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Evaluation of on-line preconcentration and flow-injection amperometry for phosphate determination in fresh and marine waters

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Abstract

Dissolved reactive phosphorus (DRP) was determined as orthophosphate (PO_4-P) in fresh and saline water samples by flow-injection (FI) amperometry, without and with in-valve column preconcentration. Detection is based on reduction of the product formed from the reaction of DRP with acidic molybdate at a glassy carbon working electrode (GCE) at 220 mV versus the Ag/AgCl reference electrode. A 0.1 M potassium chloride solution was used as both supporting electrolyte and eluent in the preconcentration system. For the FI configuration without preconcentration, a detection limit of 3.4 μg P l^{-1} and sample throughput of 70 samples h^{-1} were achieved. The relative standard deviations for 50 and 500 μg P l^{-1} orthophosphate standards were 5.2 and 5.9%, respectively. By incorporating an ion exchange preconcentration column, a detection limit of 0.18 μg P l^{-1} was obtained for a 2-min preconcentration time (R.S.D.s for 0.1 and 1 μg P l^{-1} standards were 22 and 1.0%, respectively). Potential interference from silicate, sulfide, organic phosphates and sodium chloride were investigated. Both the systems were applied to the analysis of certified reference materials and water samples.

Keywords: Phosphate; Flow-injection amperometry; Preconcentration; Bio-Rad AG-X8; DRP; Schlieren effect

1. Introduction

Phosphorus is an essential plant and animal nutrient. However, due to human activities, inadvertent addition of phosphates to watersheds has caused eutrophication, which is commonly manifested by algal bloom formation [1]. Analytical methods for determination of phosphorus species, especially readily bioavailable dissolved reactive phosphorus (DRP) are essential for investigating the sources, cycling and fate of phosphorus (P) in aquatic ecosystems.

Spectrophotometric methods for determination of DRP are commonly based on the reduction of the phosphomolybdate complex (formed from reaction between reactive phosphate and acidic molybdate) to form the intensely coloured "molybdenum blue" product. Both batch [2] and flow-based [3,4] procedures have been reported. Although the spectrophotometric method provides good sensitivity, it suffers both from interferences, e.g. silicate, turbidity [3] and refractive index (Schlieren) effects in estuarine and marine samples, which can cause large errors in quantitation [5]. Electroanalytical methods are more tolerant of these interferences, and do not suffer from the Schlieren effect. Voltammetric or amperometric determination of phosphate has been performed using a range of different electrochemical reactions including, (1) reduction of 12-molybdophosphate to molybdenum blue [6–12], (2) oxidation of molybdenum blue, which was electrochemically prereduced and adsorbed on the electrode [13,14], (3) reduction of molybdovanadophosphate [15] and (4) oxidation of $FePO_4$ after reductive accumulation of Fe(II) in the presence of phosphate [16]. Flow-injection has also been applied for electrochemical determination of

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phosphate in order to improve performance of the technique. Some electrochemical methods involving the use of enzymes for determination of DRP have also been reported [17,18]. An FI amperometric system incorporating inorganic pyrophosphatase and nucleoside phosphorylase–xanthine oxidase reactors was used for simultaneous determination of phosphate and pyrophosphate [17]. An FI amperometric system with a nucleoside phosphorylase–xanthine oxidase column was also used for determination of phosphate with a detection limit of 1.25 μM [18]. The analytical characteristics of these electroanalytical techniques are summarized in Table 1.

The three major objectives of this work, based on the reduction of 12-molybdophosphate on a glassy carbon working electrode (GCE), were to evaluate amperometric detection of phosphate in fresh and marine waters, investigate the use of an in-valve preconcentration column and develop a simple manifold suitable for application in field instruments.

2. Experimental

2.1. Instrumentation

The FIA manifolds used in this study are illustrated in Fig. 1. A laboratory-made FIA workstation, consisting of two peristaltic pumps (Ismatec CA5E, Switzerland), an

Table 1
Summary of electroanalytical techniques and their analytical characteristics for determination of phosphate

Technique	Electrochemical reactions	Working electrode	Linear range (μg P l^{-1})	Detection limit (μg P l^{-1})	Reference
Batch injection analysis—amperometry	Reduction of molybdophosphate complex	Carbon paste	30–600	10	[6]
Batch/FI—differential pulse voltammetry (DPV)	Reduction of 12-molybdophosphate in acetone–water mixture	–	–	–	[7]
Voltammetry/amperometry	Reduction of molybdophosphate complexes	Gold microdisk	30–30000	–	[8]
Voltammetry	Reduction of 12-molybdophosphate in a capillary fill device	Screen-printed carbon	150–60000	150	[9]
FI-voltammetry	Reduction of 12-molybdophosphate in a reverse FI system	Glassy carbon	150–15000	–	[10]
Batch/FI—DPV	Reduction of 12-molybdophosphate	–	–	–	[11]
FI-voltammetry	Reduction of 12-molybdophosphate	Glassy carbon	30–15000	–	[12]
Adsorptive stripping voltammetry (AdSV)	Reoxidised of the preadsorbed hetoropoly blue	Glassy carbon	0.3–30	0.3	[14]
Voltammetry (DPASV)	ASV of β-heteropolymolybdates stabilized by acetone	Glassy carbon	–	–	[13]
Cyclic voltammetry	Reduction of 12-molybdophosphate	Carbon paste	400–25000	40	[22]
SIA-amperometry	Detection of molybdophosphate	Glassy carbon	100–3000	100	[23]
Voltammetry	Reduction of molybdovanadophosphate	Hanging Hg drop	0.06–300 mg P l^{-1}	0.15	[24]
FI-voltammetry	Reduction of molybdovanadophosphate	Glassy carbon	–	30	[15]
Cathodic stripping voltammetry (CSV)	Reduction of Fe(III) phosphate	Glassy carbon	–	100	[16]
FI-amperometry	Reduction of H_2O_2 produced from enzyme reaction (pyrophosphatase, nucleoside phosphorylase, xanthine oxidase)	–	–	–	[17]
FI-amperometry	Reduction of H_2O_2 produced from enzyme reaction (nucleoside phosphorylase, xanthine oxidase)	Platinum	–	40	[18]
FI-amperometry	Reduction of 12-molybdophosphate	Platinum	–1550	0.6	[25]
FI-amperometry	Reduction of 12-molybdophosphate	Glassy carbon	50–1000	3.4	This work
FI-amperometry, with preconcentration	Reduction of 12-molybdophosphate	Glassy carbon	0.1–10	0.18	This work

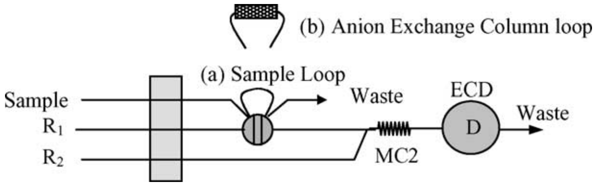

Fig. 1. FIA manifolds for the direct determination of orthophosphate: 60 cm mixing coil (MC2), $R_1 = 0.1\,\mathrm{mol\,l^{-1}}$ potassium chloride, $R_2 = 0.5\%$ ammonium molybdate in 2.5% (v/v) sulfuric acid, (a) 50 µl sample loop; (b) in-valve anion exchange column for preconcentration.

injection valve (Rheodyne 5041, USA) actuated by pressure from a compressed air-gas cylinder, and an electrochemical detector (Princeton Applied Research model 400, USA) was employed. The amperometric signal (µA) was converted to voltage (mV) within the detector, where 1 V full scale corresponds to 100 µA. PTFE tubing (0.5 mm i.d.) was used for mixing coils, and all manifold lines. The FCS computer program (A-Chem Technologies, Australia) was used to control the system and collect the data from the detector. The thin-layer flow-through cell (5 mm × 15.6 mm × 0.09 mm) consisted of a GCE (3 mm diameter, Princeton Applied Research, MP1305), a Ag/AgCl reference electrode and stainless steel cell body auxiliary electrode. The GCE was polished daily and rinsed with deionized water.

For the manifold in Fig. 1, standard/sample was injected into a stream of potassium chloride before merging with the acidic molybdate reagent. This manifold was also modified by replacing the sample loop with an ion exchange minicolumn to preconcentrate the analyte before injecting into the system. The potassium chloride served both as an electrolyte to promote electrical conduction and decrease the difference in electrical conductivity between sample and acidic molybdate and as the eluant to elute the phosphate from the ion-exchange column. The volume of standard/sample loaded onto the column was defined by the loading flow-rate and time.

2.2. Reagents

All solutions were prepared from analytical grade reagents and high purity, deionized water (Continental). The 500 ml of acidic molybdate solution (R_2 in Fig. 1) was prepared from 2.5 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (BDH) and 12.5 ml of concentrated H_2SO_4 (BDH). A 0.1 M potassium chloride solution was prepared from oven-dried KCl (BDH). Standard phosphate solutions in the concentration range of 10–1000 $\mu\mathrm{g\,P\,l^{-1}}$ were prepared daily from an intermediate solution (5 $\mathrm{mg\,P\,l^{-1}}$), diluted from a 100 $\mathrm{mg\,P\,l^{-1}}$ stock solution (0.2197 g oven-dried KH_2PO_4 (BDH) in 500 ml water). A 200 $\mathrm{mg\,l^{-1}}$ sulfide stock solution was prepared from $Na_2S\cdot 9H_2O$ (Sigma–Aldrich).

The anion-exchange mini-column was prepared by placing Bio-Rad AG1-X8 resin (Bio-Rad, USA, 200-400 mesh, chloride form) in a perspex column (5 mm × 1.5 mm i.d.), and plugging both ends with nylon mesh. The following certified reference materials were purchased from the Queensland Health Scientific Services, Australia, natural waters NLLNCT-Round 7: bottle 1, bottle 3, bottle 5 and bottle 7.

2.3. FI procedure

Potassium chloride and molybdate flow-rates in the FI system shown in Fig. 1 were 2.0 and 0.9 $\mathrm{ml\,min^{-1}}$, respectively. The potential applied to the GCE was fixed at 220 mV versus the Ag/AgCl reference electrode. The sensitivity range of the detector was 0.2 and 0.1 µA for the preconcentration system. Orthophosphate standard solutions (10–1000 $\mu\mathrm{g\,P\,l^{-1}}$) were measured in triplicate. Potential interferences including sodium chloride, sulfide, silicate and some organic phosphates were studied. The developed methods were validated using four certified reference materials. Eight water samples, which were collected from the water overlying the sediment in laboratory bioreactors were also analysed.

3. Results and discussion

3.1. Manifold without preconcentration

DRP concentrations, measured as orthophosphate (PO_4-P), were determined using the manifold depicted in Fig. 1a). The detection limit of this FI system, calculated using the criterion of 3× the standard deviation of the blank signal [19] was 3.4 $\mu\mathrm{g\,P\,l^{-1}}$. Linear calibration was obtained over the concentration range 50–1000 $\mu\mathrm{g\,P\,l^{-1}}$; the equation was: $y = 0.7834x - 2.4996$, $R^2 = 0.9995$. There was no curvature evident in the calibration curve, suggesting that even higher orthophosphate concentrations could be determined, if wastewater analysis was required. Attempts to simplify the manifold by using a single manifold line with injection of the sample into the acidic molybdate reagent resulted in a high negative blank peak due to the electrical conductivity difference between the reagent and sample streams. The detection limit in this case was 22 $\mu\mathrm{g\,P\,l^{-1}}$.

3.1.1. Signal reproducibility

The stability of the FI system was examined by measuring 50 replicate injections of 50 and 500 $\mu\mathrm{g\,P\,l^{-1}}$ phosphate standards. Fig. 2 shows that at a lower concentration of phosphate (50 $\mu\mathrm{g\,P\,l^{-1}}$), the peak-height response was very reproducible. The mean and R.S.D. of peak heights were 21.3 mV and 1.3%, respectively. More variability was observed with the higher phosphate concentration, where the mean and R.S.D. of peak heights were 182.6 mV and 9.5%, respectively. The poor reproducibility of these results at higher phosphate concentrations suggests that the reduced molybdenum blue complex may have precipitated in the flow cell.

3.1.2. Interference study

Several potentially interfering species have been studied. Quintana et al. [6], as well as many others, have reported

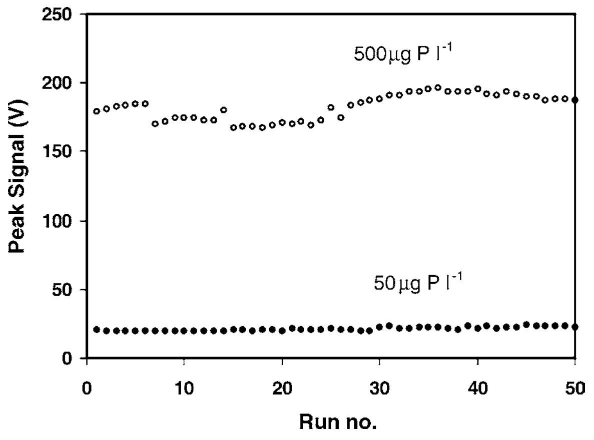

Fig. 2. Reproducibility of orthophosphate standard peak heights (50 and 500 μg P l^{-1}). Fifty replicate injections using the FI system 1a.

that silicate can cause major problems with phosphate determinations [2,6,15]. It was found here with silicate solutions of concentrations lower than 50 mg l^{-1}, no peak signal was obtained. Given that silicate is usually less than 20 mg l^{-1} in natural waters, silicate presents no significant interference for the determination of phosphate by this method.

Sulfide is another interferent in spectrophotometric phosphate determination [20]. Sulfide can be reduced at 220 mV (versus Ag/AgCl) at the working electrode and gives a negative peak. It was found that 5 mg l^{-1} of sulfide affected the peak-height responses of phosphate (50–100 μg P l^{-1}), but no interference was detected for 2 mg l^{-1} sulfide.

Potential interference by five organic phosphate species: adenosine-5-monophosphate, 2-aminoethyl phosphonic acid, glycerophosphate, phenyl phosphate and phytic acid, was also investigated. It was found that 100 μg P l^{-1} of each organic phosphate did not produce FIA peaks using the same conditions and manifolds investigated here for the determination of DRP. In natural waters, total organic phosphate concentrations do not normally exceed 50 μg P l^{-1}, although this may not be the case in sediment pore waters.

It was found that sodium chloride concentrations up to 4% (40 mg l^{-1}) did not affect peak height responses of phosphate over the range 50–1000 μg P l^{-1}. This method is therefore directly applicable to sea water samples without the need for salinity compensation. This represents a major advantage of this approach compared with the spectrophotometric FI determination of phosphate where adjustments are required to overcome the Schlieren (RI) effect [5].

3.1.3. Method validation and applications

Four reference materials: fresh waters, NLLNCT-Round 7: bottle 1 and bottle 3, and sea waters, NLLNCT-Round 7: bottle 5 and bottle 7, were analysed. The results shown in Table 2, indicate good agreement between expected and measured P concentrations, in both fresh and marine waters.

Table 2
Analysis of the certified reference materials (CRM)

Water type	CRM NLLNCT-round 7	Certified value (μg P l^{-1})	Found[a] (μg P l^{-1})
Fresh water	Bottle 1	27.0 ± 0.8	29.4 ± 1.4
	Bottle 3	97.9 ± 1.1	86.8 ± 1.1
Sea water	Bottle 5	27.7 ± 1.2	23.1 ± 0.3
	Bottle 7	11.8 ± 0.9	13.6 ± 1.7

[a] Mean of triplicate injections.

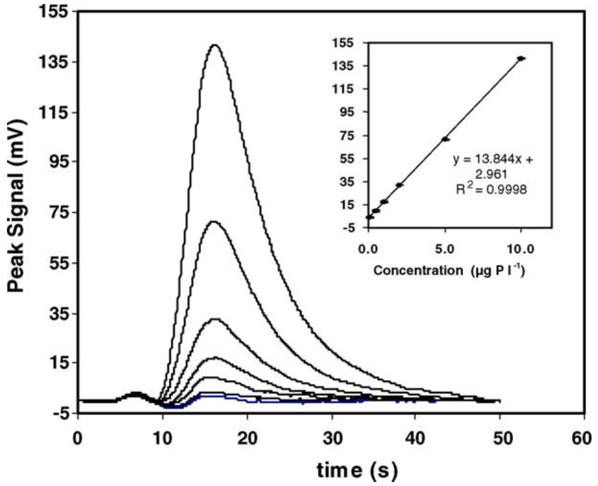

Fig. 3. FI graph and calibration graph of standard orthophosphate concentrations of 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 μg P l^{-1}: using the FI system 1b and preconcentration time = 2 min, detector range = 0.1 μA.

The method was also applied to eight saline water samples collected from the Gippsland lakes in eastern Victoria. The results, shown in Table 3, agreed well with the FI-spectrophotometric method (paired t-test, $t_7 = -0.10$, $p = 0.923$).

3.2. The FI system with preconcentration

The anion exchange minicolumn replaced the sample loop in the manifold shown in Fig. 1(b) and was used for preconcentration and subsequent determination of orthophosphate concentrations below 10 μg P l^{-1}. The calibration graph (Fig. 3) was linear between 0.1 and 10 μg P l^{-1}. Using a 2 min preconcentration time, at a loading flow-rate of 0.9 ml min^{-1}, the detection limit was 0.18 μg P l^{-1}. An

Table 3
Analysis of phosphate concentrations in sea water samples

Sample	Found (μg P l^{-1})	
	Electroanalytical procedure	Conventional spectrophotometric FIA
BB11	17	39
BB12	146	138
BB13	319	337
BB14	408	389
BB15	393	380
BB16	417	419
BB17	417	418
BB18	435	436

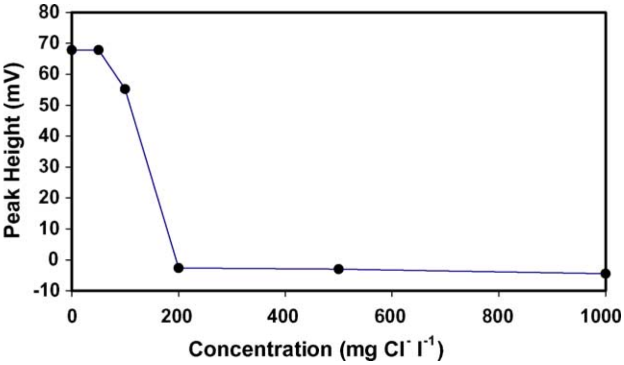

Fig. 4. The effect of chloride on peak signal response of 5 μg P l^{-1}. Preconcentration time: 2 min.

enrichment factor of 18, determined as the ratio of sensitivities with and without the preconcentration, was achieved.

Potential competition for ion-exchange sites by chloride ions present in the sample was investigated by analysing a series of 5 μg P l^{-1} standards containing chloride at a range of concentrations (0–1000 mg l^{-1}). Results indicate that chloride ion concentrations higher than 50 mg l^{-1} reduce the peak height response of 5 μg P l^{-1} (Fig. 4). At 200 mg l^{-1} of Cl^-, the phosphorus peak height response was indistinguishable from the blank signal. Similar interferences might be expected with other anions, especially divalently charged species such as sulfate with higher affinity for ion exchange sites on the column.

The preconcentration method was then applied to tap water samples. Recoveries for spiked orthophosphate standards were in the range of 92–126% (Table 4).

The viability of a single standard calibration using the preconcentration system was investigated using 5 and 10 μg P l^{-1} phosphate standards. By varying the preconcentration time (or loading time) for a sample, a linear relationship between μg P and peak height was obtained. The μg P was calculated by flow-rate (ml min^{-1}) × loading time (min) × standard solution concentration (μg P l^{-1}) × 10^{-3}. The data points using the 5 μg P l^{-1} solution coincided with the 10 μg P l^{-1} data (equation $y = 7345x + 7.47$, $R^2 = 0.996$), hence demonstrating the validity of the single standard calibration method.

Table 4
Spike recovery of phosphate by adding phosphate to tap water samples, using the preconcentration manifold (FI system 1b)

Sample	Concentration (μg P l^{-1})		% Recovery[a]
	Added	Found	
A	–	0.74[a]	–
	3.00[b]	3.50[c]	92
B	–	1.05[a]	–
	5.00[b]	6.83[c]	116
C	–	0.66[a]	–
	1.00[b]	1.92[c]	125
D	–	0.50[a]	–
	0.50[b]	1.09[c]	118

These data were calculated from the calibration equation: $y = 12.989x - 0.4932$, $R^2 = 0.999$.

[a] %Recovery = $[(c - a)/b] \times 100$.

4. Conclusion

An FI method using electrochemical detection has been developed for sensitive and rapid determination of orthophosphate. The efficacy of this method is demonstrated by a linear range (50–1000 μg P l^{-1}), covering orthophosphate concentrations commonly found in natural waters, with a detection limit of 3.4 μg P l^{-1} and sample throughput of 70 samples h^{-1}. The method is applicable to both fresh and saline samples and totally avoids the refractive index problems typically found with spectrophotometric methods. Analysis of oligotrophic freshwater samples is facilitated using the preconcentration system, where a detection-limit of 0.18 μg P l^{-1} was achieved using a 2 min preconcentration time. This method was only applicable to water samples with low chloride concentrations (<50 mg l^{-1}). It was demonstrated that a convenient, single standard calibration could be used with this preconcentration system. The analytical performance of these methods compares extremely favorably with the other studies listed in Table 1. The successful application of this simple, two line manifold to DRP determination in fresh and marine waters indicates great potential for further development into an instrument capable of field use, where artifacts engendered by sample preservation and storage can be avoided [21].

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