Talanta 133 (2015) 155–161

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

The use of on-line UV photoreduction in the flow analysis determination of dissolved reactive phosphate in natural waters

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ARTICLE INFO

Article history:
Received 3 June 2014
Received in revised form
30 June 2014
Accepted 21 July 2014
Available online 29 July 2014

Keywords:
Dissolved reactive phosphate
Flow analysis
UV photoreduction
Molybdenum blue reaction

ABSTRACT

A highly sensitive flow analysis manifold for rapid determination of dissolved reactive phosphate was developed which uses ethanol and UV light to reduce phosphomolybdic acid, instead of the reactive and short-lived chemical reductants typically employed in molybdenum blue chemistry. This reaction is impractical to perform reproducibly in batch mode, yet is very simple to handle in a flow analysis system and uses a single, very long-lived reagent solution. Interference from common inorganic anions and organic phosphorus species was minimal, and good spike recoveries for a range of sample matrices were obtained. The proposed flow analysis system is characterised by a limit of detection of 1.3 μg L^{-1} P, linear range of 5–1000 μg L^{-1} P, dynamic range of 5–5000 μg L^{-1} P, repeatability of 0.8% (1000 μg L^{-1} P, $n=10$) and 5.6% (10 μg L^{-1} P, $n=10$), and sample throughput of 57 h^{-1}. It is expected that this method will improve the feasibility of autonomous long-term environmental monitoring of dissolved reactive phosphate using inexpensive apparatus.

1. Introduction

Phosphorus is a limiting macronutrient for primary production in freshwaters, and when present in excess, eutrophication may occur. Dissolved reactive phosphate is the most bioavailable form of phosphorus, and its monitoring is used to assess water quality, the efficiency of phosphorus removal in wastewater treatment, the identification of anthropogenic phosphorus sources, and the elucidation of nutrient cycling processes [1].

The molybdenum blue reaction has long been known as an effective means of spectrophotometrically determining concentrations of dissolved reactive phosphate, and its use for this purpose is ubiquitous. Orthophosphate, in the presence of acid, binds to molybdate to form a faintly yellow heteropoly acid with the α-Keggin structure, which is subsequently reduced to an intensely coloured mixed-valence species known as phosphomolybdenum blue [2]. This blue colour is due to an intensely absorbing intervalence charge transfer (IVCT) transition involving interconversion of Mo(V) and Mo(VI). Many analytical methods using this reaction are based on Murphy and Riley's research [3], which exploited the accelerating effect of antimony on the reduction of phosphomolybdic acid by ascorbic acid, yielding a reaction product with an absorbance maximum at ca. 880 nm. This method has been modified by numerous authors [1,4–6]. The use of stannous chloride with hydrazinium sulphate as a co-reductant is also commonplace, yielding an absorption maximum around 720 nm. However, chloride is known to interfere with the $SnCl_2$ reduction method by up to 15% loss of sensitivity in seawater matrices, and this method demonstrates greater temperature dependence than the ascorbic acid reduction method [1,3]. Many flow analysis methods have been developed which use the same reductants, with the added advantage that the typically short reaction time needed in such systems reduces the likelihood and/or extent of acid hydrolysis of labile P-containing species in the sample, which has traditionally resulted in overestimation of dissolved reactive phosphate content [1,7].

A major practical problem associated with the use of these reductants, when the molybdenum blue reaction is applied to long-term automated field monitoring, is the limited reagent lifetime. Progressive oxidation of the reductants by oxygen means that the method response decreases over time, necessitating the preparation of fresh reagents and and/or frequent recalibration of the method [8]. Ascorbic acid in aqueous solution is only stable for 1 week even under refrigeration at 4 °C according to Standard Methods [9] (with the combined reagent solution stable for only 4 h), and combined with its considerable photosensitivity, its lifetime is certainly shorter when in use at ambient temperature even in the absence of other reagents. Reports by other authors

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http://dx.doi.org/10.1016/j.talanta.2014.07.058
0039-9140/

actually suggest that the lifetime of ascorbic acid is unpredictable, with reagent concentration and purity possibly having an effect as well [4]. Stannous chloride is longer-lived, and is quite popular in flow analysis methods due to its fast reaction kinetics [1], but it is also vulnerable to passive oxidation. It can apparently be stabilised indefinitely by dissolution in pure glycerol [9], although this solution is laborious to prepare, typically requires heating, and is especially difficult to use in flow analysis systems due to its high viscosity. These practical considerations suggest that a means of phosphomolybdic acid reduction that does not require short-lived reductants would be of great value in extended autonomous nutrient monitoring operations.

A number of alternative means for determining orthophosphate concentrations in natural waters have been proposed. Several are variants of the molybdenum blue reaction, relying on the formation of phosphomolybdic acid derivatives with spectrophotometric detection. The vanadomolybdate reaction is one such method, in which phosphate reacts with metavanadate and molybdate to form a V (V) substituted phosphomolybdic acid, which is typically measured at ca. 380 nm. This method is simple to carry out but is less sensitive than molybdenum blue methods, despite the use of UV detection and a number of re-optimised procedures [9–13]. The malachite green (MG) method is another spectrophotometric method for phosphate, which relies on the formation of an ion associate between a cationic dye and phosphomolybdic acid [13]. Although the sensitivity is similar to, or potentially greater than the molybdenum blue methods, this is countered by the need for a surfactant to stabilise the coloured ion associate [10], a tendency to hydrolyse organic P [10,14], and reagent precipitation problems. Ultimately, despite the potential merits of other methods, the molybdenum blue method remains the most popular for routine analysis of orthophosphate and is the basis of accepted standard methods [9,15].

The use of an unstable reductant in this reaction can be avoided if the photochemistry of molybdate is exploited instead. Whilst the molybdenum blue method for phosphate determination involving chemical reduction of phosphomolybdic acid is very widely used, the use of photochemical reduction has been rare. Several Russian researchers have reported the batch photoreduction of phosphomolybdic acid using high-powered (>400 W) Hg lamps for phosphate determination [16,17] and carried out an investigation of the reaction mechanism [18]. However, the use of this approach appears to be limited to these papers. This is probably due to problems with product stability, which are discussed below. Almost all of the attention directed towards polyoxometalate photoreduction to date has been in the context of inorganic synthesis and oxidation catalysis, notably in terms of water splitting [2,19,20].

When molybdate is irradiated with UV light in the presence of a hydrogen-bond donor, Mo=O bond excitation occurs through a ligand–metal charge transfer (LMCT) transition, forming a complex with the H-bond donor [2]. Reduction of the molybdate then occurs by a one-electron transfer from the H-bond donor, converting the latter into a radical (Fig. 1). This radical may react a second time with molybdate in order to complete its own two-electron oxidation to acetaldehyde. Species such as alcohols, amines, saccharides and carboxylic acids are all capable of performing the photoreduction [17,20–22], eliminating the need for much more reactive reagents. After further irradiation, this reaction may be repeated to yield a two-electron reduced species, albeit with decreased quantum yield [2]. It has been shown that the molar absorptivity of the two-electron reduced species is considerably greater than for the one-electron species [2,22]. The ease of photochemical reduction of these species follows their redox potentials; the phosphomolybdic acid heteropoly species is therefore much more readily reduced than the isopoly molybdate species, as is seen in the 'conventional' chemical methods described above. A considerable amount of work has been done by Yamase et al. on elucidating the mechanistic details of polyoxomolybdate photoreduction, primarily using alkylammonium ions as H-bond donors [21,23–29]. However, it appears that the quantum yield of molybdenum blue formation is greater in the presence of alcohols, and decreases if amines or carboxylic acids are used instead [22].

In molybdenum blue procedures where chemical reductants are used, the reduced heteropoly acid is typically stable at least for a number of minutes before the blue colour begins to fade, which is sufficient time for batch measurements to be carried out [3,9]. However, it has been observed that photo-reduced molybdenum blue species in solution are vulnerable to immediate re-oxidation by dissolved O_2 as soon as UV irradiation ceases, the kinetics of which are first-order in both dissolved O_2 and reduced molybdenum blue concentrations at low pH [30]. This phenomenon has been exploited in the regeneration of the oxidised molybdate species for catalytic purposes, but is potentially problematic for analytical use where a stable chromophore is desirable. The considerable product stability difference between chemical and photochemical reduction methods implies that excess reductant in solution stabilises the product against re-oxidation, as evidenced by the now-common use of hydrazinium sulphate in tandem with $SnCl_2$, without which the product formed by $SnCl_2$ alone loses its blue colour rapidly [31]. Stabilisation of photoreduced molybdenum blue has been obtained by solution degassing which, while simple to perform under laboratory conditions, is impractical for field use, especially for autonomous monitoring [32]. Whilst the rapid oxidation of photo-reduced molybdenum blue limits the application of this approach for batch analytical measurements, the same limitations do not apply where flow analytical techniques are used because the processes of sample injection, reagent mixing, irradiation, product formation and measurement are inherently controlled and reproducible.

The present paper reports on the development of a novel flow analysis method for the determination of dissolved reactive phosphate at trace levels using UV photoreduction of the phosphomolybdic acid instead of chemical reduction.

2. Experimental

2.1. Reagents

All reagents utilised in this research were used as received. Deionised water (18.2 MΩ cm, Millipore, Synergy 185, France) was used in the preparation of all aqueous solutions.

All phosphate standards were diluted as necessary from a stock solution of 1000 mg L^{-1} P, prepared by dissolving anhydrous Na_2HPO_4 (BDH, Australia), which had previously been oven-dried at 150 °C for 4 h, in 100 mL water. A certified reference material

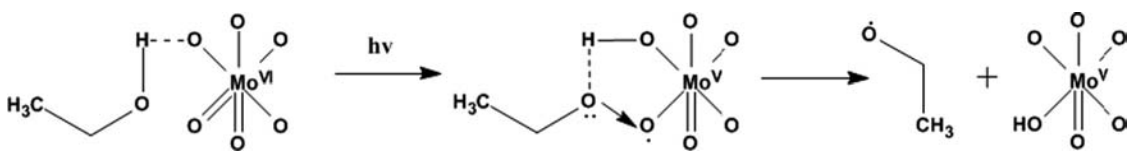

Fig. 1. Reaction scheme for the photoreduction of a molybdate species by a primary alcohol [21].

(certified value 3.40 mg L^{-1}; uncertainty- 3.53%; QC performance acceptance limits 2.94–3.84 mg L^{-1}; PT performance acceptance limits 2.89–3.91 mg L^{-1}) for orthophosphate (ERA, NIST SRM no 3186) was also used.

The reagent solution for the proposed UV photoreduction method was prepared firstly by the dissolution of ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, Ajax, Australia) in water. The requisite volume of 4.0 mol L^{-1} H_2SO_4, diluted from the concentrated acid (Scharlau, Australia), was then added, followed by the addition of anhydrous ethanol (Chem Supply, Australia). The reagent bottle was then covered with aluminium foil to prevent unwanted photoreduction of the molybdate, and was found to be stable for at least 2 months.

For the reference $SnCl_2$ molybdenum blue method, the molybdate reagent solution was prepared by the dissolution of ammonium molybdate tetrahydrate in water, followed by the addition of sulphuric acid diluted from a 4.0 mol L^{-1} stock solution. The reductant solution was prepared by the tenfold dilution of a stock solution containing 0.060% (w/v) anhydrous $SnCl_2$ (Ajax, Australia) and 0.600% (w/v) $N_2H_6SO_4$ (Ajax, Australia) and the subsequent addition of acid as for the molybdate solution. Sodium dodecyl sulphate (SDS) (Scharlau, Australia) was added to both solutions by the dilution of a 1.0% (w/v) stock solution.

Na_2SiO_3 (BDH), $Na_2HAsO_4\cdot 7H_2O$ (Sigma), NaCl (Chem Supply), Na_2SO_4 (BDH), and $NaNO_3$ (Chem Supply) were used in the interference studies. Phosphocholine chloride (calcium salt tetrahydrate, Sigma-Aldrich), phytic acid (dipotassium salt, Sigma-Aldrich) and glycerol phosphate (disodium salt hydrate, Sigma-Aldrich) were used in the organic photo-oxidation interference experiments. Seawater was obtained from Port Phillip Bay, Victoria (Australia).

2.2. Apparatus

On-line spectrophotometric measurements were performed using an optical fibre spectrophotometer (USB 4000, Ocean Optics, USA) coupled to a USB-ISS-UV-VIS light source (Ocean Optics, USA), with photometer data collection and operation controlled by the supplied OOIBase32 software (Ocean Optics, USA). A quartz flow-through photometer cell (Starna, UK) with optical path length of 1 cm was used.

For the UV photochemical reduction of phosphomolybdic acid, the UV source was a Gelman Clemco Slimline Germicidal U-tube (9002), with maximum emission at around 265 nm and ca. 40 W power consumption, encased within a home-made enclosure with added cooling fan [33]. Metal rods were added to the enclosure which surrounded the lamp, allowing the suspension of a reactor coil within the unit.

2.3. Flow manifolds

The flow injection manifold utilising UV photoreduction of phosphomolybdic acid is shown in Fig. 2a, and the reference molybdenum blue flow injection manifold, based on a system previously optimised by the authors [11], is shown in Fig. 2b.

Teflon tubing with 0.5 mm internal diameter (Supelco, USA) was used in both flow manifolds except for the waste line from the spectrophotometer, which utilised 0.3 mm internal diameter tubing to enhance back-pressure. Four-channel peristaltic pumps (Model VS4, Watson Marlow Alitea, Sweden), fitted with Tygon tubing (TACS, Australia) were used for solution propulsion. Pump flow rates were determined gravimetrically by measuring the mass of water of known temperature pumped for a predetermined period of time. A rotary injection valve (Model 5020, Rheodyne, USA) was used for sample injection in both systems. Absorbance measurements were made at 720 nm for the reference manifold and 860 nm for the UV manifold; the analytical signal was taken as the maximum absorbance of the transient absorbance peak for both systems.

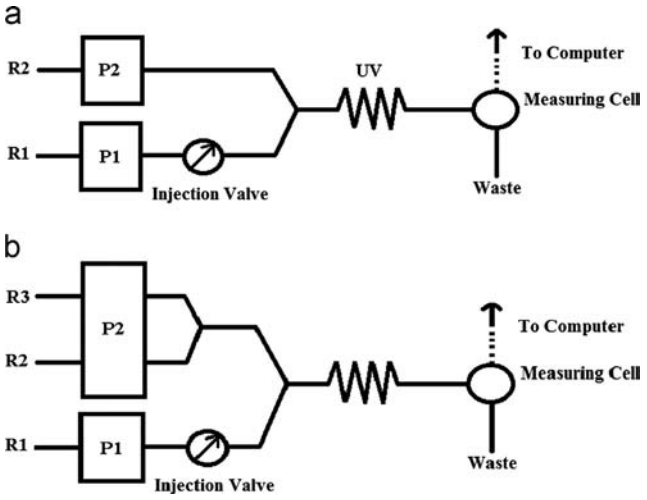

Fig. 2. (a) Schematic diagram of the UV photoreduction manifold, with photometric detection at 860 nm (P_1, P_2: carrier and reagent pumps respectively; R_1: deionised water; R_2: molybdate stream; UV: 4 m reactor coil exposed to a 40 W medium-pressure Hg lamp). (b) Schematic diagram of the flow analysis manifold for the reference molybdenum blue procedure, with photometric detection at 720 nm (P1, P2: carrier and reagent pumps respectively; R1: deionised water; R2; molybdate stream; R3: reductant stream).

2.4. UV photoreduction method

Samples containing orthophosphate were injected (500 μL) into a carrier stream of deionised water (R_1, 1.6 mL min^{-1}) which merged with the reagent stream (R_2: 0.45% (w/v) $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 1.05 mol L^{-1} H_2SO_4, 37.5% (v/v) ethanol, 0.4 mL min^{-1}), forming phosphomolybdic acid. The merged stream then passed through a reaction coil (4 m) exposed to a 40 W medium-pressure Hg lamp, which in combination with ethanol as a sacrificial reductant, formed phosphomolybdenum blue. The absorbance of the merged stream was monitored at 860 nm, the wavelength at which the product demonstrated maximum absorbance.

2.5. Reference molybdenum blue method

For comparative analyses, and for determining the presence of orthophosphate impurities or the extent of acid hydrolysis of model organic phosphate compounds, a previously reported flow method was used [11] (Fig. 2b). Samples containing orthophosphate were injected (500 μL) into a carrier stream of deionised water (R_1, 0.8 mL min^{-1}) which subsequently merged with reagent streams R_2 and R_3 (R_2: 0.5% (w/v) $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 0.1% (w/v) SDS, 0.40 mol L^{-1} H_2SO_4, 0.4 mL min^{-1}; R_3: 0.060% (w/v) $N_2H_6SO_4$, 0.006% (w/v) $SnCl_2$, 0.1% (w/v) SDS, 0.40 mol L^{-1} H_2SO_4, 0.4 mL min^{-1}) to form phosphomolybdic acid and reduce it to phosphomolybdenum blue. The combined stream then passed through a reaction coil (2 m) before its absorbance at 720 nm was measured.

2.6. Interference studies

The effects of likely interferents (silicate, arsenate, chloride, nitrate, sulphate and a seawater matrix) on the UV system performance were examined. For all anions except arsenate, standards containing 1 mg L^{-1} P and varying concentrations of the interfering anion (100 mg L^{-1}) were injected. Arsenate standards in the absence

of orthophosphate were injected to determine the sensitivity of the flow system to arsenic. The effect of a seawater matrix was examined by using seawater as the carrier (R_1), with injection of standards containing a known amount of P, made in the same seawater.

The potential of the UV photoreduction method to undesirably oxidise organic phosphorus compounds to orthophosphate was examined using three model compounds, viz; phytic acid and phosphocholine as refractory compounds, and glycerophosphate as a labile compound [34]. The associated studies involved standards containing the individual model compounds.

2.7. Analysis of natural water samples

The sensitivity of the standard reference ascorbic acid and stannous chloride spectrophotometric methods (with a 1 cm cuvette) is insufficient for analysis of samples containing low $\mu g\ L^{-1}$ reactive P, with limits of detection being typically 10 $\mu g\ L^{-1}$ P [9]. Therefore, in the absence of a viable reference procedure for comparison, method validation was performed *via* spike recovery studies on five natural water samples with additions of up to 200 $\mu g\ L^{-1}$ P as orthophosphate.

3. Results and discussion

3.1. UV reduction mechanism and reagent selection

The mechanism of molybdate reduction by UV light, as detailed by two comprehensive reviews [2,20], requires a hydrogen bond donor with a lone pair to facilitate the reaction. The mechanism begins with the excitation of a Mo=O bond via a UV-induced Mo←O ligand–metal charge transfer (LMCT) transition (Fig. 1). This is immediately followed by the transfer of H^+ from the hydrogen bond donor to a bridging O atom in the molybdate; at the same time, the lone pair on the H-bond donor interacts with the electron hole formed by the LMCT, forming a charge-transfer complex. As a result, the H-bond donor undergoes a one-electron oxidation to a radical species; this radical may then reduce the molybdate once more or alternatively oxidise water to a hydroxyl radical as its main reaction pathways [2,30]. In keeping with the corresponding reduction potentials, heteropoly molybdates are considerably easier to photoreduce than isopoly species, following the same trend as for purely chemical reduction methods; this is indeed the basis of all molybdenum blue methods for the determination of heteroanions such as phosphate and arsenate.

Amines, alcohols and carboxylic acids have all been used as H-donating reductants for polyoxometalates upon exposure to UV light. A review by Yamase [20] suggests that the most effective water-soluble reagents for this process are primary and secondary alcohols; tertiary alcohols, amines and carboxylic acids larger than formic acid are less effective. Ethanol was selected for this purpose in the present work, owing to its ready availability, water solubility, low toxicity, comparative ease of handling and inability to interfere with the acidity of the reagent solution.

3.2. Flow system optimisation

3.2.1. Reagent optimisation

The acidity of the reaction and the molybdate concentration were crucial factors in determining the extent of phosphomolybdic acid reduction, direct Mo(VI) reduction and blank signal noise; the effect of ethanol concentration was less pronounced. Sulphuric acid and molybdate concentrations were originally used as reported in a previous molybdenum blue system [11] (0.20 M and 0.13% (w/v) respectively), in which the molar ratio of [H_2SO_4] to [Mo] was 29.5:1. However, the addition of ethanol to these reagents under UV irradiation produced a significant baseline absorbance of ca. 0.15 AU. Sensitivity was somewhat enhanced with increasing ethanol concentration, although this effect became insignificant above 7.5% (v/v). Whilst increasing the ethanol concentration also increased the baseline absorbance, this effect was insignificant compared to the influence of the acid and molybdate concentrations. Therefore, an ethanol concentration of 7.5% (v/v) was taken as optimal in order to avoid excessive baseline noise at higher concentrations and to prevent possible solvent damage to the peristaltic pump tubing. By reducing the $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ concentration to 0.09% (w/v), significant reductions in baseline absorbance were obtained whilst retaining similar sensitivity. The concentration of H_2SO_4 was in most cases maintained at 0.21 M, as lower concentrations resulted in very high signal baselines, and higher concentrations decreased sensitivity, for the range of molybdate concentrations examined. Under optimal conditions (Table 1), the ratio of [H_2SO_4] to [Mo] was 39:1 and the baseline absorbance is small. It is well-established that controlling this ratio is crucial in discriminating between heteropoly – and isopoly blue formation; lower ratios allow rapid formation of isopoly blues, whereas higher ratios impede the formation of both types of species, and this behaviour is shown in Fig. 3.

3.2.2. Flow rate and reactor coil optimisation

The duration of sample and reagent exposure to the UV source was an important means of controlling sensitivity and signal noise and it was determined by the length of the reaction coil and combined flow rate of the streams R_1 and R_2 (i.e. total flow rate). Due to the refractive index difference between ethanol and water, it was found that with a 200 cm reaction coil, significant signal

Table 1
Optimal UV flow manifold parameters.

Parameter	Range tested	Optimal value
Effective [H_2SO_4] in the merged stream (mol L^{-1})	0.16–0.42	0.21
Effective % (v/v) EtOH in the merged stream	2.5–10.0	7.5
Effective % (m/v) $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in the merged stream	0.06–0.13	0.09
Total flow rate (mL min^{-1})[a]	1.2–2.4	2.0
Injection volume (μL)	100–500	500
UV reactor length (cm)	200–500	400

[a] R_1:R_2 flow rate ratio maintained at 4:1 to minimise sample dilution.

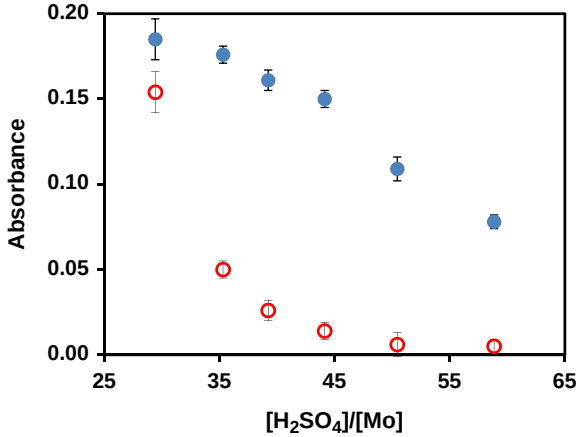

Fig. 3. Response of the manifold to different [H_2SO_4]/[Mo] ratios, shown as maximum absorbance with baseline correction at 860 nm for 1 mg L^{-1} P (●) and baseline absorbance at the same wavelength (O). Error bars indicate ± 1 SD.

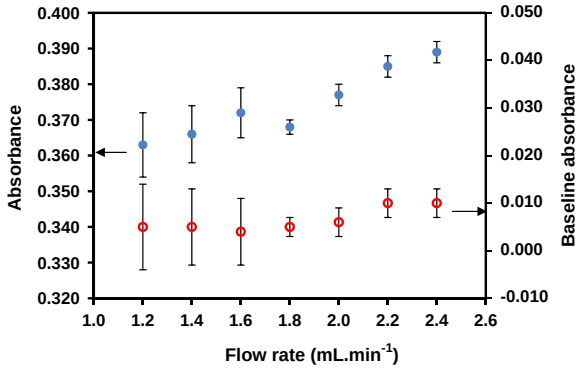

Fig. 4. Effect of the total flow rate on the maximum signal absorbance with baseline correction at 860 nm for 1 mg L^{-1} P (●) and baseline absorbance at the same wavelength (O). Error bars indicate ± 1 SD.

baseline artefacts due to the Schlieren effect were observed, even without the lamp operating. The use of a 300 cm coil decreased these effects somewhat, although a clear increase in signal was observed as flow rate decreased, suggesting incomplete photoreduction of phosphomolybdic acid. The best compromise between sensitivity and sampling rate was obtained using a 400 cm coil. With this coil, both the signal response and baseline actually increased with flow rate (Fig. 4), indicating that no further reduction was occurring with longer irradiation time, and that less re-oxidation of the product was occurring before detection at higher flow rates. Furthermore, a brief stop-flow experiment showed that the signal decreased by ca. 50% after the majority of an injected sample zone was trapped inside the UV coil for 3 min before restarting the flow. This result suggests that either excessive irradiation of phosphomolybdic acid breaks down the reduced species, or that the photochemical reduction conditions cannot prevent the re-oxidation of phosphomolybdenum blue by dissolved O_2; this behaviour is discussed further in Section 3.2.3.

3.2.3. *Product decay*

The molybdenum blue reaction involves the reaction of acidified molybdate with a tetrahedral oxoanion such as phosphate or arsenate to form a heteropoly acid with a faint yellow colour, 'molybdenum yellow'. This species is then reduced in a stepwise process to form intensely blue coloured $2e^-$ and $4e^-$ reduced species [35]. Once the reduction step of the molybdenum blue reaction occurs, the colour is often reported as stable, at least for long enough to reliably measure absorbance. In the general case, excess reductant remains in solution after the formation of molybdenum blue and is oxidised by dissolved oxygen in preference to the heteropoly blue species, thus extending its lifetime. However, in cases where there is no reductant in the solution to protect the heteropoly blue species from dissolved oxygen, such as in photoreduction reactions or catalytic applications of polyoxometalates, the reduced heteropoly blue species is vulnerable to immediate re-oxidation by dissolved O_2.

It has been shown that the kinetics of this re-oxidation at low pH are zero-order in $[H_3O^+]$, and first-order in both dissolved O_2 and reduced heteropoly acid concentrations [30]; thus, the rate of re-oxidation is proportional to how much reduced product is initially formed. Furthermore, it has been reported that phosphomolybdic acids are more difficult to re-oxidise than the corresponding phosphotungstates [30]. Because of the first-order dependence of the re-oxidation rate on the concentration of heteropoly blue, the linear calibration range of this method prior to optimisation was initially limited to between 1–100 μg L^{-1} P. Despite using the same concentrations of acid and molybdate as in the chemical reduction method previously reported by us [11], wherein the linear range extended to 1000 μg L^{-1} P, the linear range was an order of magnitude smaller when ethanol and UV light were used to reduce the heteropoly acid. After optimisation, when the $[H_2SO_4]$/[Mo] ratio was increased from 29.5 to 39, it was found that the linear range once more extended to 1000 μg L^{-1} P. The reasons for this are currently unclear. In any case, regardless of the linear range, it must be noted that the range over which this method is actually useable is significant; a quadratic relationship between absorbance and phosphate concentration is obeyed up to at least 5000 μg L^{-1} P as shown in Section 3.4. This was attributed to the much greater concentration of reductant in this method compared to standard chemical methods, where using large reductant concentrations is detrimental to baseline stability and impractical in terms of reagent stability and consumption.

Whilst a mechanistic study of phosphomolybdenum blue re-oxidation is beyond the scope of this work, it has been suggested by Hiskia [30] that O_2 forms an adduct with a $1e^-$ reduced molybdenum atom (a 7-coordinate species), which subsequently regenerates phosphomolybdic acid and produces O_2^-, which itself performs a second $1e^-$ oxidation as before and forms H_2O_2 in the presence of acid. This proposed mechanism is unclear as to whether the proton source is the bulk solution or the reduced Mo–OH group; if it is the former, then it may be mechanistically unfavourable for the photoreduction to be repeated at the same molybdenum atom, accounting for the apparent irreversibility of product re-oxidation observed and discussed in Section 3.2.2. Either way, a strong oxidant is still produced as the end result of a reaction with O_2, and the more quickly the product can be delivered to the detector following irradiation, the greater the sensitivity that can be expected. This further highlights the need for reaction control which would be utterly impractical under batch conditions, but which flow injection analysis can easily provide.

3.3. *Interference studies*

Traditionally, the two interferents of greatest concern in the molybdenum blue reaction are silicate and arsenate, owing to their ability to form heteropoly acids in a similar manner to orthophosphate. In this work, silicate produced no detectable interference at a concentration of 50 mg L^{-1} SiO_3^{2-} in the presence of 1 mg L^{-1} P, and only a 2.6% positive error was obtained with the same concentration of P in the presence of 100 mg L^{-1} SiO_3^{2-}. The system was responsive to arsenate, but with a detection limit of only 5.9 μg L^{-1} As(V). As such, waters containing less than the acceptable limit of 10 μg L^{-1} As were not considered problematic. In the presence of seawater, the system's sensitivity to orthophosphate was diminished by 20% which was attributed to the high ionic strength of the matrix and not to its spectral properties, as the UV absorptivity of seawater at and above 220 nm is insignificant [36].

UV reactors in flow manifolds are typically used for on-line sample digestion via photo-oxidation; however, such a process is undesirable in the proposed method, as it would lead to potential overestimation of orthophosphate due to photo-oxidation of organic phosphorus compounds. Analysis of one labile and two refractory model compounds by the reference method indicated only a small mole percentage of orthophosphate (Table 2), present as impurities and/or generated by acid hydrolysis. It should be noted that the optimal acidities of the reference and UV methods are almost identical. The actual degree of photoconversion of these compounds to orthophosphate in the UV system was also minimal (Table 2), even in the case of glycerophosphate, which is considered a labile organic phosphorus species.

Table 2
Photo-oxidation behaviour of three model organic phosphorus compounds.

Organic-P compound	Organic-P compound concentration (mg L^{-1} P)	Mole per cent of orthophosphate detected		
		Reference method	UV method	Extent of photoconversion
Phytic acid	10	0.16%	0.51%	0.67%
Phosphocholine chloride	10	3.10%	3.60%	0.50%
Glycerophosphate	10	0.06%	1.17%	1.23%

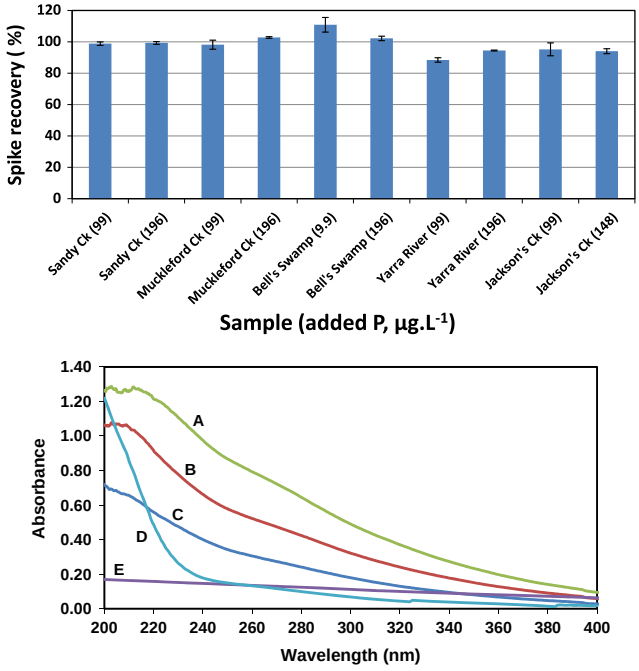

Fig. 5. Sample spike recoveries and corresponding UV absorbance spectra for NOM in real samples (A: Bell's Swamp, B: Muckleford Ck, C: Sandy Ck, D: Jackson's Ck, E: Yarra River). Error bars indicate ± 1 SD for triplicate measurements. Values in brackets indicate orthophosphate spike concentrations in $\mu g\ L^{-1}$ P.

3.4. Analytical figures of merit

The limit of detection of the proposed method was 1.3 $\mu g\ L^{-1}$ P, calculated using three standard deviations of the blank signal ($n=3$) [37]. The linear range was 5–1000 $\mu g\ L^{-1}$ P and the dynamic range was 5–5000 $\mu g\ L^{-1}$ P. The repeatability of the method was determined to be 0.8% (1000 $\mu g\ L^{-1}$ P, $n=10$) and 5.6% (10 $\mu g\ L^{-1}$ P, $n=10$), and sample throughput was 57 h^{-1}. The calibration equations for the linear and dynamic ranges were found to be $A=(4.329\mathbf{C}+39.32)\times 10^{-4}$ ($R^2=0.9996$) and $A=(-2.020\times 10^{-8})\mathbf{C}^2+(4.549\mathbf{C}+21.09)\times 10^{-4}$ ($R^2=1.000$), respectively, where A is the absorbance and $\mathbf{C}$ is the concentration of P as orthophosphate in $\mu g\ L^{-1}$. The P concentration in the diluted CRM determined by the proposed method was $3.47\pm 0.02\ \mu g\ L^{-1}$ (RSD=0.5%, $n=10$) which is in good agreement with the nominal P concentration of 3.40 $\mu g\ L^{-1}$.

3.5. Natural sample analysis

Given that phosphomolybdic acid has a broad absorption band at ca. ≤ 300 nm corresponding to the Mo←O LMCT transition required for photoreduction, it is possible that the absorption of UV by sample matrices in this spectral region, particularly in samples containing higher concentrations of natural organic matter (NOM), could interfere with the photoreduction process. The UV–vis spectra of five samples were obtained and the samples were then spiked with orthophosphate before analysis. Spike recoveries were good, and no relationship between UV absorbance and recovery was evident (Fig. 5).

4. Conclusions

A highly sensitive flow analysis method for rapid dissolved reactive phosphate determination has been developed which uses ethanol and UV light to reduce phosphomolybdic acid to molybdenum blue, instead of the reactive and short-lived chemical reductants typically employed. The proposed flow system is a simple two-line manifold which is very simple to handle, and the only reagent solution used (i.e. acidified molybdate in ethanol) is very long-lived. Interference from common inorganic anions and organic phosphorus species was minimal, and good spike recoveries on a range of complex sample matrices were obtained. The proposed method was characterised by a limit of detection of 1.3 $\mu g\ L^{-1}$ P, linear range of 5–1000 $\mu g\ L^{-1}$ P, dynamic range of 5–5000 $\mu g\ L^{-1}$ P, repeatability of 0.8% (1000 $\mu g\ L^{-1}$ P, $n=10$) and 5.6% (10 $\mu g\ L^{-1}$ P, $n=10$), and sample throughput of 57 h^{-1}. These parameters indicate the potential of this system to reliably measure dissolved reactive phosphate concentrations with excellent temporal resolution over long periods of time, owing to the stability of the only reagent solution used.

Acknowledgements

We are grateful to the Australian Research Council (ARC Linkage Project LP100100800) for financial support. E. Nagul is grateful to the University of Melbourne for the award of a postgraduate scholarship.

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