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Determination of dissolved reactive phosphorus (DRP) and dissolved organic phosphorus (DOP) in natural waters by the use of rapid sequenced reagent injection flow analysis

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Abstract

A FI system for rapid sequential determination of dissolved reactive and organic phosphate is described. It utilizes on-line UV photo-oxidation for digestion of dissolved organic phosphate (DOP) with detection of the phosphate produced as phosphomolybdenum blue at 690 nm after reduction of phosphomolybdate with tin(II) chloride. Two injections are performed in the analysis of each sample: the first of sample solution alone enables DRP determination, while the second is of sample plus alkaline peroxydisulfate solutions, which under the photo-oxidation conditions used converts DOP to DRP. The DOP content is evaluated from the difference of the two injections. The digestion efficiency for DOP, evaluated using a range of model organic P compounds of varying stability was greater than 97%. Calibrations were linear over the range of 0.01–6.00 mg P l^{-1} for both DRP and DRP + DOP graphs, with a detection limit (3 s) of 0.01 mg P l^{-1} for both species. Relative standard deviations were 0.3% ($n = 11$, 0.50 mg P l^{-1}) for the DRP determination and 1.0% ($n = 11$, 0.50 mg P l^{-1}) for the DRP + DOP determination. Injection throughput of 22 h^{-1} was achieved. The proposed system was validated by analysis of the certified reference materials and comparison with the previous flow injection system. Additional advantage of this system is that it requires the use of only small amounts of the oxidant, with the result that nuisance gas bubble formation is correspondingly minimized.

Keywords: Dissolved reactive phosphorus; Dissolved organic phosphorus; UV photo-oxidation; Peroxydisulfate; Flow injection

1. Introduction

Phosphorus is a limiting nutrient for algal growth in aquatic ecosystems, and in high levels has been recognized to be a factor in eutrophication and the development of algal blooms [1,2]. Phosphorus occurs in water as inorganic phosphate (i.e., orthophosphate and condensed phosphate) and organic phosphates in both dissolved and particulate forms [2,3]. While it is accepted that the DRP fraction approximates the most available form of phosphorus in aquatic ecosystems, there is growing interest in the transport, transformation and bioavailability of organic phosphorus [4]. The development of rapid and relatively selective methods for the detection of dissolved organic phosphate (DOP) is therefore important. The determination of phosphorus compounds in waters commonly involves measurement of the operationally defined fractions,

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dissolved reactive phosphorus (DRP), dissolved acid hydrolysable phosphorus (DAHP), which estimates the condensed phosphorus fraction, and total dissolved phosphorus (TDP). Dissolved organic phosphorus is often referred to as: [DOP] = [TDP] − [DRP], although more strictly this should be: [DOP] = [TDP] − [DAHP] − [DRP] [3]. Determination of phosphorus in these fractions depends on the complete conversion of phosphorus compounds present into orthophosphate, which can be directly reacted with molybdate to produce phosphomolybdenum blue after reducing the phosphomolybdic acid with a suitable reducing agent. Conversion of the DAHP fraction is accomplished using acid hydrolysis, while the DOP and/or TDP determinations require the application of various digestion methods, such as wet chemical digestion, high temperature combustion, or fusion, microwave digestion, or UV photo-oxidation [3,5,6]. A number of flow injection methods, which involve flow-through on-line digestion systems for the determination of TDP have been described. These systems used microwave digestion with nitric acid [7], or perchloric acid and peroxydisulfate [8], sulfuric acid and peroxydisulfate [9], and a heated capillary digester containing a Pt wire catalyst with peroxydisulfate [10].

UV batch photo-oxidation systems using high wattage UV lamps (≥1000 W) and long exposure time (3–8 h) have commonly been used for the conversion of TDP species to orthophosphate. The elevated temperatures that are generated and the gradual acidification of the sample that occurs as peroxydisulfate decomposes to sulfuric acid ensure that any condensed phosphates present are hydrolysed to orthophosphate. Flow injection systems incorporating low wattage UV lamps for UV photo-oxidation with thermal digestion [11], or UV photo-oxidation with acidic peroxydisulfate [12–14] have also been shown to provide effective TDP digestion.

However, it has been reported that UV photo-oxidation performed under alkaline conditions does not result in the conversion of condensed phosphates to orthophosphate [15], and this provides the basis for a selective measurement of DOP. When applied in a flow injection system that used a simple PTFE photoreactor wrapped around a 40 W medium pressure mercury lamp [16], complete photo-oxidation of DOP was achieved within 90 s. Model compound studies showed that even refractory compounds such as 2-aminoethylphosphonic acid, which in batch mode would require irradiation times in excess of 2 h [17] could be quantitatively converted to orthophosphate.

Previous flow injection methods for the determination of DOP have suffered from two important disadvantages, viz; that DRP must be measured separately, e.g., with the UV lamp switched off, so that DOP could be calculated from the difference $(DOP + DRP)_{UV\ ON} - (DRP)_{UV\ OFF}$, and that the consumption of the continuously pumped alkaline peroxydisulfate reagent (typically 40 g l^{-1}) is quite high. In this work, we propose the use of rapid sequenced reagent injection of oxidant in such a way that DOP + DRP and DRP can be determined on the same sample for determination of DRP and DOP in water samples. We investigated the efficiency of the UV photo-oxidation system used in this configuration using a range of model organic phosphorus compounds. The proposed FIA system has been validated using certified reference materials and applied to natural water samples.

2. Experimental

2.1. Apparatus

The FIA system (Fig. 1) consisted of two peristaltic pumps (MS/CA4-E/08/Synchro, Ismatec), a Teflon rotary injection valve (Rheodyne 5020), a three-way solenoid valve (Lee, LVFA series), and a spectrophotometric detector (Applied Biosystems, Spectroflow 757).

A UV reactor consisting of a 300 cm length of 0.3 mm, i.d. Teflon tubing wound in a figure eight configuration around a Gelman Clemco Slimline Germicidal UV-U-tube (40 W, model 9002) was housed in a light–tight, with fan-vented box. All mixing coils and injection loop were made of 0.5 mm, i.d. Teflon tubing. Bubble-removal (BR) was achieved using a 10 cm length of microporous tubing (Accurel PP, type S 6/2, 0.2 μm pore size, Enka AG).

A personal computer with a LabView program (Version 6.0, National Instrument) was employed to control the valve and pump switching (Fig. 2), as well as data collection and processing. The interface used was a custom designed 12 bit DAQ board with 8 digital outputs that were used for controlling switching of pumps and valves, as per the timing sequence shown in Fig. 2.

2.2. Reagents

All solutions were prepared in deionized water (with 18 MΩ/cm, Mobulab Analytical Research Grade RO/Polishing system, Continental® Water Systems Corporation, San Antonio).

Alkaline peroxydisulfate solution was prepared by dissolving potassium peroxydisulfate, 4.0% (w/v) (BDH, AnalaR) in sodium tetraborate, 3.4% (w/v) (BDH, AnalaR).

Acid molybdate solution was prepared by dissolving ammonium molybdate, 0.01 mol l^{-1} (BDH, AnalaR) in sulphuric acid, 0.63 mol l^{-1} (BDH, AnalaR).

Tin(II) chloride solution was prepared by dissolving tin(II) chloride, 0.001 mol l^{-1} (Fisons, Australia) and hydrazinium sulfate, 0.015 mol l^{-1} (Fisons, Australia) in sulphuric acid, 0.50 mol l^{-1} (BDH, AnalaR).

A stock standard solution of phosphate was prepared by dissolving potassium dihydrogen orthophosphate, 100 mg P l^{-1} (purity 99.5%, BDH, AnalaR). Working solutions were freshly prepared by dilution of the stock solution with water.

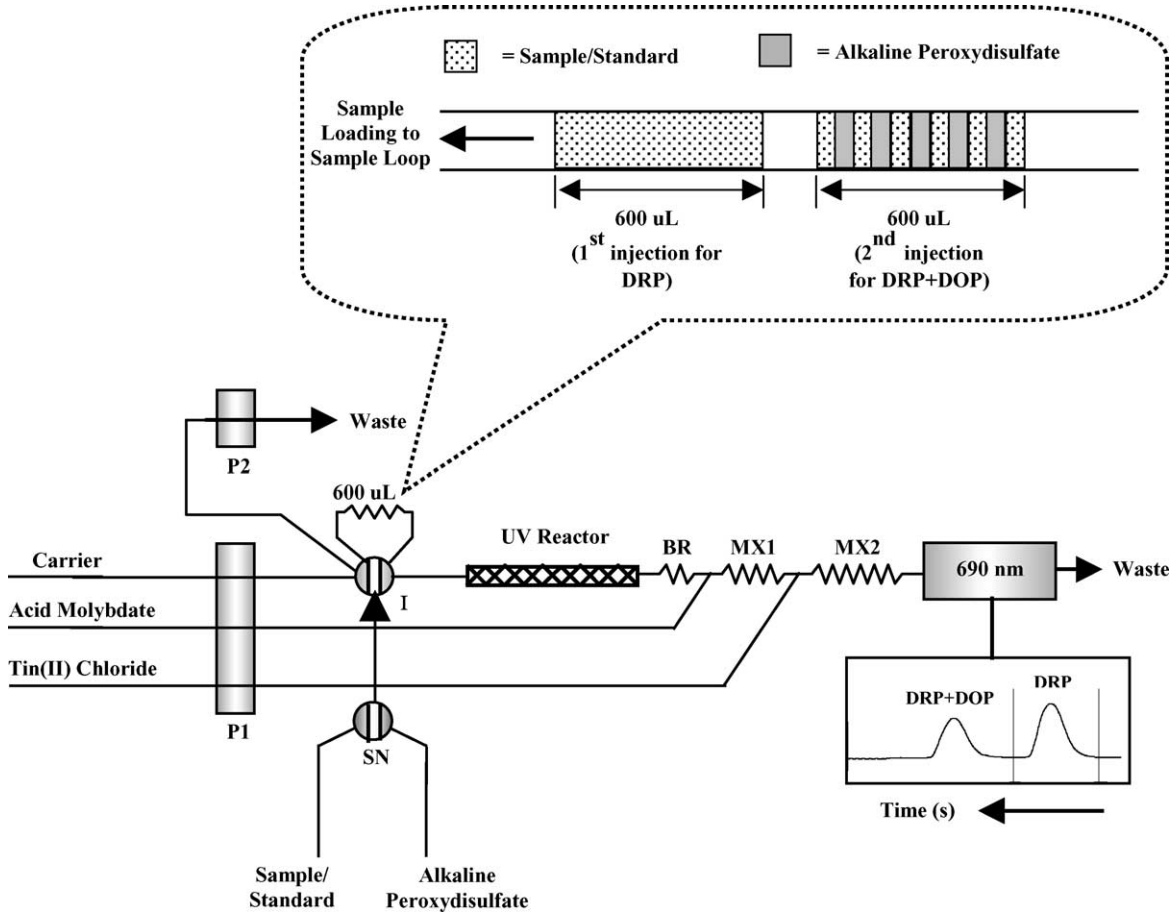

Fig. 1. Flow injection manifold used for the determination of DRP and DOP: carrier: deionized water (flow rate = 2.9 ml min^{-1}), acid molybdate = 0.01 mol l^{-1} ammonium molybdate + 0.63 mol l^{-1} sulfuric acid (flow rate = 1.1 ml min^{-1}), tin(II) chloride = 0.001 mol l^{-1} tin(II) chloride + 0.5 mol l^{-1} sulfuric acid (flow rate = 1.1 ml min^{-1}), alkaline peroxydisulfate = 4.0% (w/v) potassium peroxydisulfate + 3.4% (w/v) sodium tetraborate, P1: peristaltic pump 1, P2: peristaltic pump 2 (flow rate = 4.4 ml min^{-1}), I: rotary injection valve, SN: solenoid valve, UV reactor: U-shape UV lamp wound with Teflon tubing (300 cm × 0.3 mm, i.d.) in a figure of eight, BR: bubble-removal tubing (10 cm long), MX1: mixing coil 1 (90 cm × 0.5 mm, i.d.) and MX2: mixing coil 2 (90 cm × 0.5 mm, i.d.).

2.3. Model phosphorus compounds

A range of organic and condensed phosphorus compounds, containing PO_4^{3-}, P–O–P, C–O–P and C–P bonds as recommended by Kerouel and Aminot [18], were chosen for digestion efficiency testing experiments.

Stock standard solutions (100 mg P l^{-1}) of all model phosphorus compounds were prepared by using dried chemicals, viz., potassium dihydrogen orthophosphate (OrthoP, 99.5%, BDH), phytic acid (Phytic, 78%, Aldrich), adenosine-5′-monophosphate (AMP, Boehringer Mannheim), DL-α-glycerophosphate disodium salt (GRP, 95%, Sigma), phenylphosphate disodium salt (PhP, 95%, Sigma), 2-aminoethylphosphate disodium salt (AminoP, 97%, Sigma), phosphonoformic acid trisodium salt (PF, Sigma), sodium pyrophosphate (PyroP, 99%, Sigma), trisodium trimetaphosphate (MetaP, 95%, Sigma), and sodium tripolyphosphate (PolyP, Ajax). All working standard solutions were freshly prepared daily by appropriate dilution of these stock standard solutions.

2.4. Interference study reagents

Stock solutions (1000 mg P l^{-1}) containing interfering species, viz., Al(III), Ca(II), Cu(II), Fe(III), Zn(II), Mg(II), NO_3^- and Cl^-, were prepared from aluminium sulfate (M&B Laboratory Chemicals), calcium chloride (BDH), copper sulfate (BDH), ferric sulfate hydrate (Ajax), zinc sulfate (Sigma), magnesium chloride hexahydrate (Sigma), potassium nitrate (BDH), sodium chloride (BDH) and methanol (Fluka).

2.5. FI manifold and procedure

A schematic diagram of the manifold configuration is shown in Fig. 1. Two consecutive injections of standard or sample solutions were made into the carrier stream (deionized water). The first injection (600 μl) for DRP determination consisted only of sample, while the second for DRP + DOP determination comprised a series of adjacent zones of sample and alkaline peroxydisulfate oxidant occupying the 600 μl in-

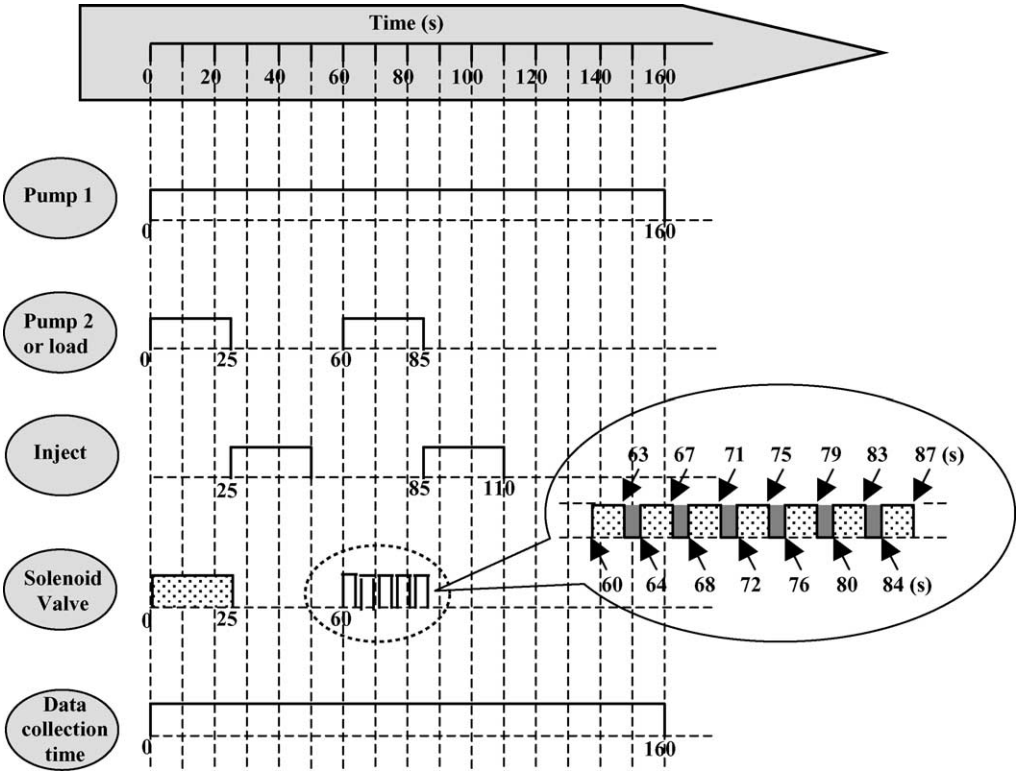

Fig. 2. Timing diagram for one analytical cycle by the system, for control of FI components by LabView Program; pump 1: controlling the flow rates of carrier and reagent streams; pump 2: controlling the sample loading into sample loop; inject: controlling the sample injection into the carrier stream; solenoid valve: controlling amounts of sample and alkaline peroxydisulfate into sample loop; data collection time: time period for collection of detector signal. (□): open; (▨): sample solution; and (■): alkaline peroxydisulfate solution.

jection loop. Both injected samples were pumped through the UV reactor with the UV lamp powered on. Any gas bubbles produced by the injection containing peroxydisulfate due to photo-oxidation were removed using microporous Accurel tubing. Orthophosphate in each injected sample zone reacted with acid molybdate and tin(II) chloride solutions to produce doublet phosphomolybdenum blue peaks corresponding to DRP and DRP + DOP, respectively, which were monitored at 690 nm.

The operation cycle controlled by the LabView program is represented in Fig. 2. Pump 1 was operated continuously. For the first injection to determine DRP, pump 2 was switched on for 25 s while the solenoid valve was positioned to load sample or standard solution into the sample loop of the injection valve. After injection into the system, a second injection to determine DRP + DOP was made, in which pump 2 was turned on for 25 s while the solenoid valve was alternately switched for 3 s to sample/standard and 1 s to alkaline peroxydisulfate solutions, to provide a sequence of short sample/standard and alkaline peroxydisulfate segments in the sample loop. Switching the injection valve led to the mixing of these samples and oxidant segments which were then passed through the UV reactor to convert DOP to DRP. Under the selected conditions, the first injection was separated from the second injection for 35 s. The data collection time for the two injections was 160 s, resulting in an injection throughput of approximately 22 double peak measurements per hour.

3. Results and discussion

3.1. Optimization of the FIA system for DRP determination

A standard solution 1.00 mg P l^{-1} of orthophosphate was used in the optimization of the system for DRP determination. The concentration of ammonium molybdate solution in 0.63 mol l^{-1} of sulfuric acid was varied between 0.004 and 0.02 mol l^{-1}, while the tin(II) chloride concentration (prepared in 0.015 mol l^{-1} hydrazinium sulfate and 0.5 mol l^{-1} sulfuric acid) was between 0.0006 and 0.0018 mol l^{-1}. The effects of sample injection volumes were studied between 400 and 1000 μl, and the lengths of mixing coils MX1 and MX2 were varied between 10–120 and 10–120 cm, respectively. The optimum values found for each variable are presented in Table 1.

3.2. Optimization of UV digester

Manifold conditions for the detection of DRP are summarized in Table 1 and the UV digester as described in Section

Table 1
The selected conditions used for DRP determination of the proposed FIA method

Parameters	Selected condition
Reagents	
Alkaline peroxydisulfate	4% (w/v) potassium peroxydisulfate + 3.4% (w/v) sodium tetraborate
Acid molydate	0.01 mol l^{-1} ammonium molybdate + 0.63 mol l^{-1} sulfuric acid
Tin(II) chloride	0.0010 mol l^{-1} tin(II) chloride + 0.015 mol l^{-1} hydrazinium sulfate + 0.5 mol l^{-1} sulfuric acid
Flow rates of	
Carrier line (ml min^{-1})	2.9 ± 0.1
Acid molybdate line (ml min^{-1})	1.1 ± 0.1
Tin(II) chloride line (ml min^{-1})	1.1 ± 0.1
Sample (pump #2) (ml min^{-1})	4.4 ± 0.1
Sample loop (μl)	600
MX1 (cm × mm, i.d)	90 × 0.5
MX2 (cm × mm, i.d.)	90 × 0.5
BR (cm)	10

2.1 was used. The conditions required to achieve the highest digestion efficiency for UV digestion were investigated by comparison of signals obtained from injections of phytic acid (one of the more refractory model organic phosphorus compounds) with those obtained for orthophosphate at the same concentration (1.00 mg P l^{-1}).

3.2.1. Ratio of sample and oxidant

The volume ratio of sample and sequenced oxidant (alkaline peroxydisulfate reagent) injections was investigated by varying switching time of the solenoid valve. For higher sample volumes, the digestion efficiency tended to decrease, while for larger oxidant injection volumes, acceptable conversion was achieved but the peak height was diminished. A sample ratio of 3:1, corresponding to volumes of 450:150 μl, was found to be the most suitable, because it gave both maximum signal response and conversion efficiency. The use of a sequence of alternating sample and reagent injections also minimized the amount of alkaline peroxydisulfate used, and decreased the volume of gas-bubbles produced, compared with the situation where alkaline peroxydisulfate reagent is continuously pumped through the UV photoreactor.

3.2.2. Photo-oxidation period and oxidant concentration

The photo-oxidation period and the concentration of peroxydisulfate are important factors affecting the digestion. The photo-oxidation period is inversely proportional to carrier stream flow rate. The influence of oxidant concentration on conversion efficiency of the photo-oxidation system was investigated at carrier flow rates of 1.1 and 2.9 ml min^{-1} for peroxydisulfate concentrations ranging from 1 to 4% (w/v). It was found that at the lowest carrier flow rate of 1.1 ml min^{-1} all model compounds except phenylphosphate were completely converted to orthophosphate at all of the oxidant concentrations tested (Fig. 3(a)). However, at a carrier flow rate of 2.9 ml min^{-1} (Fig. 3(b)), 4% (w/v) peroxydisulfate was required to achieve ca. 100% digestion efficiency for phytic acid. Thus, there is a compromise between minimizing the oxidant concentration and maximizing both conversion efficiency and sample throughput, and for this reason we have selected the higher carrier flow rate (equivalent to about 9–10 s of digestion time) and oxidant concentration for all subsequent work (Table 1).

The high selectivity of this photo-oxidation method for organic phosphates with respect to condensed phosphates is also highlighted by the results in Fig. 3(a) and (b), which show negligible conversion of condensed phosphate represented by PyroP, MetaP and PolyP, an observation which is in accord with that reported previously [20].

3.3. Analytical performance of the proposed FIA method

Under the optimized manifold conditions (Table 1), DRP + DOP calibration graphs for both orthophosphate and phytic acid standard solutions in the concentration range of 0.10–6.00 mg P l^{-1} were very similar (for orthophosphate: $y = 140.28x - 1.3949$; $r^2 = 0.9998$ and for phytic acid: $y = 137.15x - 3.1367$; $r^2 = 0.9995$). This indicates that the photo-oxidation efficiency remains adequate over a concentration range that will likely be encountered for measurement of waters, sediment and soil extracts, and porewaters and wastewaters. The observed relative standard deviations were 0.2–1.9% (0.1–1.0 mg P l^{-1} orthophosphate, $n = 11$) for DRP determination and 0.5–5.0% (0.1–1.0 mg P l^{-1} phytic acid, $n = 11$) for DRP + DOP determination. Detection limits (3 s) of 0.01 mg P l^{-1} for both of DRP and DRP + DOP were achieved, with an injection throughput of 22 h^{-1}.

3.4. Interference study

Peat et al. [14] have described three likely types of interference that may occur in the photo-oxidation method for DOP determination. These include interferences in the detection chemistry from anionic species such as silicate and arsenate that can compete with phosphate in the formation of phosphomolybdate. Secondly, cations such as Al(III), Ca(II), Cu(II), Fe(III), Zn(II) and Mg(II) may form insoluble complexes with either orthophosphate or organic phosphates such as phytic acid, some of which may not be amenable to photo-oxidation. Interferences of this type may have an effect on either or both the photo-oxidation and detection steps. Lastly, the presence in the sample of any species, such as bicarbonate, that may acts as a radical scavenger in the photo-oxidation process is likely to result in decreased conversion efficiency of organic phosphorus.

In this study, Al(III), Ca(II), Cu(II), Fe(III), Zn(II) and Mg(II) were added to 0.50 mg P l^{-1} of orthophosphate solution and the solution was injected into the system for DRP determination. It was found that the method tolerated (defined as a relative error of <5% in the signal of 0.50 mg P l^{-1} orthophosphate) a 1000-fold excess of Ca(II), a 4-fold excess of Cu(II), a 40-fold excess of Zn(II) and a 200-fold of excess of

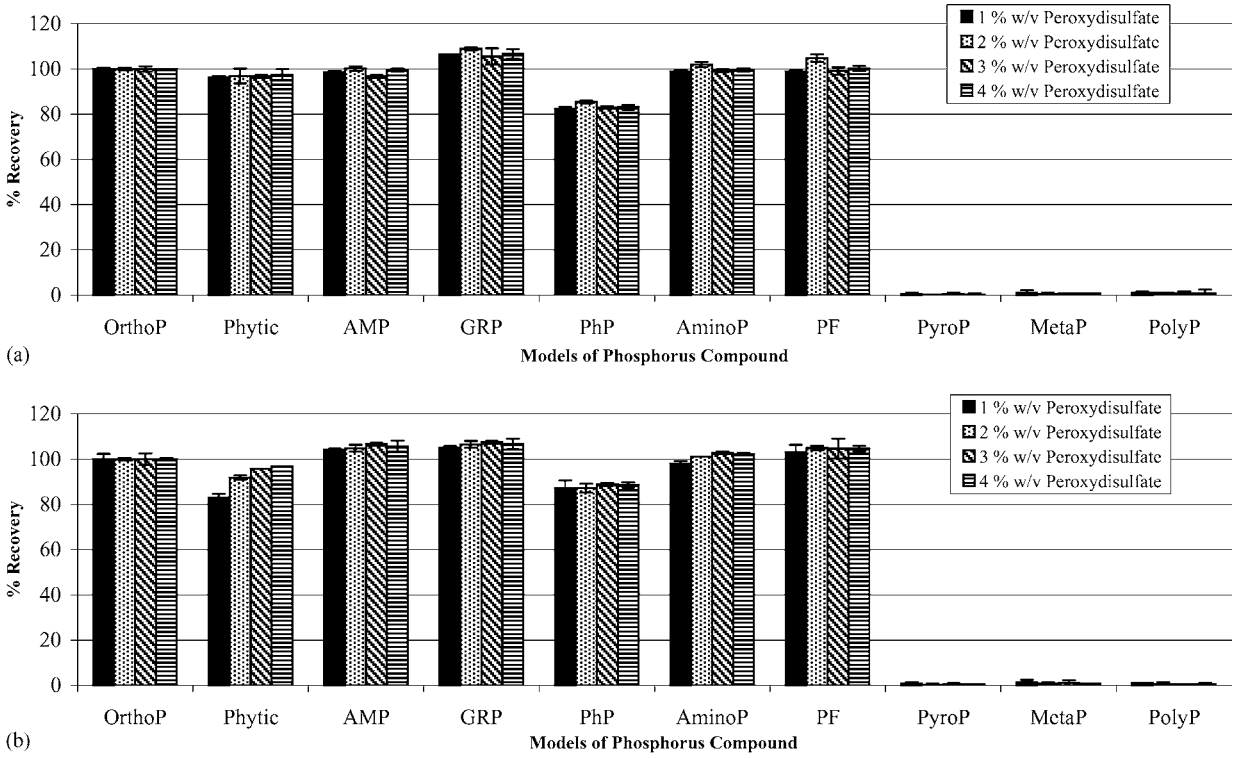

Fig. 3. Effect of potassium peroxydisulfate concentration (1–4%, w/v) on recovery of organic and condensed phosphorus model compounds (10.0 mg P l^{-1}) at: (a) flow rate of carrier = 1.1 ml min^{-1} and (b) flow rate of carrier = 2.9 ml min^{-1}. The % recovery is defined by comparison of 10.00 mg P l^{-1} of each model compound with that for 10.0 mg P l^{-1} of potassium dihydrogen orthophosphate. Error bars: one standard deviation (n = 3).

Mg(II), Al(III) and Fe(III) interfere seriously in this method, but they could be overcome by incorporating a strong cation exchange column before the injection valve [14].

The effect of Cl^- (e.g., in saline water samples) was tested by adding of 5000–30,000 mg Cl^- l^{-1} into 0.05, 0.10, 0.50 and 1.00 mg P l^{-1} of orthophosphate and into 0.05, 0.10, 0.50 and 1.00 mg P l^{-1} of phytic acid solutions. It was found that increased Cl^- concentration decreased the calibration slopes of both phytic acid and orthophosphate standards. This effect is less pronounced for the determination of DRP (Fig. 4), and is a well known artifact of the tin(II) chloride reduction of phosphomolybdate [3]. However, the effect of Cl^- is more evident on the slope of the phytic acid calibration, and this suggests that Cl^- also interferes in the UV digestion procedure, probably by oxidation of chloride. This observation is at odds

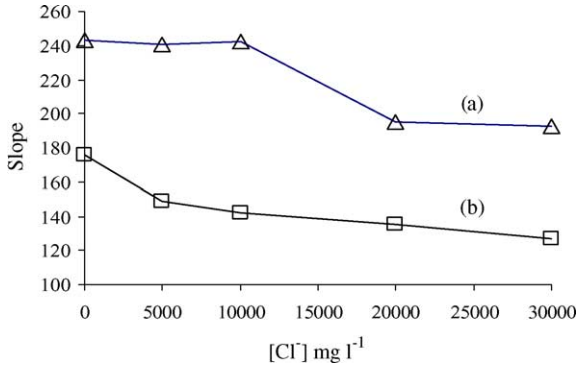

Fig. 4. Effect of chloride on slope of calibration graphs for: (a) DRP determination and (b) DOP determination. Standard solutions used were in the range of 0.05–1.00 mg P l^{-1} for both orthophosphate and phytic acid.

Table 2
Comparison of phosphorus concentrations obtained using the proposed FIA method with the specified concentrations of the certified reference materials (CRM) (Queensland Health Scientific Services, Australia)

No.[d]	DRP (mg P l^{-1})			DRP + DOP (mg P l^{-1})		
	Proposed FIA	CRM[a] value	% Recovery	Proposed FIA[c]	CRM[b] value	% Recovery
1	0.03 ± 0.02	0.027 ± 0.005	111	0.04 ± 0.03	0.037 ± 0.011	108
2	0.08 ± 0.02	0.098 ± 0.004	82	0.09 ± 0.02	0.106 ± 0.018	85
3	0.03 ± 0.02	0.028 ± 0.004	107	0.04 ± 0.02	0.040 ± 0.018	100
4	0.03 ± 0.02	0.012 ± 0.004	250	0.04 ± 0.02	0.034 ± 0.018	118

[a] CRM value for DRP: the certified value is the mean value of "filterable reactive phosphorus" as P (mg P l^{-1}).
[b] CRM value for DRP+DOP: the certified value is the mean value of "total dissolved phosphorus" as P (mg P l^{-1}).
[c] Phosphorus value (as DRP + DOP) found by this proposed FIA method should be total dissolved phosphorus.
[d] No. 1, 2, 3 and 4: the CRM (fresh water sample) numbers of NLLNCT—Round 7, bottles 1, 3, 5 and 7, respectively.

Table 3
Phosphorus contents in synthetic samples, determined by the proposed method (triplicate results)

No.	Added phosphorus standard (mg P l^{-1})					DRP (A) (mg P l^{-1})		DRP + DOP (B) (mg P l^{-1})		DOP[b] (mg P l^{-1})	
	OrthoP	Phytic	AMP	AminoP	PyroP	Added	Found	Added	Found	Added	Found
1	–	–	–	–	–	0.00	ND[a]	0.00	ND	0.00	ND
2	0.10	–	–	–	–	0.10	0.10 ± 0.03	0.10	0.10 ± 0.05	0.00	ND
3	–	0.40	–	–	–	0.00	ND	0.40	0.35 ± 0.05	0.40	0.32 ± 0.03
4	0.10	0.40	–	–	–	0.10	0.10 ± 0.03	0.50	0.46 ± 0.05	0.40	0.36 ± 0.02
5	–	–	0.40	–	–	0.00	ND	0.40	0.40 ± 0.04	0.40	0.38 ± 0.01
6	0.10	–	0.40	–	–	0.10	0.10 ± 0.03	0.50	0.50 ± 0.05	0.40	0.40 ± 0.02
7	–	–	–	0.40	–	0.00	ND	0.40	0.40 ± 0.05	0.40	0.37 ± 0.02
8	0.10	–	–	0.40	–	0.10	0.10 ± 0.03	0.50	0.50 ± 0.05	0.40	0.40 ± 0.02
9	–	–	–	–	0.40	0.00	ND	0.00	ND	0.00	ND
10	0.10	–	–	–	0.40	0.10	0.10 ± 0.02	0.10	0.10 ± 0.04	0.00	ND

[a] ND: not detected.
[b] DOP = $B - A$.

Table 4
Comparison the results for the determination of phosphorus in water by the proposed FIA and the previous FIA methods (triplicate results)

No.	DRP (A) (mg P l^{-1})		DRP + DOP (B) (mg P l^{-1})		DOP[b] (mg P l^{-1})	
	Proposed FIA	Previous FIA[a]	Proposed FIA	Previous FIA[a]	Proposed FIA	Previous FIA[a]
1	0.19 ± 0.02	0.19 ± 0.01	0.22 ± 0.03	0.22 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
2	0.19 ± 0.02	0.20 ± 0.01	0.22 ± 0.03	0.23 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
3	0.20 ± 0.02	0.20 ± 0.01	0.23 ± 0.03	0.23 ± 0.02	0.04 ± 0.01	0.03 ± 0.01
4	0.33 ± 0.03	0.33	0.39 ± 0.01	0.36	0.06 ± 0.01	0.03
5	0.32 ± 0.03	0.33	0.38 ± 0.01	0.34	0.06 ± 0.01	0.01
6	0.30 ± 0.03	0.31	0.36 ± 0.01	0.34	0.06 ± 0.01	0.03
7	0.31 ± 0.03	0.32	0.38 ± 0.01	0.34	0.07 ± 0.01	0.02

[a] Phosphorus values obtained from the previous FIA method [16].
[b] DOP = $B - A$.

with the previously observed salinity tolerance of the photo-oxidation procedure involving continuously pumped oxidant through the photoreactor [19]. Thus, in its present form, this method is not suitable for analysis of DOP in seawater, which contains approximately 19,200 mg Cl^{-} l^{-1} [20]. Similar loss of oxidation efficiency was also noted when methanol, which was used as a solvent for phospholipid samples, was present at greater than 2% (v/v), for presumably the same reasons.

3.5. Applications

The developed method was validated using certified reference materials (CRMs) for fresh water provided by Queensland Health Scientific Services, Australia. All the CRMs were filtered through a 0.45 μm pore size membrane filter before injecting into the system. The results are summarized in Table 2. Percent recoveries of phosphorus species obtained for all samples were close to 100%, except for sample No. 4, for which the DRP content was close to the method detection limit.

The proposed method was applied to a range of synthetic sample and natural water samples. Ten synthetic samples prepared by spiking standard solutions, viz., orthophosphate as DRP, phytic acid, AMP and AminoP as DOP, and PyroP as DAHP, into tap water were injected into the system. From the results obtained (Table 3), it was found that the concentrations found of DRP, DRP + DOP, and DOP were all close to the added ones for all samples.

Furthermore, seven samples of fresh water taken from Ornamental lake, Royal Botanic Garden of Melbourne were also analyzed by this proposed FIA method and by the previously reported FIA method [16]. All samples were filtered through a polyethersulphone membrane filter of 0.2/0.8 μm pore size (Pall, Gelman Sciences, USA). The data in Table 4 show that the DRP, DRP + DOP, and DOP contents determined by both methods are all in good agreement. The *t*-test method indicates that there was no difference between the results obtained from both procedures (at the 95% confidence level).

4. Conclusion

A new FIA method for the rapid sequential determination of DRP and DOP was developed. The performance of the FI system with UV photo-oxidation was tested for digestion of a range of model organic phosphorus compounds of varying refractivities. The digestion efficiency of better than 97% for DOP was achieved for all but phenylphosphate, and was selective for DOP species, even in the presence of condensed phosphate species. The effects of a range of interferents are reported. Chloride at concentration levels found in estuarine and sea water samples interfered in the digestion step, so the

method cannot be applied to the determination of DOP in such samples. The proposed method was validated by using CRMs and applied to fresh water samples. Compared with the previously reported FIA system [16], this proposed system is simpler and consumed much smaller volumes of reagents for digestion (150 μl in stead of about >2 ml per injection).

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