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Determination of orthophosphates using a macro segmented flow analyzer (MSFA) based on colorimetric detection

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Abstract

Fabrication of a macro segmented flow analysis (MSFA) system based on reconfiguration of the manifold by adjustment of the sample/reagent ratio, has been found to produce a sensitive method for orthophosphate analysis based on colorimetric detection at 880 nm. Optimization of sample tube length, reaction temperature and molybdate concentration in the carrier solutions has been carried out. The larger sample tube internal diameter led to the combined advantages of better sensitivities, wider working range and higher sample throughput over most existing methods. Using the optimized conditions of 50.0 cm sample tube length (1.6 mm i.d.), 37.0 °C reaction temperature and 0.0113 M molybdate concentration in the carrier solution, the calibration model for orthophosphate standard solutions was found to be linear ($y = 0.04895x + 0.003561$; correlation coefficient, $r^2 = 0.9970$) over the working range 0.01–2.00 mg l^{-1} orthophosphate. The volume of the sample injected was 1.396 ml at a flow rate of 6.0 ml min^{-1}. The sample throughput of this MSFA method was 40 samples per an hour, with a detection limit of 4.0 μg l^{-1}, and %R.S.D.'s below 5%. The MSFA method was successfully applied to analysis of water and wastewater samples.

Keywords: Orthophosphate; Segmented flow analysis; Colorimetric detection

1. Introduction

Orthophosphates are generally a limiting factor in aquatic environments. At concentrations above 0.1 mg l^{-1} in water bodies, orthophosphates usually leads to increased eutrophication [1–3].

Wet chemistry methods of analysis were previously used for the determination of orthophosphates in water and wastewater [3]. The chemical basis of many methods for determination of orthophosphate in water lies in the reaction between orthophosphate with molybdate in acidic medium to form a heteropolyacid of molybdophosphate (see Eq. (1)). Mo(VI) in this complex is readily reduced by mild reducing agent, ascorbic acid, to Mo(V) (see Eq. (2)) that has an intense blue color. In most recent methods, detection is undertaken either on the molybdophosphate reduction product e.g., the molybdenum blue method [4,5], or on the yellow vanadomolybdate complex [4]. The former approach is widely adopted in the official methods [4] due to higher sensitivity. However, lack of reagent stability e.g., of the combined stock solution, sometimes limits application of this approach in long term monitoring of orthophosphates.

$$H_3PO_4 + 12H_2MoO_4 \rightarrow H_3P(Mo_3O_{10})_4 + 12H_2O \quad (1)$$

$$\text{Mo(VI)} + \text{L-ascorbic acid} \rightarrow \text{Mo(V)} \quad (2)$$

The standard methods used for determination of phosphorus in the form of orthophosphates include the batch vanadomolybdate and molybdophosphate methods and flow injection method based on the molybdophopshate precursor [4]. The above methods are limited to particular orthophosphate concentration ranges. For example, the batch vanadomolybdophosphoric acid method is most suitable for routine analysis in the range 1–20 mg l^{-1} orthophosphate. This method therefore, can only be applied to wastewater samples with typical orthophosphate concentrations in this range. Respective researchers have suggested that careful attention to procedures might allow application of these methods to very low concentration levels of orthophosphate, such as those found in freshwaters [4,5].

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Detection of the end products of the reactions summarized in Eqs. (1) and (2) is based on colorimetry. As a result, colorimetric detection has been widely used for determination of orthophosphate in flow injection analysis or sequential injection analysis of aqueous samples [5–9]. A micro flow injection analysis method has been developed based on lithographic techniques and etching methodologies for determination of orthophosphate. However, the sample throughput of such a method was relatively low [10,11]. Also, etching procedures are complicated and the etched devices are mostly unavailable and expensive to acquire.

Other workers evaluated three spectrophotometric methods for determination of orthophosphates by sequential injection and concluded that the molybdophosphate method was a most sensitive and reproducible method than the stannous chloride and malachite green methods [12]. Using molybdenum blue chemistry it was reportedly possible to achieve lower detection limits than those reported in the standard methods [5,6]. Other workers presented a sequential injection method utilizing molybdenum for complex formation, and subsequent detection at 660 nm. This method was sensitive with a linear range 0.2–7.0 mg l^{-1} for orthophosphates [13]. The problem with this method was that the range was still above most orthophosphate concentrations, found in freshwater samples. Also another major problem with this method is the difficulty of zone interpenetration when several reagents are used in sequential injection analysis [13].

Other methods used for determination of orthophosphates in water include the ion-exclusion chromatography coupled to inductively coupled plasma mass spectrometry [14] and conventional ion chromatography [4]. The sample throughput of such methods can at times be low, when compared to colorimetric methods.

By considering the basic principles of both chromatographic and flow injection analysis (FIA) methods, segmented flow analysis (SFA) where the reaction stream is segmented with air bubbles or nitrogen to reduce inter sample dispersion, could be a fast and robust method for orthophosphate analysis in aqueous samples. It has previously been reported [15] that the segmentation of the stream, unlike FIA and SIA can be highly reproducible, precise, and lead to low inter sample contamination. In this paper we present a macro segmented flow method for the determination of orthophosphate at trace concentrations (0.01–2 mg l^{-1}), based on the classical and well-known chemical reactions (see Eqs. (1) and (2)). For the first time, in the method outlined, it is possible to use large bore diameter sample tubing with a shorter length to maximize sensitivity. The method exploits the inherent advantages of large bore internal diameter tubes, simpler SFA manifolds, high sample throughput, high precision, and low detection limits not provided by the many available methods for analysis of natural water samples. The method outlined also provided a wider calibration range not provided by previous methods for determination of orthophosphate.

2. Experimental

2.1. Reagents

All the reagents used were obtained from Sigma Aldrich GmbH Chemie (Steinheim, Germany) and were of analytical reagent grade except where otherwise specified. Doubly deionised water prepared using a Milli-Q Ultrapure Water Purification System (Millipore, Billerica, MA, USA) was used throughput.

The MSFA combined carrier solution consisted of: 0.075 g potassium antimonyl tartarate dissolved in 150 ml doubly deionised water to which 33.8 ml of concentrated H_2SO_4 (95–97%) was carefully added with shaking. On cooling 3.50 g ammonium molybdate was added and the resultant solution diluted to 250 ml with doubly deionised water. Colorimetric color-developing reagent (also the SFA reaction reducing agent) consisted of 0.85 g L-ascorbic acid dissolved in 250 ml doubly deionised water.

Two water reference materials were used to validate the method; certified reference material SABS 04 (low orthophosphate concentration) and certified reference material SABS 05 (high orthophosphate concentration), both obtained from South African Bureau of Standards (SABS), Johannesburg, South Africa.

2.2. Apparatus

A macro segmented flow analysis system was fabricated using the following components: An 8-port Symatec MV peristaltic pump (Struers, Copenhagen, Demark), Large bore tubings (Swords Company Technicon ltd, Dublin, Ireland) Technicon Autoanalyser II S.C. spectrophotometer (Swords Company Technicon ltd, Ireland) equipped with a 1.50 cm optical path flow cell, and a Rec 80 Servograph chart recorder (Radiometer, Copenhagen, Denmark). Dual wavelength spectrophotometry was used to minimize the Schlieren effect and blank values associated with suspended matter in aqueous solutions [16].

2.3. Segmented flow manifold

The components of the MSFA system were arranged as illustrated in Fig. 1. All the tubing was made of PVC Technicon flow rated quality. Internal diameters of the tubing used were much larger than those for conventional SFA systems. This was aimed at facilitating injection of large analyte volumes and hence, improve method sensitivity. The larger bore diameter tubings were expected to provide low dispersions, when otptimized to shorter lengths. This would allow the use of higher flow rates than those normally used [4,5] and therefore, increasing sample throughput of the method. The internal diameters for the sample, carrier solution, air, and L-ascorbic acid tubings were 1.60, 1.00, 0.32, and 0.32 mm, respectively. These internal diameters, used for both reagents

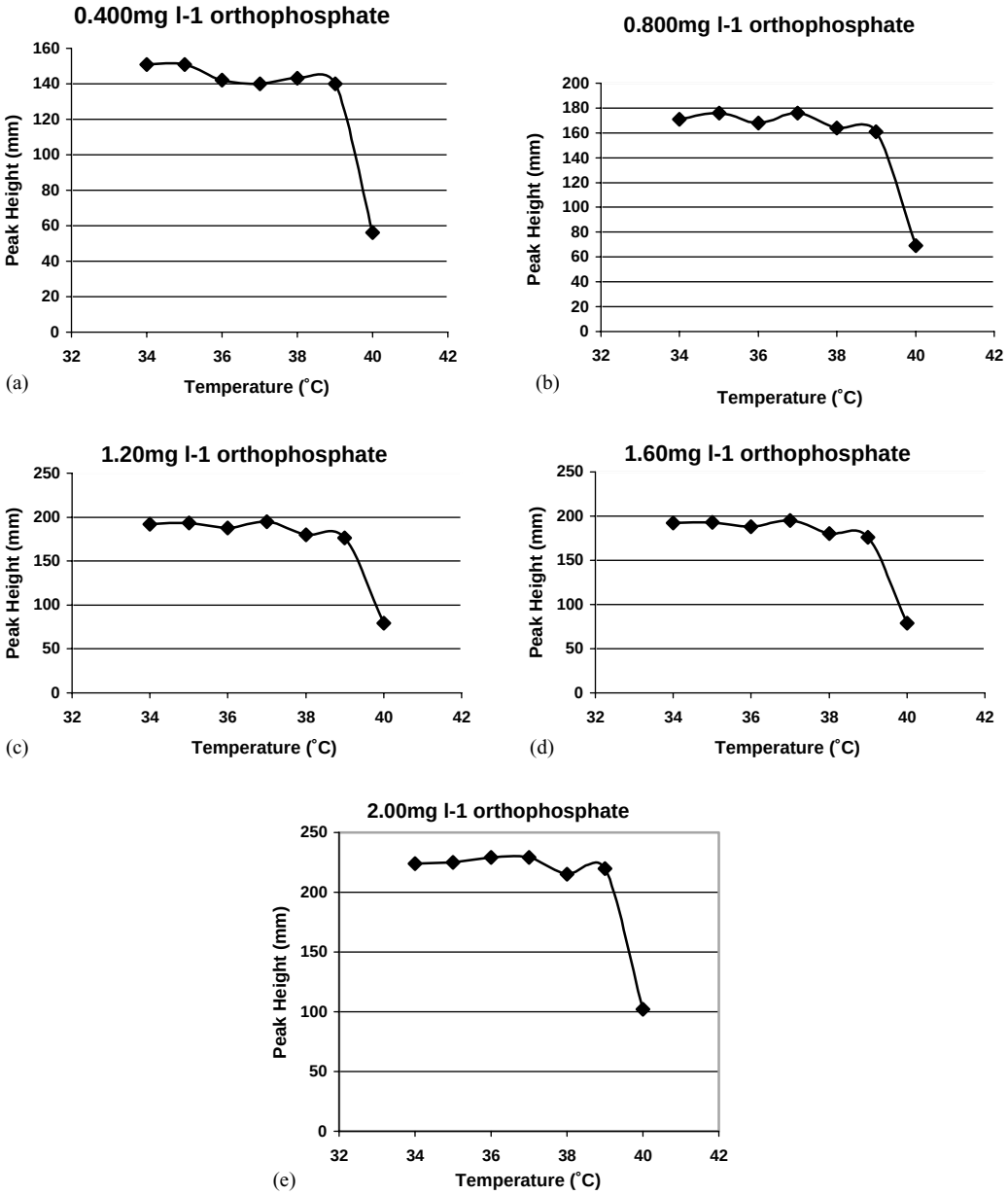

Fig. 1. A Schematic diagram of an MSFA manifold used for the determination of orthophosphate: (1) auto sampler, (2) peristltic pump, (3) air, (4) acidic molybdate, (5) ascorbic acid, (6) from flow cell, (7) to waste, (8a) mixing coil (nine turns), (8b) reaction coil, (8c) reaction coil in water bath (nine turns), (8d) holding coil (seven turns), (9) spectrophotometer flow cell, (10) waste, (11) chart recorder.

and samples, were larger than for the conventional tubings [4]. The flow rates for the respective tubes were: sample tube 1.60 $cm^3\,min^{-1}$, carrier solution tube 1.00 $cm^3\,min^{-1}$ L-ascorbic acid tube 0.32 $cm^3\,min^{-1}$ and air tubing 0.42 $cm^3\,min^{-1}$.

The first reaction coil (8a in Fig. 1) was for the reaction between the analyte containing solution and the acidic molybdate. Molybdate was air segmented before mixing with the orthophosphate containing solution. The resultant segmented reaction mixture (molybdophosphate) was then reduced by L-ascorbic acid and mixed further in the second reaction coil (8b in Fig. 1). The reduced reaction product was further passed through a reaction coil (8c in Fig. 1) immersed in a water bath maintained at a known temperature. A holding coil (8d in Fig. 1) was used to ensure and allow the reaction to reach completion. The reduced Mo(V) complex was then detected, as it traversed through the flow cell, using a colorimeter set at 880 nm.

2.4. Procedures

The SFA system was rinsed with 50 ml of 2.0 M H_2SO_4, to remove any left over end products of previous reactions. The system was then rinsed with 100 ml doubly deionized water to stabilize the baseline on the chart recorder before commencement of analyte measurements.

Orthophosphate standard and sample solutions were reacted with acidic molybdate reagent and the reaction product reduced by L-ascorbic acid. The reduced product was detected by a colorimeter set at an analytical wavelength of 880 nm. The absorption peak data obtained from the chart recorder were then computed into Microsoft Excel program. Regression data for calibration curves were obtained by use of Linear Regression software (2001–2 version, Orlando, USA).

Optimization of experimental conditions was undertaken using orthophosphate standard solutions and the synthetic water reference materials SABS 04 and SABS 05.

2.5. Optimization procedures

Optimization of experimental conditions for reaction temperature, sample tube length, and carrier solution concentration was undertaken. This was because large bore diameter tubing was used and important parameters affecting method sensitivity could differ from the conventional experimental conditions [4,5].

2.5.1. Reaction temperature

This was to determine the optimum reaction temperature necessary for complete reduction of Mo(VI) to Mo(V) using the fabricated MSFA manifold. Orthophosphate standard solutions of 0.400, 0.800, 1.20, 1.60, and 2.00 mg l^{-1}, were reacted with acidic molybdate reagent and the products reduced with ascorbic acid. The reduction reaction occurred in a reaction coil kept in a water bath at temperatures which varied from 32 to 40 °C. The optimum temperature was the one, at which the highest absorption peak (at 880 nm) was observed for the varying concentrations of orthophosphate, indicating complete reduction of Mo(VI) to Mo(V) or a steady-state condition of the reduction reaction.

2.5.2. Sample tube length

The sample tube length determines the volume of sample analyzed and hence, indirectly, the sensitivity of the method. With the large internal diameter tubes used in this SFA method, sample tube length was optimized in order to establish the highest sensitivity that can be achieved with the method. Varying concentrations of orthophosphate (as in Section 2.5.1) were used. Sample tube length of 50.0, 75.0, 117.5, and 150 cm were used at a constant flow rate of 6.00 ml min^{-1} and at the optimized reaction temperature. Absorption peak heights at 880 nm were used to determine sensitivity of the method.

2.5.3. Molybdate concentration in carrier solution

For the fabricated MSFA manifold, experiments were necessary to establish the optimum concentration of molybdate in the carrier solution needed to react with orthophosphate in the sample solutions at 37 °C, flow rate of 6.00 ml min^{-1} and sample tube length 50.0 cm (1.60 mm i.d.). Molybdate concentration in the carrier solution was expected to be lower than concentrations normally used; decreasing, therefore, the amount of Molybdate consumed in this MFSA method.

A stock solution of molybdate (0.113 M) was prepared and diluted to 5.00% (0.00567M), 10.0% (0.0113 M), 20.0% (0.0226 M) and 50.0% (0.0567 M) using doubly deionized water. The resultant solutions were used as carrier solutions in the analysis of 2.00 mg l^{-1} orthophosphate standard solution.

2.6. Analysis of real samples

To obtain calibration curves, orthophosphate standard solutions of 0.01, 0.05, 0.100, 0.500, 1.00, and 2.00 mg l^{-1} prepared by diluting the stock solution were analyzed at the optimized conditions.

Two synthetic reference materials, SABS 04 and SABS 05, obtained from South African Bureau of Standards (Johannesburg, South Africa) were analyzed to validate the SFA method and to ascertain precision of the method. The reference materials were also analyzed using optimized conditions.

Real samples analyzed were natural surface water from Gaborone dam, Notwane river, Boro river (in Okavango Delta), and storm water samples from wastewater drains in Gaborone city. All samples were collected by grab sampling and filtered through a 0.45 μm glass fiber filter membrane. When necessary, samples were diluted with doubly deionised water to fit the calibration range. Potassium antimonyl tartrate was used in the carrier solution to suppress any possible interference due to silicate in the samples [8].

3. Results and discussion

Having optimized temperature, sample tube length and molybdate concentration in the carrier solution, othorphosphate in natural water and stormwater samples was determined using the fabricated MSFA manifold. Validation of the method, precision, and accuracy of the method were ascertained by analysing SABS certified reference materials.

3.1. Optimization of reaction temperature

Reaction coil (8c in Fig. 1) was kept in a water bath at variable temperatures between 34 and 40 °C.

Fig. 2a–e represent temperature profiles for orthophosphate standard solutions of 0.400, 0.800,1.20, 1.60, and 2.00 mg l^{-1}. Similar shaped graphical plots were obtained for each respective orthophosphate standard solution. Any slight deviations could be attributable to interference's from e.g., arsenate, which is able to contribute to the reaction end product of the molybdenum blue chemistry [13] hence, increasing the detected absorption peak height. A study of the effect of temperature between 34 and 40 °C showed that the absorption peak height increases with an increase in temperature, up to 37 °C. Thereafter, the absorption peak height

decreased. In all cases maximum absorption peak height was obtained at 37 °C, subsequently chosen as the optimum reaction temperature for this MSFA method. At this temperature the reaction pathway appears completely stabilized, as previously reported by Ruzicka and Hansen [17] who studied the reaction using different Sequential injection and flow injection manifolds. The optimized temperature, 37 °C, was similar to the one used before, indicating that even when the internal diameter of the tubing is increased the reaction temperature remains constant.

At temperatures lower than 34 °C the efficiency of the reaction, as indicated by the absorption peak height, was very low. At temperatures above 40 °C, efficiency of the reaction was again very low and air bubbles, possibly due to the faster kinetics of the reaction, were introduced in the MSFA system, this greatly interfered with colorimetric detection.

3.2. Optimization of sample tube length

Sample tube length if considered separately, affects the dispersion coefficient, D, (Eq. (3)) of the SFA system [18].

$$D = \frac{C^0}{C} \tag{3}$$

where D is the dispersion coefficient, C^0 the original concentration of the analyte in standard/sample solution, C the concentration of analyte in the dispersed solution zone.

The influence of the sample volume on dispersion is one which has been studied most frequently, and the decrease of dispersion by increasing the injected sample volume is well documented [18]. Lower values of D are desirable, ensuring that all the initial concentration of the analyte (C^0) reaches the detector. Decreasing the sample tube length increases the volume of sample reaching the detector, thereby increasing sensitivity of the method. Decreasing sample tube length lowers the value of D. Even though varying the tube length, is a convenient way of manipulating dispersion, the dispersion coefficients cannot vary by more than a factor of 3–4 [18]. Thus, putting a limitation to how low tube length can be decreased. Hence, the option to widen tube internal diameter.

A sample tube with a wider internal diameter (i.d.) of 1.60 mm varied from a length of 50.0 to 150 cm for each orthophosphate standard solution concentration of 0.400, 0.800, 1.20, 1.60, and 2.00 mg l^{-1}, respectively. At each orthophosphate concentration, as the tube length progressively decreased the absorption peak height increased (see Fig. 3). The shorter the sample tube length, the better the sensitivity

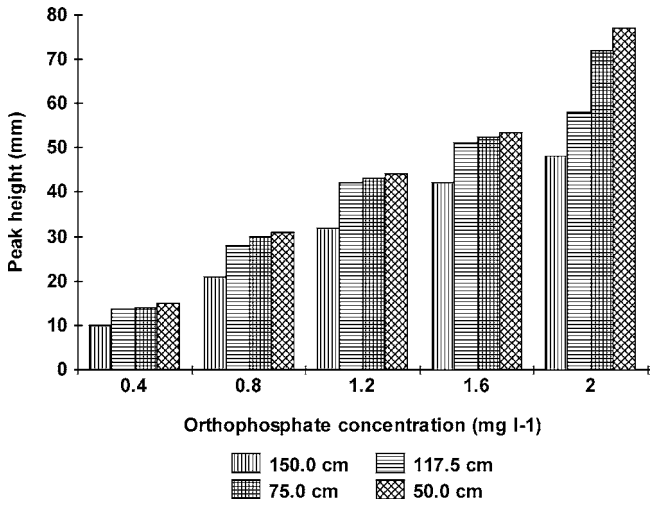

Fig. 3. Optimization of sample tube length, at flow rate 6.00 ml min^{-1}, temperature of reaction 37 °C and molybdate concentration, 0.01133 M.

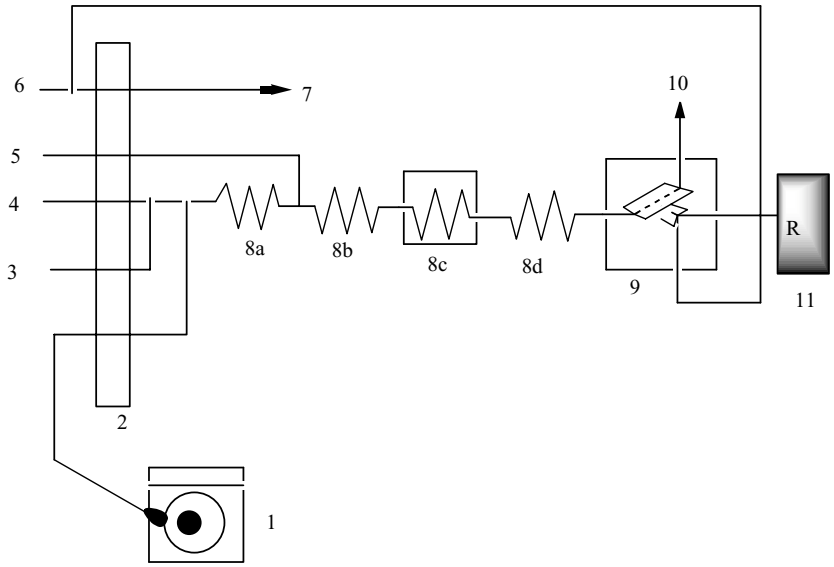

Fig. 2. Optimization of reaction temperature using (a) 0.400 mg l^{-1}, (b) 0.800 mg l^{-1}, (c) 1.200 mg l^{-1}, (d) 1.600 mg l^{-1} and (e) 2.000 mg l^{-1} orthophosphate standard solutions.

of the method. The reproducibility in results was poor with sample tube lengths longer than 117.5 cm, e.g., for a 150 cm sample tube length, poor reproducibility was shown by the low precision (%R.S.D. of 8.16, $n = 3$, for a 2.00 mg l^{-1} orthophosphate standard solution). The shorter sample tube (50.0 cm) was more efficient than the longer sample tube (150 cm). However, the geometrical features of the manifold, especially the autoinjector, did not allow sample tube lengths below 50 cm to be used. The sample tube length of 50 cm was found most suitable and it also gave low dispersions.

Better sensitivities were obtained with a sample tube length of 50 cm at all concentrations of orthophosphate standard solutions. Although smaller sample tube internal diameters are usually employed, a larger sample tube internal diameter (i.d.) of 1.60 mm was found most suitable. The sample tube length of 50 cm at the given i.d. was chosen as the optimized tube length for this MSFA system. The universality of this sample tube length was also ascertained by successfully using it on a different auto analyzer system.

Optimization of the sample tube length enhanced sensitivity of the method allowing for analysis of low concentrations of orthophosphate in aqueous samples. Unlike with FIA and SIA methods, it was possible to use sample tubing with a large internal diameter without the risk of increasing the dispersion coefficient, *D*. Most of the methods of SIA and FIA used very small internal diameter tubings but still dispersion remained the limiting factor in most of these methods. The use of large diameter tubings is demonstrated for the first time in this MSFA method and dispersion was minimized by use of air segmentation. In this MSFA method due to the use of coils and air segmentation, *D* was drastically reduced thereby increasing sensitivity of the method.

3.3. Optimization of carrier solution concentration

Since molybdate is the primary component of the orthophosphate complexation reaction (see Eq. (1)), its concentration has a direct effect on the sensitivity of the method. Macro segmented flow analysis (MSFA), in contrast to flow injection analysis (FIA) allowed for use of large sample volumes in the larger bore internal diameter tubing, and allowed a longer time period for the reaction to reach steady state conditions, this contributed to the high sensitivity of the method as the reaction was allowed to reach its completion or steady state conditions.

Molybdate concentration in the carrier solution was varied by, diluting the molybdate stock solution as indicated in sub Section 2.5.3. Orthophosphate (2.00 mg l^{-1}) analysis was undertaken at a flow rate of 6.0 ml min^{-1}, temperature of 37 °C, and sample tube length of 50 cm.

Absorption peak heights increased from concentrations 0.00567 M to 0.0113 M molybdate in the carrier solution and thereafter decreased as the molybdate concentration was increased (see Fig. 4). Molybdate concentration in the carrier solution was optimum at 0.0113 M as at this concentration

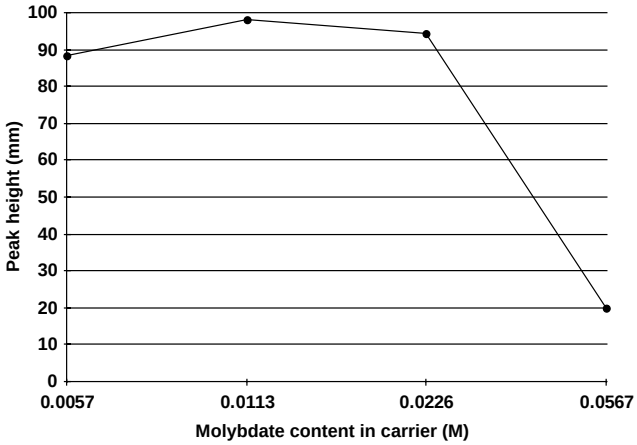

Fig. 4. Optimization of molybdate concentration in the carrier solution for analysis of 2.00 mg l^{-1} orthophosphate standard solution.

the highest absorbance peak height was obtained, suggesting a completion in the complexation reaction of orthophosphate with molybdate. This molybdate concentration was lower than that used before [12] and allowed an increase in the sensitivity of the MSFA method.

Lowest absorption peak heights were obtained at 0.0567 M molybdate concentrations in the carrier solution. Preparation of the MSFA combined carrier solution was suitable with the exclusion of ascorbic acid in contrast to the procedure used in the standard method [4]. If Ascorbic acid is present, any trace amount of orthophosphate can increase the blank signal. At molybdate concentrations in the carrier solution higher than 0.0113 M, reaction products adhered to the walls of the reaction tubes. Also at high molybdate concentrations in the carrier solution, the chart recorder baseline increased by an average of 5.0 mm, indicating an increase in the background absorption. This caused an error by decreasing the absorbance peak heights.

3.4. Validation of the method

Two certified reference materials SABS 04 (low orthophosphate concentration) and SABS 05 (high orthophosphate concentration), obtained from the South African Bureau of Standards, were analyzed using the fabricated MSFA manifold using the optimized experimental conditions, see Table 1.

The certified reference materials were also concurrently analyzed using the conventional colorimetric method [4]. For both methods the certified reference materials were, as recommended by the SABS, diluted 10 times with doubly deionized water before analysis. Table 2 (a and b) show the results obtained.

Using the paired Student's *T*-test [19] at $P = 0.05$, a comparison of the results obtained was made. Comparing the results by the MSFA method to the certified values of the reference materials, the calculated *T*-values were much lower than the critical *T*-value of 2.31, at the 95% confidence

Table 1
Optimized experimental conditions for the analysis of water samples using the fabricated MSFA manifold

Parameter/Equipment	Optimized values
Rinse of loops	50.0 ml of 2.00 M H_2SO_4
Pump rate	6.00 ml min^{-1}
Volume of sample injected	1396 μL
Temperature of reaction (Water bath)	37.0 °C
Concentration of molybdate	0.0113 M
Sample tube length	50.0 cm
Colorimeter settings	Standard calibration 3,8 Baseline: $5^{3/4}$ turns Range: 0.40 Abs
Chart recorder	Range: 2.00 mV cm^{-1} Speed: 10.0 min cm^{-1}

level. This indicates that there were no statistically significant differences between the certified values and the ones obtained using the developed MSFA method. Hence, good accuracy in the results was obtained by the MSFA method. Successful analysis of the high concentration (upper limit) and low concentration (lower limit) orthophosphate certified reference materials demonstrated the versatility of the method to a wider concentration range.

Comparing the results obtained by the MSFA method to those obtained by the conventional colorimetric method, the calculated *T*-value was lower for SABS 04 but higher for SABS 05 than the critical *T*-value of 2.31 at the 95% confidence level. This indicates that for SABS 05, the results obtained by the two methods were significantly different. Also for SABS 05, the values obtained by the conventional colorimetric method were significantly different from the certified values of the reference material. The conventional colorimetric method being operator dependent is prone to many errors in analysis. This could have caused the statistically significant differences in SABS 05 results, by the two methods. Overall, in comparison to the conventional colorimetric method, the MSFA methods gave equally precise and more accurate results. This being demonstrated at the sub-mg l^{-1} and mg l^{-1} levels by analysis of SABS 04 (0.481 ± 0.024 mg l^{-1} orthophosphate) and SABS 05 (2.38 ± 0.317 mg l^{-1} orthophosphate) certified reference materials respectively.

Table 2
SABS 04 and SABS 05 certified reference materials analyzed by (a) MSFA method and (b) conventional colorimetric method [4]

Reference material	MSFA method ($n = 8$)	Certified value	Calculated *T*-value
(a)			
SABS 04	0.48 ± 0.02	0.53 ± 0.08	0.90
SABS 05	2.4 ± 0.3	2.4 ± 0.2	0.15
Reference material	MSFA method ($n = 8$)	Conventional colorimetric method ($n = 8$)	Calculated *T*-value
(b)			
SABS 04	0.48 ± 0.02	0.424 ± 0.006	1.2
SABS 05	2.4 ± 0.3	2.3 ± 0.3	2.4

mg l^{-1} orthophosphate.

3.5. Analysis of real samples

Optimized experimental parameters were used for the analysis of real water and wastewater samples. The analysis time for each sample was on average 1.5 min. This is a shorter analysis time than that of other methods [9,12,14], demonstrating the speed of this MSFA method. For analysis of real samples, a linear calibration graph was plotted over the concentration range 0.0100–2.00 mg l^{-1} orthophosphate. This wide linear calibration range was possible due to use of a shorter sample tube with a wider internal diameter. The regression equation obtained was $y = 0.04895x + 0.003561$ with a coefficient of regression, $r^2 = 0.997$, where y is the relative peak height and x is the concentration of orthophosphate in mg l^{-1}. Samples above this calibration range required dilution using doubly deionized water. Table 3 indicates the results obtained.

Orthophosphate was detected in the natural water and wastewater samples analyzed. Notwane river samples generally had the highest orthophosphate concentration in comparison to other samples, mainly due to pollution of the river water by poultry industries, agrochemicals from domestic gardens and the sewage treatment plant in the proximity of the river. Boro river samples also had high orthophosphate concentrations possibly because they were sampled at the time of nutrient influx into the river, due to

Table 3
Concentration of orthophosphate determined by segmented flow analysis (SFA) in water and wastewater samples collected from Gaborone dam, Notwane river, Boro river in Okavango Delta, and stormwater from drains in Gaborone

Sampling Location	Sample Type	Concentration (mg l^{-1}, $n = 4$) ± S.D.
MCF/C2	Dam surface water	0.13 ± 0.009
MCF/C3	Dam surface water	0.13 ± 0.004
MCF/C4	Dam surface water	0.0010 ± 0.0002
MCF/C6	Dam surface water	0.086 ± 0.0001
OT's place	Notwane river water	25.0 ± 0.3
Chinese garden	Notwane river water	15.0 ± 0.2
Oodi-Matebele bridge	Notwane river water	14.0 ± 0.1
Ramotswa border	Notwane river water	1.2 ± 0.08
Ramotswa poultry	Notwane river water	0.28 ± 0.01
Botala farms	Notwane river water	0.14 ± 0.0002
Cemetry	Storm water	8.7 ± 0.810
W.A headquarters	Storm water	1.1 ± 0.2
BNPC	Storm water	2.8 ± 0.1
Grand palm	Storm water	16.8 ± 0.9
Boro 06:00 h	Boro river water	4.9 ± 0.3
Boro 12:00 h	Boro river water	4.0 ± 0.05
Boro 16:00 h	Boro river water	5.0 ± 0.009
Boro site 1	Boro river water	8.9 ± 0.08
Boro site 2	Boro river water	7.8 ± 0.1
Boro site 3	Boro river water	6.7 ± 0.06
Boro site 4	Boro river water	6.4 ± 0.09
Boro site 5	Boro river water	4.3 ± 0.6

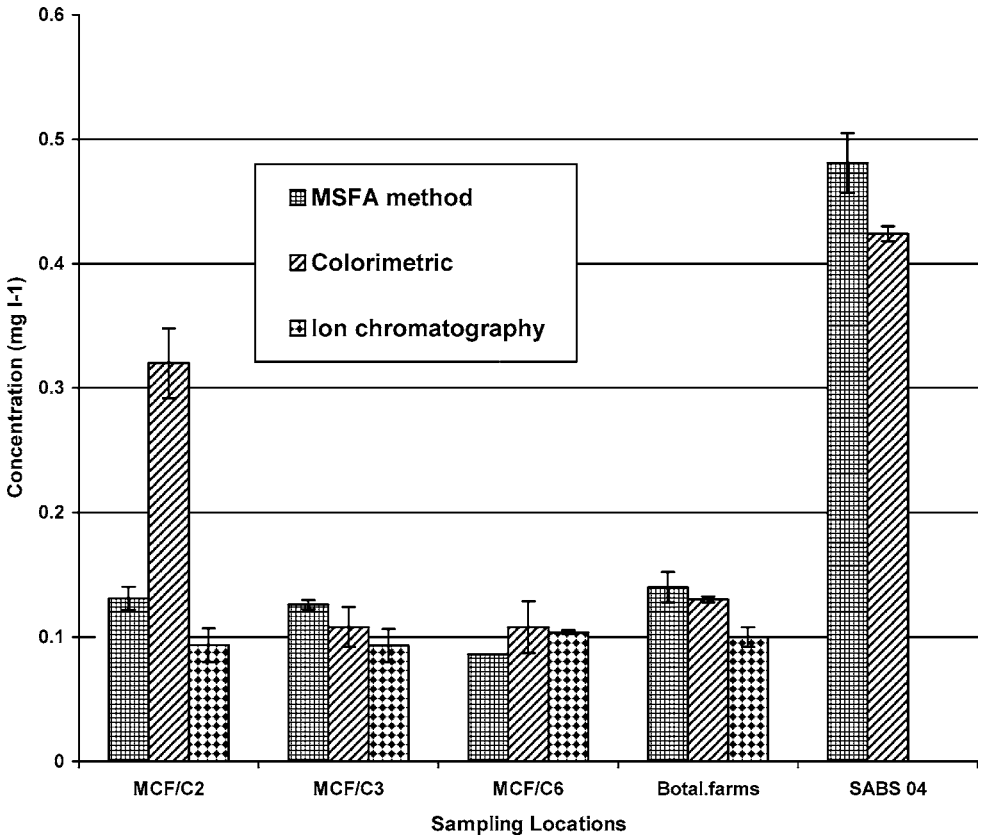

Fig. 5. Orthophosphate content in real water samples determined by the developed MSFA method, conventional ion chromatography [4], and conventional colorimetric method [4].

increased water inflows into the Okavango Delta in June 2003.

Orthophophoshate content in stormwater varied. Locations where high concentrations of orthophosphate were detected could be due to point source pollution, and where low orthophosphate concentrations were detected could be due to non-point sources of pollution. Orthophosphate content will be lower at locations of non-point source pollution because of the dilution when pollutants traverse down the river.

Using the optimized conditions of this MSFA method about 40 samples could be analyzed in one hour. This sample thoughrouput is appreciably good since with the conventional colorimetric method, one can only analyze few samples in an hour, with comparably the same sensitivity and reproducibility. The %R.S.D. for this MSFA method was less than 5.00% (1.47%, $n = 4$, for 0.0400 mg l^{-1} orthophosphate standard solution), with a method detection limit of 0.00400 mg l^{-1} for orthophosphate (3X standard deviation of the mean for the lowest concentration standard solution).

3.6. Comparative performance of the MSFA method

Real water samples and SABS 04 certified reference material were concurrently analyzed for orthophosphate using the developed MSFA method, conventional ion chromatography [4], and the conventional colorimetric method [4]. The results obtained (see Fig. 5) were compared using the Students *T*-test, at $P = 0.05$ level.

For most samples, results obtained by the three methods were not significantly different. The developed MSFA method gave equally precise results as those obtained by conventional ion chromatography. When compared to conventional colorimetric method, equal or better precision in results was obtained using the MSFA method.

With the developed MSFA method higher numbers of samples could be analyzed in comparison to the low sample throughput when using ion chromatography or the conventional colorimetric method.

4. Conclusions

The current study has demonstrated the use of a macro SFA system in which use of short sample tube length with larger bore internal diameter provided enhanced sensitivity and a wider working concentration range for orthophosphate analysis. The manifold is simple, with high sample throughput (ca. 40 samples per an hour) and permits the use of larger bore diameter tubings. The method detection limit was lower than that of most other methods.

The optimum reaction temperature was found to be 37 °C. This temperature was similar to that reported by other workers [9,12–15] using different FIA and SIA manifolds. 0.0113 M was the optimum molybdate concentration in the carrier solution for complete complexation of orthophosphate in aqueous solutions, within the optimized working range.

The macro SFA method was comparable in performance to the conventional ion chromatography and conventional colorimetric methods for orthophosphate determination in aqueous samples. Using the paired Student's *T*-test, the values obtained in analysis of SABS04 and SABS05 certified reference materials using the SFA method were not significantly different to the certified values of the reference materials, and to those obtained when using conventional ion chromatography. The SFA method gave equal or better precision in results in comparison to the conventional colorimetric method for analysis of water and wastewater samples.

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