premixed rhodamine B and ammonium molybdate solution (flow rate 1.12 mℓ/min) and 0.1% polyvinyl alcohol solution (flow rate 0.16 mℓ/min). The resulting stream was homogenised and processed in a mixing coil (960 mm x 0.5 mm i.d.) at a constant temperature of 35°C. The product was detected at 586 nm in a 80 µℓ Hellma type flow cell.

Linearity

The linearity of the four flow injection-spectrophotometric methods was studied under optimum conditions. Figure 5 illustrated the calibration curve obtained for the determination of trace amounts of phosphate using the $SnCl_2$ method. The relationship obtained for peak height vs. phosphate concentration was:

$$y = 0.006753x + 0.05892;\ r = 0.9993$$

where y = peak height and x = phosphate concentration in µg/ℓ. The correlation coefficient (r) indicated that the method was linear for a phosphate concentration ranging between 1 and 400

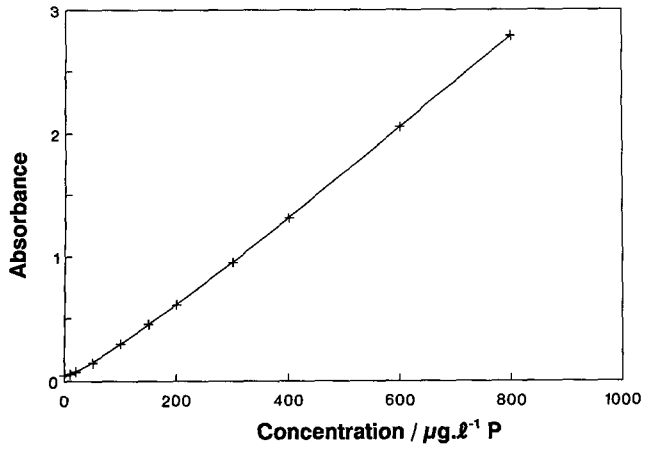

Figure 6
Calibration curve obtained for the determination of trace amounts of phosphate using the ascorbic acid method

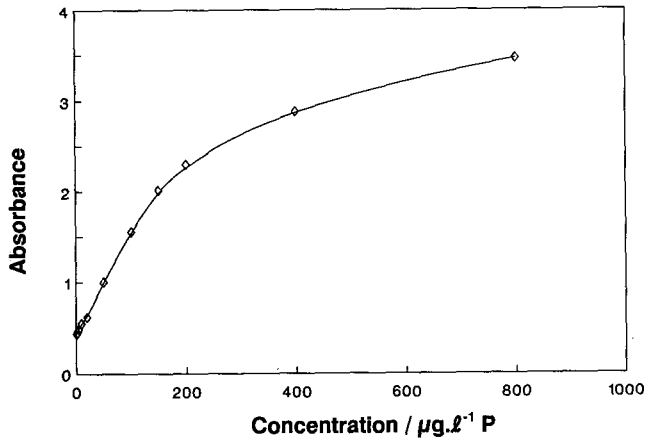

Figure 7
Calibration curve obtained for the determination of trace amounts of phosphate using the malachite green method

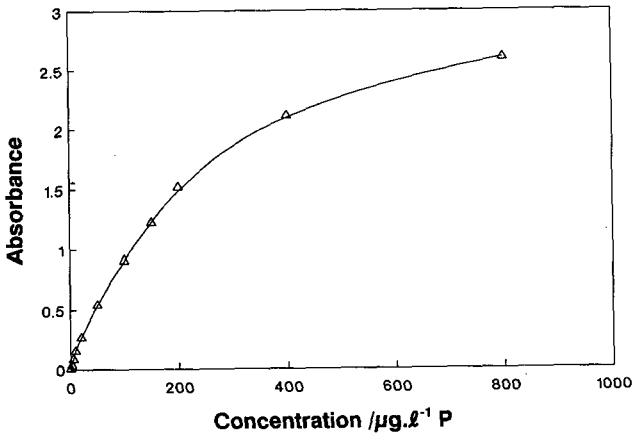

Figure 8
Calibration curve obtained for the determination of trace amounts of phosphate using the rhodamine B method

µg/ℓ. The calibration curve obtained for the determination of trace amounts of phosphate using the ascorbic acid method is outlined in Fig. 6. According to Fig. 6 the sensitivity decreased on average from a relatively high concentration range until a concentration of 20 µg/ℓ is reached. Below 20 µg/ℓ the slope of the calibration curve changed slightly. This was clearly indicated when comparing the difference in gradient of the equations when looking at the relationship between the peak height and phosphate concentration for the two concentration ranges. The equations were:

$$y = 0.003453x - 0.02633; r = 0.9987$$

for the concentration range between 1 and 800 µg/ℓ and:

$$y = 0.003513x - 0.05695; r = 0.9993$$

for the concentration range between 20 and 800 µg/ℓ where y = peak height and x = phosphate concentration in µg/ℓ. The correlation coefficient (r) clearly showed that the calibration curve is more linear between 20 and 800 µg/ℓ. The equations, furthermore, showed that when linearity was applied the calibration curve intercepted the y-axis at a negative concentration. This is, however, not the case as seen from Fig. 6. The curve should, however, only be applicable between 20 and 800 µg/ℓ. For themalachite green method the calibration curve is outlined in Fig. 7. In this case the calibration curve was only linear between 1 and 100 µg/ℓ with a relationship between peak height and phosphate concentration of:

$$y = 0.01134x + 0.4277; r = 0.9999$$

with y = peak height and x = phosphate concentration in µg/ℓ. It is obvious from the calibration curve (Fig. 7) that a relatively large background was encountered with the particular procedure. This is also clear from the intercept in the equation above. However, due to the fact that both standard solutions and samples were subjected to exactly the same conditions in the flow injection system, the method was applicable in the concentration range 1 to 100 µg/ℓ. For the rhodamine B method the calibration curve is illustrated in Fig. 8. The results obtained showed a linearity between 0 and 10 µg/ℓ with a relationship between peak height and phosphate concentration of:

$$y = 0.01493x + 0.01171; r = 0.9994$$

where y = peak height and x = phosphate concentration in µg/ℓ.

Evaluation of the four methods

The four flow injection-spectrophotometric analytical systems were evaluated according to accuracy, precision, detection limit, range of linearity, sample interaction (carry-over), interferences, sampling rate and general problems experienced. The results obtained are summarised in Table 1. All the water samples and standards were preserved with 20 mg/ℓ mercury(II)chloride throughout the investigation. It is, however, very difficult to preserve phosphate in trace amounts over long periods. Different water samples were therefore used in the evaluation of the four methods.

Accuracy

Accuracy was determined in terms of recovery using real samples in the presence of interfering ions normally found in the samples

TABLE 1
SUMMARY OF THE RESULTS OBTAINED IN THE EVALUATION OF THE FLOW INJECTION-SPECTROPHOTOMETRIC ANALYTICAL SYSTEMS

Method	$SnCl_2$	Ascorbic acid	Malachite green	Rhodamine B
Linear range (µg/ℓ⁻¹ P)	1 - 400	20 - 800	1 - 100	0 - 10
Detection limit (µg/ℓ⁻¹ P)	2	15	2.5	1.24
%RSD	3.7	10	3.5	2.7
Accuracy (%)	96	95	95	94
Carry-over (%)	1.05	0.50	1.3	1.3
Sampling rate (samples·h⁻¹)	55	51	51	55
%RSD at 20 µg/ℓ				

used. The accuracy of the four methods was determined by spiking borehole water samples. The recovery was determined by analysing a sample to determine its phosphate content, after which a known amount of phosphate was added and the sample re-analysed. Recovery was determined as follows:

$$\text{Recovery (\%)} = \frac{\text{(determined phosphate)}}{\text{(expected phosphate)}} \times 100$$

It was necessary to dilute some borehole samples to fall within the linear range of the specific method. As seen from Table 1 a recovery of 96% was obtained for the $SnCl_2$ method (range 1 to 400 µg/ℓ), a recovery of 95% for the ascorbic acid method (range 20 to 800 µg/ℓ), a recovery of 95% for the malachite green method (range 1 to 100 µg/ℓ) and a recovery of 94% for the rhodamine B method (range 0 to 10 µg/ℓ).

Precision

The precision of the four methods was determined by 14 repetitions of phosphate standard solutions in the linear ranges of the specific methods as well as 14 repetitions of samples within the ranges. A comparison for the four methods at 20 µg/ℓ is summarised in Table 1. The %RSD was 3.7 for the $SnCl_2$, 10 for the ascorbic acid, 3.5 for the malachite green and 2.7 for the rhodamine B. The best precision was obtained by the rhodamine B method and the worst by the ascorbic acid method.

Detection limit

All four methods were capable of reaching very low detection limits. The detection limits for the different methods were calculated using the formula:

$$\text{Detection limit} = \frac{3 \times (S_k)}{100} \times K$$

where S_k is the lowest concentration of the specific method and K the signal value of the corresponding lowest concentration. The values obtained are given in Table 1. The results illustrated that the lowest detection limit was obtained from the rhodamine B method with the ascorbic acid giving the highest detection limit of 15 µg/ℓ.

Sample interaction

Sample interaction was determined using the equation:

$$\text{Interaction} = \frac{(A_3 - A_1)}{A_2} \times 100$$

where :

A_1 = the true peak height of a sample with a low phosphate concentration, in other words, the peak height obtained from a stable baseline

A_2 = the true peak height of a sample containing ten times more phosphate

A_3 = the peak height for an interacted sample containing the same amount of phosphate as A_1.

The carry-over effect between consecutive samples for the four flow injection-spectrophotometric methods compared well as given in Table 1. At a sample frequency of about 50 to 55 samples per hour a carry-over of less than 1.5% was obtained for all four systems.

Interferences

Polyphosphates, mercury(II), CO_3^{2-} and pH as possible interferences were evaluated. The phosphate determination in all four methods can tolerate at most one equivalent of polyphosphate and polyphosphate started to interfere when the ratio of polyphosphate:phosphate was 1:1. Mercury(II) started to interfere when the ratio of Hg(II):phosphate became more than 10:1 for both the $SnCl_2$ and ascorbic acid methods. Hg(II) did not interfere with the malachite greenand rhodamine B methods at all. CO_3^{2-}-ions did not interfere with the $SnCl_2$ and ascorbic acid methods but started to interfere in the rhodamine method when the ratio of CO_3^{2-}- ions:phosphate rose above 1:1 and for the malachite green method above 20:1. pH did not interfere with the $SnCl_2$ method but very low (pH 2.5) and very high pH (pH 11) values interfered with the ascorbic acid, malachite and rhodamine B methods.

Sampling rate

As seen from Table 1 all four methods were capable of giving a reasonable sampling rate of about 50 to 55 samples an hour. For the low level of phosphate concentration determined, the sampling rate achieved was good.

General problems experienced

A number of general problems were experienced with each method. The $SnCl_2$ solution is subject to oxidation by O_2 in acid solution. The $SnCl_2$ solutions should be prepared under N_2 atmosphere, freshly made, preferably day by day, and protected from O_2. Blockages occurred due to the high concentrations of

$SnCl_2$ used when the flow system is allowed to stop. It was necessary to clean the system once a day by flushing for about 10 min with diluted hydrochloric acid, followed by sodium hydroxide and distilled water. Among the disadvantages for the ascorbic acid and rhodamine B methods, is baseline drift, which is not the case with the other two methods. A tendency that occurred with the malachite green and rhodamine B methods is coating of the tubing, accompanied by a pressure build-up and eventual blocking of the manifold. This is, however, prevented by flushing the system with acid which was only necessary once a day. As seen from Fig. 8, the accuracy of the rhodamine B method was relatively low for higher concentrations of phosphate (>10 µg/ℓ), due to the exponential curve.

Conclusion

It seems from the discussion and when looking at Table 1 that every one of the four methods had certain merits. Of all four the methods the $SnCl_2$ analytical system, however, showed the best overall results. A low detection limit (2 µg/ℓ) combined with a relatively large linear working range (1 to 400 µg/ℓ) made this method superior to the others. The relatively unstable nature of $SnCl_2$ solutions together with the blockages that occurred due to high concentration ranges are, however, factors to be considered when employing the method in routine laboratories.

References

BASSON WD, VAN STADEN JF and CATTIN PM (1981) Determination of phosphorus (P_2O_5) as molybdovanadophosphoric acid in phosphate rock with a flow injection procedure. *Fresenius' Z. Anal. Chem.* **307** 373 - 377.

BOLTZ DF (1958) *Colorimetric Determination of Non-metals. Vol. 8: Chemical Analysis.* Interscience Publishers, New York.

DEMING SN and MORGAN SL (1973) Simplex optimization of variables in analytical chemistry. *Anal. Chem.* **45** 278A - 283A.

DEMING SN and MORGAN SL (1987) *Experimental Design: A Chemometric Approach.* Elsevier, Amsterdam.

FOGG AG and BSEBSU NK (1984) Sequential flow injection voltammetric determination of phosphate and nitrite by injection of reagents into a sample stream. *Analyst* **109** 19 - 21.

FOGG AG and BSEBSU NK (1981) Differential-pulse voltammetric determination of phosphate as molybdovanadophosphate at a glassy carbon electrode and assessment of eluents for the flow injection voltammetric determination of phosphate, silicate, arsenate and germanate. *Analyst* **106** 1288 - 1295.

FOGG AG and BSEBSU NK (1982) Flow injection voltammetric determination of phosphate: Direct injection of phosphate into molybdate reagent. *Analyst* **107** 566 - 570.

JACKSON ML (1962) *Soil Chemical Analysis.* Constable and Company, Ltd, London, UK.

KARLBERG B and PACEY CE (1989) *Flow Injection Analysis. A Practical Guide.* Elsevier Science. Amsterdam.

LACY J (1965) Automatic procedures for the determination of calcium, potassium, magnesium and phosphorus in soil extracts. *Analyst* **90** 65 - 70.

MALINOWSKI ER and HOWERY DG (1980) *Factor Analysis in Chemistry.* John Wiley and Sons, New York.

MASSART DL, DIKSTRA A and KAUFMAN L (1978) *Evaluation and Optimization of Laboratory Methods and Analytical Procedures - A Survey of Statistical and Mathematical Procedures.* Elsevier, Amsterdam.

MASSART DL, VANDEGINSTE BGM, DEMING SN, MICHOTTE Y and KAUFMAN L (1988) *Chemometrics: A Textbook.* Elsevier, Amsterdam.

MEIER PC and ZÜND RE (1993) *Statistical Methods in Analytical Chemistry.* John Wiley and Sons, New York.

MARSHALL GD and VAN STADEN JF (1992) Computer-aided flow-analysis for laboratory use and process analysis. *Anal Instr.* **20** 79 - 100.

MOELLER J (1988) *Flow Injection Analysis.* Springer Verlag. Heidelberg.

MOORE GL (1991) The Optimisation of Inductively Coupled Plasma Spectroscopy. Ph.D. Thesis, University of Pretoria.

MOORE GL and BÖHMER RG (1991) Software for the modified simplex optimization of inductively coupled plasma-atomic emission spectrometry and other multivariate processes. *Chemometrics and Intelligent Laboratory Systems.* **11** 98 - 100.

PAUER JJ, VAN VLIET HR and VAN STADEN JF (1988) Determination of phosphate at low concentrations in surface waters by flow-injection analysis. *Water SA* **14** 125- 130.

RUZICKA J and HANSEN EH (1988) *Flow Injection Analysis* (2nd edn.) Wiley, New York.

RUZICKA J and HANSEN EH (1975) Flow Injection Analysis. Part I. A new concept of fast continuous flow analysis. *Anal. Chim. Acta* **78** 145 - 157.

VALCARCEL M and LUQUE DE CASTRO MD (1987) *Flow Injection Analysis. Principles and Applications.* Ellis Horwood. Chichester.

UENO K and KINA K (1983) *Introduction to Flow Injection Analysis.* Kodansha Scientific. Tokyo.