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Sequential Injection Analysis for the Monitoring of Riverine Phosphorus and Iron Inputs into the Lagos Lagoon Sediments

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Abstract

Sequential injection analysis (SIA) protocols for the determination of phosphorus and mineral iron (Fe) in the Lagos Lagoon sediment were developed by optimizing some existing standard methods. Sandwiched sample was found to offer higher sensitivity than a 2-zone stack for P SIA. The injection volumes of sample and reagent for P analysis were 40 μL and 20 μL (split into two 10-μL zones) respectively while 150 μL of sample and 10 μL of reagent were injected in Fe analysis. The sampling rates were 31 h^{-1} and 75 h^{-1} for P and Fe SIA protocols respectively. Quantitative digestion of total P was achieved by a sediment-to-persulfate ratio of 1:3 by mass in 2.0 mL of 0.3 M NaOH. Average recovery of bioavailable P in matrix samples was 91.7 % and average recoveries of total P were 111.5 % and 96.6 % in matrix samples and blanks respectively. Average recovery of mineral iron in matrix samples was 94.3 % while average recovery in blanks was 97.4 %. The method detection limits were 1.4 mg P kg^{-1} and 2.6 mg P kg^{-1} for bioavailable-P and total-P methods respectively while the method detection limit for mineral iron method was 0.44 mg Fe kg^{-1}. The combination of neutralization and dilution of bicarbonate extract prevented CO_2 interference during SI absorbance scanning. The performance of these methods indicated their suitability for sediment P and Fe monitoring of the Lagos Lagoon system.

Keywords: Sediment P and Fe, alkaline persulfate oxidation, sequential injection-spectrophotometry, method validation

1. Introduction

Determination of Fe and P forms in sediments requires an initial partial or total extraction step. P species extracted by sodium bicarbonate is labile P as it comprises free phosphates and P loosely associated with soil or sediment through adsorption on soil particles and absorption into interstitial water. This P species is generally accepted as Olsen P or more commonly called bioavailable P (BAP) [1,2]. BAP, the portion of total P (TP) available for uptake by living organisms, could comprise dissolved inorganic and organic P as well as particulate P [3,4].

Although it has been established in soil analysis that the <0.45 μm fraction is the most BAP [4], the <2.5 μm P fraction has also been determined as the plant-available P in soils [5]. Although in water analysis, the truly dissolved P has been defined to be between <0.1 μm and <0.2 μm fraction, there is also widespread acceptance that P is linked with a seamless range of <0.45 μm sized particles and colloids [6,7]. In sample preparation, 0.45 μm filter is still widely used while 1.0 μm and 0.7 μm filters have also been used for dissolved P fraction [3,8]. It is worthy of note that sediment analysis like soil can be significantly affected by particle size [9].

A method of colorimetric analysis of dissolved reactive P involves the reaction of ammonium molybdate with orthophosphate in an acid medium to form a phosphomolybdate complex, which is reduced by ascorbic acid in the presence of antimony tartrate to form phosphomolybdenum blue that is spectrophotometrically measured [10]. Determination of mineral iron on the other hand can be achieved by extracting the soil or sediments with 0.5 M HCl followed by colorimetric measurement [11]. A colorimetric method for Fe involves reduction of Iron in solution to ferrous ion by boiling with acidic hydroxylamine solution and treatment with 1,10-phenanthroline solution at pH 3.2 – 3.3 where an orange-red complex is formed and spectrophotometrically measured [10]. Currently, programmable flow and green chemistry derived from SIA carried out in the lab-on-valve (LOV) are influencing automation of many adaptable methodologies. Micro-SIA-LOV offers a stopflow mode for slow reactions and high sample throughput comparable to flow injection analysis (FIA) [12,13]. FIA and SIA of P and Fe are usually reported for water matrix [e.g., 14,15] and few publications are available for sediment P and Fe analysis using SIA. Hence, the need for the development of these methods.

The coastal Lagos Lagoon increasingly receives substantial sediment-laden runoff and treated and untreated sewage in addition to effluents from the deluge of industries scattered around the Lagoon metropolitan watershed. In this study, colorimetric reagent concentrations and reaction time for orthophosphate determination were optimized and interference

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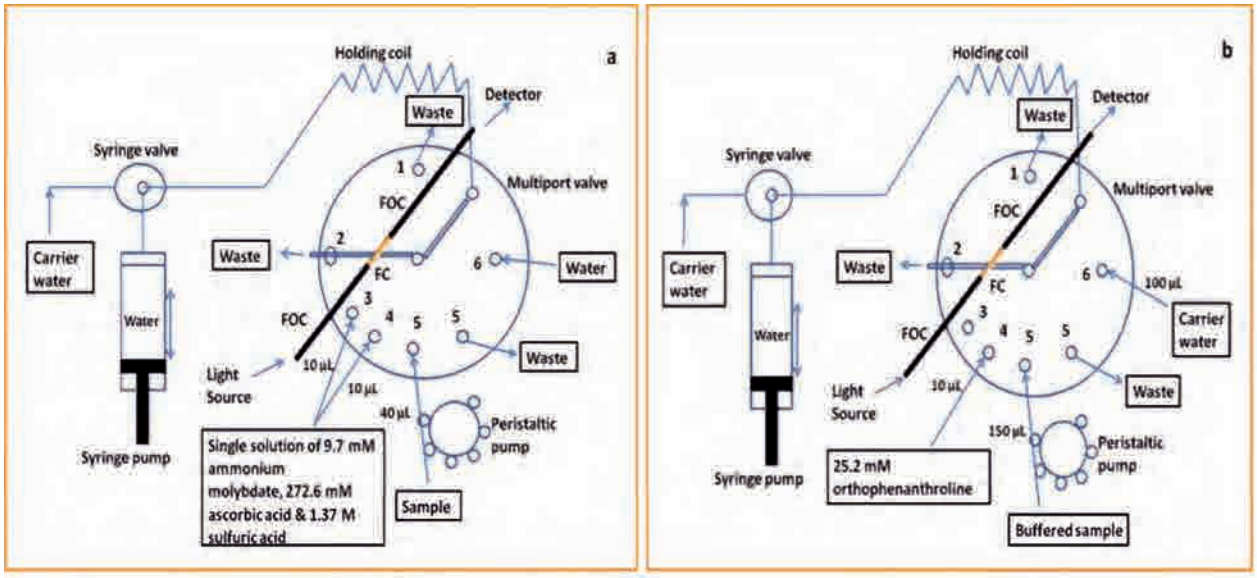

Fig. 1 a. Optimized manifold for SI analysis of dissolved P in stopflow mode. FC: flowcell (pathlength 7 mm), FOC: fibre optic cable. **b.** Optimized manifold for SI analysis of dissolved Fe in flow mode.

of aluminum, iron and silicon on the molybdenum blue method was studied. Conditions of sodium bicarbonate extraction of BAP were also optimized and interference due to CO_2 gas generation from the reaction of bicarbonate with sulfuric acid in the colorimetric reagent during spectrophotometric measurement of SIA was investigated. Alkaline persulfate digestion for TP was optimized. SIA-LOV protocols were developed for determining orthophosphate and Fe. The developed methods were validated for BAP, TP and mineral Fe in Lagos Lagoon sediment.

2. Experimental

The micro-SIA-LOV used was FIAlab-3500 (FIAlab Instruments, USA) and the spectrometer was USB 2000 (Ocean Optics, USA). The halogen light source was connected to the spectrometer through two fiber optics separated by 7 mm path length.

2.1 P procedures

SI protocol for dissolved P: P analysis was carried out as shown in the micro-SIA-LOV manifold (Fig. 1.a.). The SI analysis was looped thrice and in each analysis, 10 µL of the single reagent composed of 272.6 mM ascorbic acid, 9.7 mM ammonium molybdate, 0.43 mM potassium antimony tartrate and 1.368 M H_2SO_4 was aspirated at 25 µL s^{-1}. This was followed by aspirating 40 µL of sample. The sample zone was sandwiched in the holding coil with another aspirated 10 µL of the single reagent using ports 3 and 4 for the same reagent and flow-through outlet port 5 for sample. Forty µL of the combined zones was dispensed to flowcell at 25 µL s^{-1} and delayed for 100 s for colour development and absorbance was simultaneously read. The sampling rate was 31 h^{-1}.

Optimization of manifold parameters: The stacking of sample and reagent zones in the holding coil was optimized to determine whether the combination of reagent/sample zones or a sandwich of reagent/sample/reagent zones would give greater sensitivity. For these tests, 40-µL samples of 200 and 500 µg P L^{-1} standards and 20-µL reagent were used. The selection of volumes was guided by FIAlab operation manual (2005). For the 2-zone stack, a 20-µL reagent zone was aspirated followed by a 40-µL sample zone. For the 3-zone sandwich, a sample zone was sequentially stacked in between two 10-µL reagent lines in the holding coil. The tests were performed using the SI protocol for P. Using a 2.0 mg P L^{-1} standard and 10 µL of the single reagent, eight different sample volumes were aspirated with a range of 10 to 140 µL in determining the optimum sample volume. Optimization of volume dispensed to flowcell (delay point) for the stopflow mode of sample sandwich (10 µL/40-µL sample/10 µL) was performed. For this test, 40-µL of 2.0 mg P L^{-1} standard and two 10-µL reagent zones were aspirated. Twelve different volumes of the sandwiched zones in the range 1 to 60 µL were dispensed to the flowcell and delayed for the absorbance reading.

Optimization of P colorimetric reagents and reaction time: The simplex procedure was used to simultaneously optimize acid-molybdate concentration, ascorbic acid concentration and reaction time (delay time) [16,17]. Ascorbic acid was allowed to vary between 0.0228 M and 0.9089 M and ammonium molybdate was allowed to vary between 0.0016 M and 0.0323 M. An $[H^+]$:$[MoO_4^{2-}]$ molar ratio of 40.3 was used in this work.

The corresponding [H^+] concentrations prepared for the molybdate reagent had a range of 0.4509 M to 9.103 M. Reaction time was allowed to vary between 30 and 151 s. A 2.0 mg P L^{-1} standard was used for this multi-factor optimization.

Interference studies: Three mixed standards of Al and P each containing 1.0 mg P L^{-1} and 2.0 mg Al L^{-1}, 10.0 mg Al L^{-1} and 20.0 mg Al L^{-1}, six mixed standards of Fe and P each containing 1.0 mg P L^{-1} and 0.5 mg Fe L^{-1}, 2.0 mg Fe L^{-1}, 10.0 mg Fe L^{-1}, 20.0 mg Fe L^{-1}, 100 mg Fe L^{-1} and 150 mg Fe L^{-1}, and five mixed standards of Si and P each containing 1.0 mg P L^{-1} and 0.5 mg Si L^{-1}, 2.0 mg Si L^{-1}, 10.0 mg Si L^{-1}, 20.0 mg Si L^{-1}, and 100 mg Si L^{-1} were prepared to determine the effect of interfering ions on the P analysis. These interferent-ion spiked P standards and unspiked 1.0 mg P L^{-1} standard were analyzed for P using the SI procedure. The difference in absorbance between the spiked standard and the unspiked standard was presented in this study as percentage of the absorbance of unspiked P standard.

Optimization of extraction conditions: A series of nine concentrations (0.0 to 0.5 M) of $NaHCO_3$ (pH 8.5) were used to extract 1.0 g of the same homogenized, air-dried sediment (sieved through 2 mm). Twenty milliliters of each $NaHCO_3$ solution was added to the sediment and shaken at 200 rpm and 25^0C for 30 min using thermostated mechanical shaker (SHZ-88, Jiangsu Taicang Lab) [5]. The extracts were filtered through Whatman No 42 papers. Three replicate extractions were obtained for each $NaHCO_3$ concentration and analyzed for P. Simplex algorithm was used to simultaneously optimize extractant volume, extraction speed and time for extraction. Extractant volume was varied between 20 and 120 mL, extraction speed between 160 and 240 rpm and extraction time between 5 and 100 min. In every experiment, 1.0 g of the same homogenized sediment was extracted with 0.5 M $NaHCO_3$ and extraction temperature of 25^0C was used. The extracts were filtered through Whatman No 42 papers in three replicates and analyzed for P. Because of the changing extractant volume which resulted in different sediment-to-solution ratios, the response here was calculated as absorbance times volume divided by mass of sediment (i.e., $A \cdot V/m$).

Optimization of alkaline persulfate digestion: The alkaline persulfate digestion method was optimized for quantitative recovery of TP. For this, the mass of potassium persulfate used for digesting a constant mass of sediment was increased from 0.3 g to 1.8 g. Sediment with high organic matter (3.89 %) was selected. In each experiment, 0.5 g of sediment was accurately weighed into a 100-mL digestion flask (Boro 3.3, Samduk). Two milliliters of 0.3 M NaOH solution and 0.3 g potassium persulfate were added to it. Allihn condenser was fit to the digestion flask and the upper end of the condenser was stopperred to have a closed apparatus for digesting and refluxing the mixture and the cooling water flowing through the condenser was at approximately 2 - 3^0C and pumped by a multifunction vacuum pump (Zhengzhou Greatwall, SHB-III). The digestion was carried out on a preheated magnetic stirrer (Jincheng Guosheng, 78-1) at 110^0C for 2 h.

Validation of P methods: To validate BAP method, four aliquots (4.0 g) of a sediment sample previously analyzed to contain 19.3 mg P kg^{-1} (as BAP) were each spiked with 0.5 mL of 60 mg PO_4^{3-}-P L^{-1} standard. They were stirred, allowed to stand for 2 h and were analyzed for BAP. In the validation of TP method, laboratory-fortified matrix samples and blanks were prepared. For the matrix sample set, three aliquots (1.0 g) of a sediment sample previously analyzed to contain 1,372.1 mg P kg^{-1} (as TP) were separately spiked with 0.1 mL of 4000 mg P L^{-1} tricyclohexyl phosphine (THP), 4000 mg P L^{-1} adenosine 5'-diphosphate disodium (ADP) and 2500 mg P L^{-1} KH_2PO_4. THP (1.8110 g) was dissolved in 2.5 mL ethanol (99.7 %) by sonication and diluted to 50 mL with ultrapure water. They were allowed to stand for 1 day before analysis for TP. For the blank set, three aliquots (1.0 mL) of ultrapure water were separately spiked with 0.1 mL of 400 mg P L^{-1} D-glucose-1-phosphate dipotassium (G-1-P), 400 mg P L^{-1} ADP and 250 mg P L^{-1} KH_2PO_4 and were analyzed for TP.

2.2 Fe Procedures

Procedure for Fe reduction: The mixture comprising 25 mL of sample, 0.4 mL of concentrated HCl and 0.4 mL of 10% $NH_2OH{\cdot}HCl$ was heated for 7 min on a preheated magnetic stirrer. It was cooled and diluted back to 25 mL with water. Subsequently, 1 mL of buffer solution (pH 3.96) composed of 1.87 M ammonium acetate and 6.13 M glacial acetic acid was added to 4 mL of the sample solution before SI analysis of dissolved Fe.

SI protocol for dissolved Fe: Fe analysis was carried out as shown in the micro-SIA-LOV manifold (Fig. 1.b.). The SI analysis was looped thrice and in each analysis, 10 µL of the 25.22 mM orthophenanthroline reagent (port 4) and 150 µL of buffered sample (port 5) were sequentially aspirated at 25 µL s^{-1}. This was followed by aspirating 100 µL of carrier water. The stacked zones were then propelled to the flowcell and data was collected. The sampling rate was 75 h^{-1}.

Optimization of manifold variables: Reagent concentrations for this optimization were adapted from APHA [10] and 25.22 mM 1,10-phenanthroline was prepared to achieve a high sensitivity. Aspirated sample volume was optimized by testing ten different sample volumes in the range 10 to 170 µL using a 5.0 mg Fe L^{-1} solution. Sample was taken through the Fe reduction procedure and analyzed for Fe as outlined in the SI protocol. The ratio of sample volume to buffer volume was optimized by testing eight different buffer volumes in the range 0.0 to 3.0 mL at a fixed sample volume of 3.0 mL using a 5.0 mg Fe L^{-1} solution.

Validation of Fe methods: Mineral Fe was extracted from 2.0 g of sediment with 20 mL of 0.5 M HCl at 200 rpm and 25 ^{0}C for 6 h using thermostated mechanical shaker (SHZ-88, Jiangsu Taicang Lab). Extract was filtered through 0.45 µm cellulose acetate membranes. For the matrix sample set, four aliquots (2.0 g) of a sediment sample previously analyzed to contain 1,535 mg Fe kg^{-1} were each spiked with 0.3 mL of 500 mg Fe L^{-1}

$FeCl_3 \cdot 6H_2O$. They were stirred, allowed to stand for 2 h and analyzed for mineral Fe. For the blank set, three aliquots (0.3 mL) of 500 mg Fe L^{-1} $FeCl_3 \cdot 6H_2O$ were dispensed into three 100-mL conical flasks and were analyzed for mineral Fe through the HCl extraction step**.**

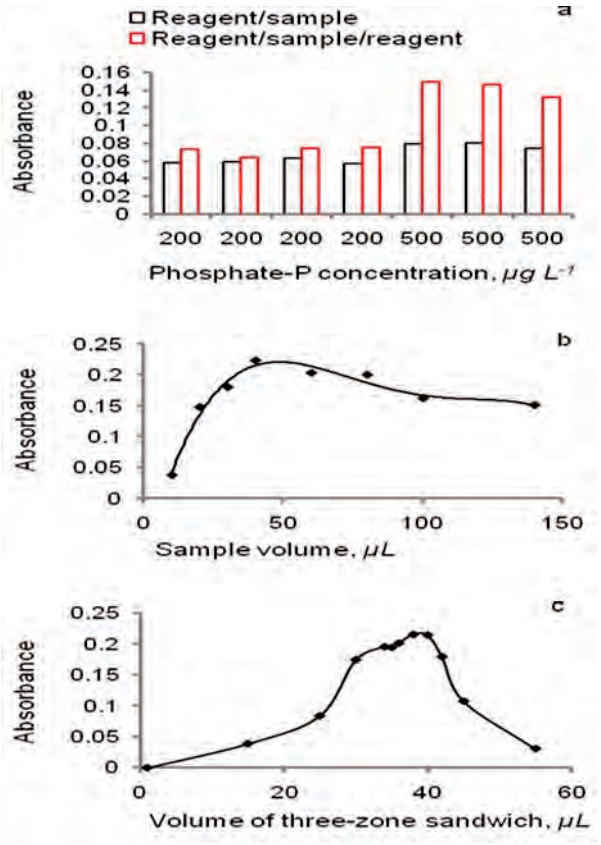

Fig. 2 a. Improved sensitivity of a sandwiched sample over reagent/sample combination in molybdenum blue method for phosphate analysis. **b.** Variation of aspirated sample volume of a 2.0 mg P L^{-1} standard in search of the optimum with 10 µL of reagent in both sandwiching zones. **c.** Profile of zone overlapping in a three-zone sandwich for the optimization of volume dispensed to flowcell in stopflow analysis of a 2.0 mg P L^{-1} standard.

3. Results and Discussion

3.1 P manifold optimization

Influence of 3-zone stacking over 2-zone in the holding coil: The results of stacking optimization are presented in Fig. 2.a**.** At both 200 and 500 µg P L^{-1}, a sandwich of 40-µL sample in between two 10-µL reagent lines did not only give a higher response than a 20-µL reagent/40-µL sample combination, but the difference in their responses widened with increasing phosphate concentration as shown in Fig. 2.a. This was due to greater mixing between sample and reagent through two reagent overlaps as against one reagent overlap and one carrier-water overlap inherent in the reagent/sample combination whereby sample dilution by the carrier water was normal. In addition, reagent concentration available to the sandwich was higher than that available to the two-zone stack. Therefore, sample sandwiching was adopted for this study.

Influence of aspirated sample volume on molybdenum blue sensitivity: Variation of sample volume was performed to determine the optimum value so that sample would not be too diluted by an excessive reagent volume and vice versa. The results are illustrated in Fig. 2.b. Below 40 µL, sample became too small and too diluted by excessive reagent, therefore, the response had a sharp decrease to the left of 40 µL in the line graph (Fig. 2.b**.**).

Above 40 µL, the reagent became increasingly slightly diluted by excessive sample volume as shown by the gentle slope to the right of 40 µL. This was because the longer the sample zone, the further its dispersion approaches 1 at its center and the reagent concentration moves close to zero at that point of sample concentration maximum. The optimum sample volume was 40 µL and was adopted for this study.

Influence of volume dispensed to flowcell (delay point) for sample sandwiching: The results of dispensed volume optimization are displayed in Fig. 2.c**.** It was discovered that the optimum point was approximately 40 µL (38-40 µL) at the 67 % position of the total sandwich length (60 µL). This point did not coincide with the center of the sandwiched sample where sample concentration was supposedly maximum. The response at the center (30 µL) of sample zone was 0.175 while that at the three-quarter point (40 µL) of sandwich was 0.215. The competition for optimum response between the central point of assumed concentration maximum and the three-quarter point was shown by a disappearing second peak left of the major peak in Fig. 2.c. The general profile slightly resembled a doublet separated by a minimum at 35 µL, or better, a small plateau was first reached extending from 30 µL to 36 µL and then a small rise to a hill in between 38 and 40 µL (Fig. 2.c**.**). In further analyses, 40 µL of the sandwich was the dispensed volume adopted.

3.2 P colorimetry optimization

Influence of P colorimetric conditions: The results of optimization of P colorimetric conditions are shown in Fig. 3.a. Each response was the absorbance of an experiment comprising three factors undergoing simultaneous variation, namely, ascorbic acid concentration, ammonium molybdate concentration and reaction time. In Fig. 3.a**.**, the simplex moved up to climb the tallest ridge at the third experiment. It went down and up again probing through a vast space forming many small ridges from the fifth experiment to the tenth one in search of the optimum point. The simplex finally descended through experiments 11 and 12. After those many experiments,

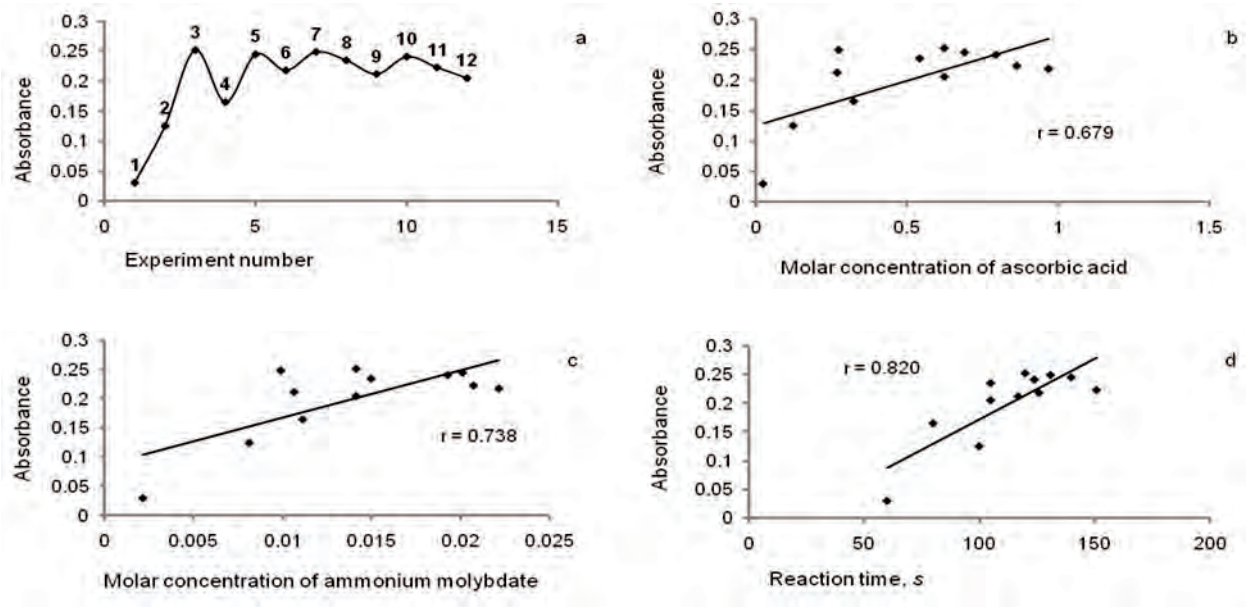

Fig. 3 a. Multivariate search of the optimum colorimetric conditions for phosphate analysis. Each absorbance was a triplicate average of the analysis of a 2.0 mg P L^{-1} standard. **b.** Significant contribution of ascorbic acid concentration to molybdenum blue development in univariate interpretation of the 3-factor experiments. **c.** Significant contribution of ammonium molybdate concentration to molybdenum blue development in univariate interpretation of the 3-factor experiments. **d.** Significant contribution of reaction time to molybdenum blue development in univariate interpretation of the 3-factor experiments.

it was not economical to explore more region as it appeared the optimum had been reached on the third experiment. The seventh experiment with conditions 0.2758 M ascorbic acid, 0.0099 M ammonium molybdate and 131 s gave the next highest response after the third experiment with conditions 0.6246 M ascorbic acid, 0.0141 M ammonium molybdate and 120 s and therefore, the conditions of the seventh experiment were adopted for the methodology of this study because it gave a very close response and it consumed much smaller concentrations of reagents. However, 0.2726 M ascorbic acid, 0.0097 M ammonium molybdate and 1.368 M H_2SO_4 were the final concentrations in the single reagent used in all further analyses with a reaction time of 100 s.

The multivariate response was further broken down into the individual single-factor scatter plots (Fig. 3.b. – 3.d.) to assess their significance to the response or molybdenum blue development. Increase in ascorbic acid concentration, acidic molybdate concentration and reaction time significantly increased ($p < 0.01$) response but the most important factor of the three was reaction time whose linear association with response was 0.820 (Fig. 3.d.) as determined by statistically significant Pearson correlation r. In addition, acidic molybdate concentration affected response more than ascorbic acid concentration and this is indicated by the higher association (r = 0.738, Fig. 3.c.) of the former than that (r = 0.679, Fig. 3.b.) of the latter. This was expected because ascorbic acid can only reduce 12-molybdophosphoric acid which is a first-step reaction product that depends on acidic molybdate reactivity and availability. While acidic molybdate can react with as much phosphate ions as possible, ascorbic acid reaction is limited by the quantity of that reaction product.

Effects of Interferent ions: Interference investigation of acid cations especially aluminum and iron was necessary due to the acidic pH of the sediment samples under study. Arsenic interference has been widely documented to be rare because of the very low concentration of arsenic (v) in the aquatic environment [6,18]. Fig. 4.a. displays the results of the interference studies. Between 0.5 and 2.0 mg Fe L^{-1}, ferric-ion spiked solution absorbed slightly above the unspiked standard with a difference of + 4.2 %. At 20 mg Fe L^{-1}, its response decreased largely to 0.139 from 0.161. When the new response was compared to the unspiked standard, it was different by -10.9 %. At very high concentrations, 100 to 150 mg Fe L^{-1}, ferric spiked solution steadily absorbed above the unspiked solution with a difference of + 17.9 % to 19.9 % respectively (Fig. 4.a.). The complex nature of positive and negative errors by iron may be due to two different occurrences. While the negative interference might be the result of a lowered phosphate concentration because iron could precipitate phosphate, it is difficult to attribute all the large positive interference at high iron concentrations to light absorption by the faint reddish brown colour of ferric ion or some other Fe complex because this occurrence did not increase significantly with increasing ferric ion concentration. If it is assumed that the initial positive difference (+ 4.2) was due to random error because it was small, the second and third differences were large and they stood for some effects. Aluminum-ion spiked

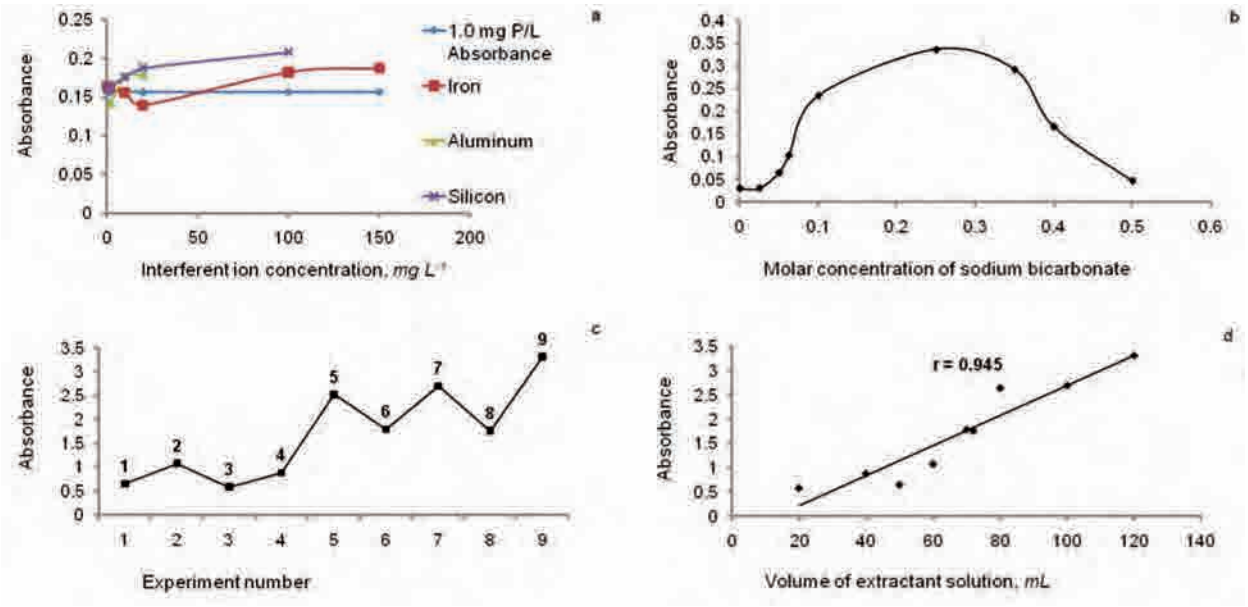

Fig. 4 **a.** Verification of the extent of aluminum, iron and silicon interferences in phosphomolybdenum blue method of phosphate analysis. Absorbance curves of the interferent ions cluster around the straight absorbance line of the 1.0 mg P L^{-1} standard which is parallel to concentration axis. **b.** Depletion of molybdenum blue absorbance by CO_2 gas in the confined manifold. Each absorbance was the average of three extractions for each extractant concentration. **c.** Multi-factor variation of bioavailable P extraction conditions in search of the optimum. Each absorbance plotted was calculated as instrumental absorbance times extractant volume divided by mass of sediment. **d.** Significant linear relationship between extractant solution volume and phosphate extraction capacity in univariate interpretation of the 3-factor optimization.

solution also absorbed below and above the standard with its response curve stretching across the standard line (Fig. 4.a**.**). At a low level of 2.0 mg Al L^{-1}, its response was - 9.2 % below the standard and at higher concentrations, 10 and 20 mg Al L^{-1}, its responses were + 13.5 % and + 14.7 % respectively above the standard. It is likely the negative interference was due to alum precipitation of phosphate, but the positive interference was strange, and it could not be light absorption by colour because aluminum solution was colourless.

Silicon behaved differently to some extent having only positive errors. At 0.5 mg Si L^{-1}, its response was slightly above the standard line with a difference of + 2.6 %. At 2.0 mg Si L^{-1}, it was different by + 5.4 % and as Si concentration increased, its response increased as shown by the gently rising slope in Fig. 4.a**.** It has been described to form molybdenum blue with ammonium molybdate in the same way as phosphate. At 100 mg Si L^{-1}, its positive interference was + 32.7 %. Benson *et al*. reported similar positive and negative interference especially for silicon in phosphomolybdenum blue chemistry [18]. The 0.5 M $NaHCO_3$ (pH 8.5) that was used to extract phosphate from the sediment was slightly alkaline. Under this pH, most multi-valent metals including iron and aluminum usually form insoluble hydroxides [19, 20] and were expected not to be extracted or their particulates be filtered out during extract filtration. Therefore, their interference might not be important. In the total-P sample preparation, after digestion, samples had their acidity neutralized with 1.5 M NaOH to a faint pink at pH 8.3 using phenolphthalein indicator. Again this pH could keep dissolved iron and aluminum ions coming from the digest in their insoluble hydroxide form and be eliminated during filtration.

Similarly, at concentration up to 2.0 mg Fe L^{-1}, which is unlikely to occur in the extract, the absorption of + 4.2 % can be tolerated as it is within random error. Only in acidic conditions can an aluminum level of 2.0 mg L^{-1} exist in the extract and as stated above, the sample preparation conditions were finally at pH 8.3 to 8.5. Therefore the negative absorption of 9.2 % due to Al cannot occur in reality. The positive interference of + 2.6 % and +5.4 % due to silicon at 0.5 mg Si L^{-1} and 2.0 mg Si L^{-1} respectively can also be tolerated. Tartrate was used in formulating the acidic molybdate-ascorbic acid reagent to speed up the rate of phosphomolybdenum blue complex and reduce interference by silicate. In real samples, silicate levels are unlikely to cause large positive interference owing to a number of conditions, namely, its available concentration, pH and chemical state because silicon does not react with molybdate in at least one of its forms, which is called molybdate-unreactive silica [6].

3.3 Carbon dioxide interference on molybdenum blue sensitivity

$NaHCO_3$ was employed for the extraction of bioavailable P but CO_2 generation from the reaction of $NaHCO_3$ with sulfuric

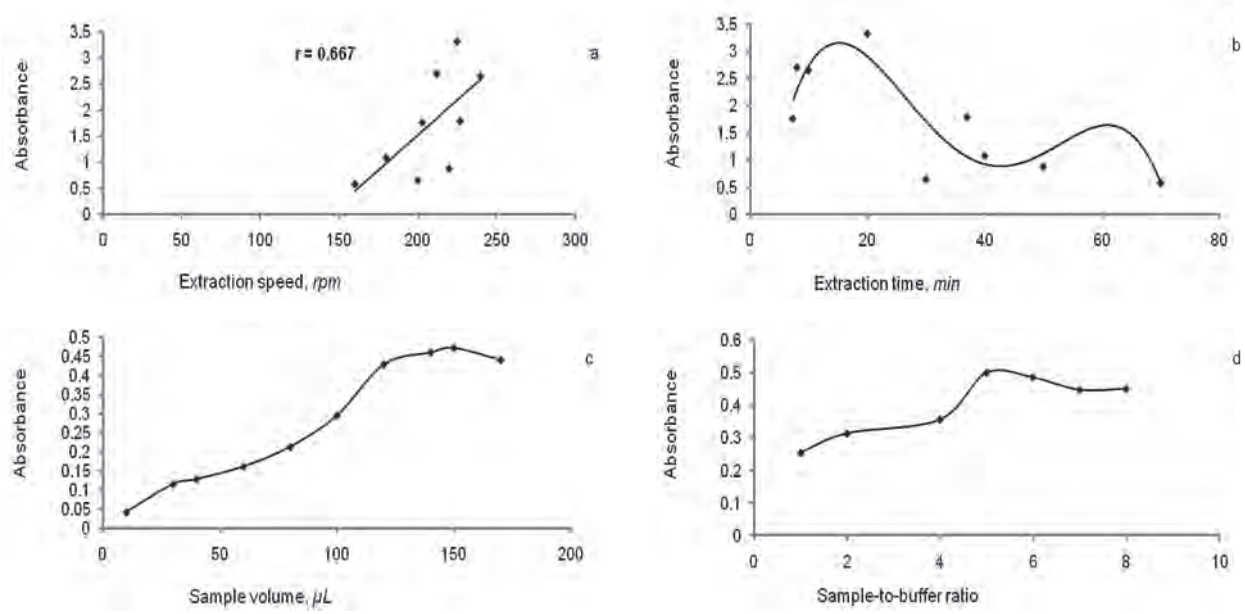

Fig. 5 a. Significant linear relationship between extractant speed and phosphate extraction capacity in univariate interpretation of the 3-factor optimization. **b.** Non-linear relationship between extractant time and phosphate extraction capacity. **c.** Variation of aspirated sample volume in search of the optimum using a 5.0 mg Fe L^{-1} solution at a sample-to-buffer ratio of 5:1. **d.** Variation of sample-to-buffer ratio in search of the optimum Fe-phenanthroline orange-red development using a 5.0 mg Fe L^{-1} solution.

acid in the colorimetric reagent interfered during spectrophotometric measurement in SIA and greatly reduced absorbance causing very low results. The effects of the interference on the measurement are illustrated in Fig. 4.b. At high concentrations of bicarbonate between 0.3 and 0.5 M, gas generation was high reducing light absorption by the blue product due to molybdenum blue dispersion/dilution by the gas. The absorption approached zero at about 0.5 M preventing the direct use of 0.5 M $NaHCO_3$. At low bicarbonate concentrations below 0.3 M, response was falling with decreasing molar concentration mainly because the phosphate extraction capacity was reducing (Fig. 4.b.). Therefore, to overcome this interference, the bicarbonate extract was carefully neutralized with 0.5 M H_2SO_4 to approximately pH 7.6 and some CO_2 gas was eliminated by this step. It was then diluted 12.5 folds with deionized water to further suppress the interference. This combination proved effective.

3.4 Optimization of extraction conditions

Influence of extraction conditions on BAP: The results of optimization of extraction conditions are shown by the line graph (Fig. 4.c.). Each response was the absorbance of an experiment comprising three factors undergoing simultaneous variation, namely, extractant solution volume, extraction speed and extraction time. The simplex probed around a vast region from experiment 1 to 4 and made three big jumps to experiment 9 amidst two falls which had the highest response. The tenth experiment could not be carried out due to violations of predetermined boundary conditions by the new results of the tenth vertex. Therefore, optimization was stopped. The ninth experiment comprised extractant volume of 120 mL, extraction speed of 225 rpm and extraction time of 20 min. However, extractant volume of 50 mL, extraction speed of 220 rpm and extraction time of 20 min were adopted in all further analyses because in case a sediment sample contains very low phosphate, too high extractant volume such as 120 mL can bring the phosphate concentration to an undetectable level.

Univariate interpretations of the 3-factor experiments are discussed with the aid of scatter plots (Fig. 4.d., 5.a. and 5.b.). Volume of extractant solution and extraction speed significantly affected ($p < 0.05$) response at a constant sediment mass (1.0 g), constant extractant concentration (0.5 M) and 25 ^{0}C. The response here was calculated as absorbance times extractant volume divided by sediment mass (i.e., $A \cdot V/m$). While increase in extractant volume and extraction speed led to higher response (Fig. 4.d. and 5.a**.**), higher extraction time appeared to lower response (Fig. 5.b.). The most important factor influencing the extraction response was extractant volume as shown by the significant Pearson correlation $r = 0.945$ (Fig. 4.d.) and this was followed by the extraction speed ($r = 0.667$, Fig. 5.a.). Positive association initially appeared between extraction time and response from 7.3 min to 20.0 min but later the association turned negative and positive again with increasing time (Fig. 5.b.). Long extraction times were therefore not ideal for BAP extraction. The association between extraction time and extraction response was appropriately defined by the quartic curve displayed in Fig. 5.b**.**

Table 1 Validation of P methods by recovery studies

Recovery studies of BAP		Recovery studies of TP			
		Matrix sample			
Spike level / mg P kg^{-1}	Found concentration plus 19.3 mg P kg^{-1} in original sediment	Reference compound	Spike level / mg P kg^{-1}	Found concentration plus 1,372.1 mg P kg^{-1} in original sediment	Recovery / %
7.5	25.9	THP	400.0	1834.0	115.5
7.5	26.5	ADP	400.0	1796.1	106.0
7.5	26.2	KH_2PO_4	250.0	1654.6	113.0
7.5	26.1	Average recovery			111.5
Average recovery / %	91.7				
Precision / %	3.6	Fortified blank			
		Reference compound	Spike level / mg P L^{-1}	Found concentration / mg P L^{-1}	Recovery / %
		KH_2PO_4	250.0	261.6	104.6
		G-1-P	400.0	387.5	96.9
		ADP	400.0	353.6	88.4
		Average recovery			96.6

Table 2 Validation of Fe methods by recovery studies of mineral iron using $FeCl_3 \cdot 6H_2O$

Matrix sample			Fortified blank		
Spike level / mg Fe kg^{-1}	Found concentration plus 376.1 mg Fe kg^{-1} in original sediment	Recovery / %	Spike level / mg Fe L^{-1}	Found concentration / mg Fe L^{-1}	Recovery / %
75.0	451.7	100.9	500.0	487.0	97.4
75.0	443.9	90.5	500.0	496.5	99.3
75.0	446.3	93.6	500.0	477.0	95.4
75.0	445.1	92.0			
Average recovery / %		94.3			97.4
Precision / %		4.9			

Influence of potassium persulfate mass on digestion efficiency: As the mass of potassium persulfate used for digesting a constant mass (0.5 g) of a brown-coloured sediment was increased from 0.3 g to 1.8 g in 0.3-g increment, the brown colour of the sediment was still observed at the end of the first, second, up to fourth experiments implying the presence of undigested organic matter, although it was fading with the increase in persulfate mass since every succeeding experiment had 0.3 g more $K_2S_2O_8$ than the preceding experiment. At the end of the fifth experiment consisting of 1.5 g $K_2S_2O_8$, amounting to a sediment : persulfate ratio of 1:3, the sediment became colourless and a clear solution was obtained implying the complete digestion of the sample. In all further digestion, a sediment-to-potassium persulfate ratio of 1:3 (or 1.0 g sediment/3.0 g $K_2S_2O_8$) was adopted for TP analysis. In addition to visual inspection, after each digestion experiment, the total P concentration of the solution was determined according to P protocol given above and the absorbance results were 0.456 for the first experiment and 0.511 – 0.529 for the remaining four experiments indicating that all TP in the sediment had been released into solution from the second experiment. Therefore, the slight variation in TP responses from the second to the fifth experiment was mainly due to heterogeneity in subsamples digested and analytical random errors.

3.5 P method performance

Limits of detection and quantitation for P analysis: In spiked deionized water, the method detection limit (MDL) was determined to be 0.019 mg P L^{-1} using the 3s criterion and the linear calibration range was 0.05 to 3.00 mg P L^{-1}. In contrast to the lower MDL in water, in spiked $NaHCO_3$ solution (spike level 0.5 mg P L^{-1}) taken through the bioavailable P procedure, the MDL increased to 0.109 mg P L^{-1} due to matrix effects. When this MDL value was multiplied by the sediment-to-solution ratio of 12.5 arising from the phosphate extraction step using 50 mL 0.5 M $NaHCO_3$ per 4.0 g sediment, the method detection limit for the bioavailable P method became 1.4 mg P kg^{-1}. In spiked water (spike level 0.2 mg P L^{-1}) taken through the alkaline persulfate digestion procedure for total P, the MDL was found to be 0.026 mg P L^{-1}. When this MDL value was multiplied by the sediment-to-solution ratio of 100 arising from the digestion step using 100 mL final solution volume per 1.0 g sediment, the MDL for total P became 2.6 mg P kg^{-1}.

Recovery studies for P method validation: The average recovery of bioavailable P was 91.7 % and precision was 3.6 % (Table 1). The average recovery of total P in laboratory-fortified-matrix samples was 111.5 % while average recovery in laboratory-fortified blanks was 96.6 % (Table 1). The over-recovery (111.5 %) of matrix samples indicated that the matrix of the samples caused a small positive error. The results demonstrate the good accuracy of the P methods including quantitative dissolution of organic and condensed P by the sediment-to-persulfate ratio of 1:3 without undesired waste of the reagent.

3.6 Fe method optimization

Influence of sample volume and sample-to-buffer ratio on Fe colorimetric sensitivity: Fig. 5.c. shows the influence of aspirated sample volume on the sensitivity of iron – phenanthroline orange-red method where peak height increased gradually with increasing aspirated sample volume until 150 µL was reached. After 150 µL, sensitivity dropped slightly. Therefore, 150 µL was adopted for all analyses because this was the point of minimal sample dispersion by the mixing phenanthroline reagent.

At sample pH below 2.9, much lower results will be obtained from the Fe^{2+} - phenanthroline reaction [9], therefore, samples must be rightly buffered above 3.0. When no buffer was added to the sample, the response was extremely low 0.065, because sample pH was measured to be 0.49, a value that was below 2.9. When 3.0 mL of buffer was added to 3.0 mL of sample to give a 1:1 ratio, the response jumped to 0.254. As the sample : buffer ratio increased, that is, as the sample volume increased relative to buffer volume, the peak height increased from ratio 1 to 5. Figure 5.d. explains the results. After ratio 5, the peak height dropped gently and was almost constant from ratio 7 to 8. Although ratio 5 gave the highest sensitivity, ratio 4 was used in all analyses so as to properly buffer samples.

3.7 Fe method performance

Limits of detection and quantitation for Fe analysis: The MDL in deionized water was determined to be 0.037 mg Fe L^{-1} using the 3s criterion and the linear calibration range for Fe – phenanthroline colorimetry was 0.09 to 9.00 mg Fe L^{-1}. In spiked HCl solution (spike level 0.1 mg Fe L^{-1}) taken through the mineral Fe procedure, the MDL increased to 0.044 mg Fe L^{-1} due to matrix effect. When this MDL value was multiplied by the sediment-to-solution ratio of 10 arising from the iron extraction step using 20 mL of 0.5 M HCl per 2.0 g sediment, the MDL for the mineral iron method became 0.44 mg Fe kg^{-1}. The method had good accuracy as average recovery of mineral iron in matrix samples was 94.3 % while average recovery in blanks was 97.4 % (Table 2).

4. Conclusion

The SIA protocols for P and Fe analyses offered high sample throughputs and the combination of neutralization and dilution of bicarbonate extract prevented CO_2 interference during SI absorbance measurement. The initial alkaline digestion offered by the method adopted for this study would be advantageous in case of materials stable to acid oxidation. The efficiency of the developed methods indicated that they are suitable for sediment P and Fe monitoring.

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