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A paper-based device for measurement of reactive phosphate in water

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ABSTRACT

The preparation and evaluation of a simple paper-based device for the determination of reactive phosphate in natural and soil waters based on the formation of phosphomolybdenum blue is reported. Hydrophilic reagent zones were defined by printing filter paper with a hydrophobic paper-sizing agent using an inkjet printer. The molybdate/potassium antimony(III)-tartrate reagent and ascorbic acid reductant merged together from adjacent hydrophilic zones after spotting the liquid sample onto the paper. The colour intensity of the phosphoantimonylmolybdenum blue complex produced was measured using a flat bed scanner. Under optimal conditions, the paper-based device is characterised by a working range of 0.2–10 mg L^{-1} P, limits of detection and quantitation of 0.05 and 0.16 mg L^{-1} P, respectively, and repeatability of less than 2% RSD at 1 and 5 mg L^{-1} P (n=10 and 8). There was no significant difference between the results obtained using the paper-based device and reference methods (t=0.0135, P ($T \leq t$) two-tail=0.9892, df=38). In its optimum form, the paper-based device was stable for up to 15 day under ambient conditions, and up to 122 day when stored at ≤ -20 °C. The small dimensions, minimal reagent consumption, low cost (a few cents), ease of operation and favourable analytical performance make the proposed paper-based device attractive for on-site environmental monitoring and analysis.

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1. Introduction

Interest in the determination of reactive phosphate in aquatic systems stems from the realisation in the late 1960s to mid 1970s that phosphorus was a critical nutrient in eutrophication of lakes [1,2] and a control of primary production in streams [3]. Phosphorus in natural waters consists of inorganic (ortho- and condensed) and organic phosphates in both dissolved and particulate forms. Orthophosphate is the most bioavailable form of phosphorus [4], and for that reason is most frequently measured in natural, waste and process waters as a means of assessing phosphorus impact on the trophic status of receiving waters. Free inorganic phosphate, predominantly in the orthophosphate form, is commonly measured by spectrophotometry as a phosphomolybdenum blue complex [5,6] or more specifically by ion chromatography [7].

The development of convenient, fast and inexpensive analytical methods for on-site determination of reactive phosphate would be a boon for monitoring nutrient removal and discharge compliance in wastewater treatment processes, and for environmental monitoring of waters and soil waters by farmers and community groups. The paper-based device described here offers that facility.

While there are a number of proprietary test kits available that are based on the measurement of PMB which are suitable for the rapid determination of reactive phosphate, these require the operator to use prepackaged reagents and necessitate a degree of sample and reagent manipulation, with determination either by visual comparison with a disc comparator or a colour card, or by use of a dedicated photometer.

One revolutionary approach for conducting inexpensive analysis is based on the use of microfluidic devices that are disposable and amenable to use by unskilled operators [8]. Lab-on-Chip (LoC) and micro Total Analysis Systems (μ-TAS) devices, where flow channels for liquid reagents and samples are integrated with one or more detectors on a small glass or polymeric substrate, have become well established in recent decades. However preparation of these devices often entails expensive tooling and fabrication techniques, and they frequently require bulky external power sources and signal detection units (e.g., fluorescence microscope), making them inherently unsuitable for field use.

Paper-based devices have been developed as a viable alternative to LoC and μ-TAS microfluidic devices [9]. In a paper-based device, the cellulose fibres form a hydrophilic pathway for liquid transport using only capillary action without the need for any other driving force. This hydrophilic pathway is defined by patterning the

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paper with a hydrophobic agent, and the resulting hydrophilic zones or channels provide the means for transport of reagent and sample solutions to the detection zone, which may entail chemical reaction en route.

Early paper-based fluidic devices used photolithography to define hydrophilic channels, which involved soaking paper in a photoresist solution, attaching a mask with the pattern of the hydrophobic regions, and exposing it to UV light [9]. Abe et al. [10] used an inkjet printing method which entailed soaking the paper in a solution of polystyrene in toluene, and forming the hydrophilic regions by printing with toluene. Bruzewicz et al. [11] used modified *X*–*Y* plotter to print the hydrophobic pattern on filter paper with polydimethysiloxane (PDMS). Lu et al. [13] developed a printing technique involving the printing of wax onto paper followed by heating to enable the wax to penetrate the paper to define the hydrophobic barriers to flow. Olkkonen et al. [12] used a method based on flexographic printing to prepare liquid guiding barriers of polystyrene on paper.

The recent development of simple, low-cost printing methods using hydrophobic materials [9,10,12,13] or paper sizing agents [14–16] to prepare paper-based devices has stimulated interest in their use for low-cost, rapid and portable analysis. Whilst most research in this area has emphasised diagnostic and healthcare applications [9,10,14,16–18], there has latterly been some focus on food and environmental analysis [16,18–21]. The inkjet printing method of Li et al. [15] has been used in the present work because it is a quick and simple approach that requires only a simple computer drawing programme and a budget-priced printer for preparation of the paper-based devices.

The molybdenum blue reaction is the most common basis for the manual and automated wet chemical methods of phosphate analysis [6,8]. In this method a mixture of ammonium molybdate and potassium antimony(III) tartrate reacts with orthophosphate in acidic medium to produce an antimony–phosphomolybdate complex ($PSb_2Mo_{10}O_{40}^{3-}$) which is subsequently reduced to form the phosphoantimonylmolybdenum (PAMB) blue complex.

The objective of the work described in this paper was to develop a paper-based device based on this reaction suitable for the determination of reactive phosphate in waters and soil waters.

2. Materials and methods

2.1. Reagents

A stock solution of 100 mg L^{-1} P containing sodium dihydrogen orthophosphate (BDH) was used to prepare working standards. The molybdate/antimony reagent used for the detection of orthophosphate was prepared from 0.126 M ammonium heptamolybdate tetrahydrate (Fisons) and 6 mM potassium antimony(III)-tartrate hydrate ($\geq$ 99%, Sigma-Aldrich) dissolved in 6.6 M sulphuric acid (95–98%, Chem Supply–Scharlau). The reducing reagent consisted of 0.5 M L-ascorbic acid (Ajax Finechem). A stock solution of silicate (100 mg L^{-1} Si) was prepared from sodium metasilicate pentahydrate (Ajax Chemicals) and used to prepare standards to test for silicate interference in the reactive phosphate determination.

2.2. Design and fabrication of the paper-based device

2.2.1. Channel and zone printing

Paper-based devices were designed using the drawing facility in Microsoft Word™, and printed on Whatman Grade 4 filter paper (0.205 mm thick) using a Canon™ iP4700 inkjet printer fitted with a custom-filled printer cartridge containing 4% (*v*/*v*) alkenyl ketene dimer (AKD, Precis 900, Hercules Chemicals Australia) in *n*-heptane. The printed paper was heated at 105 °C for 30 min during which time the AKD was distributed over the cellulose fibre surface by processes such as capillary wicking, evaporation–redeposition and surface diffusion to form a well defined hydrophilic pattern. The measured water contact angle of the printed area was ca. 110°, which is consistent with the hydrophobicity of the applied AKD [22].

2.2.2. Design and preparation of paper-based devices

Two different designs for paper-based devices for phosphate determination were assembled and evaluated (Fig. 1). The prototype device comprised a hydrophilic channel (2 mm width, 30 mm length) printed with an inkjet printer (Fig. 1(a)) with an ascorbic acid reductant zone (8 mm diameter, 0.8 μL reagent) midway along its length, and another zone of acidic molybdate/antimonyl tartrate (4 mm diameter, 0.4 μL reagent) at the end of the channel. Both reagents were added to these zones using micropipettes. The two reagent zones were separated in an attempt to maximise the stability of the device because in mixed form, these reagents are known to be viable for less than 24 h [6]. Sample added at the opposite end is transported by capillary forces along the channel through the ascorbic acid zone and thence to the molybdate/antimonyl tartrate zone. Maximum formation of PMB occurred approximately mid-way between the two reagent zones, which was presumably the point at which both reagent concentrations were maximal. Although the architecture of this device is similar to that previously described for a successful paper based device for other analytes [9,23], where the sample is transported along the hydrophilic channel to the reaction and detection zone, this prototype gave variable product formation and was quite insensitive, with a limit of detection of ca. 10 mg L^{-1} P. In part, this was due to the need for the sample to migrate relatively large distances before reacting with both reagents, and to the effects of evaporative losses of sample and of sulphuric acid from the molybdate reagent. The selectivity and kinetics of the PAMB reaction are very pH-dependent [5], and any evaporation of acid results in poor repeatability and sensitivity due to autoreduction of the molybdate reagent.

Given these limitations, the 2-D paper based device design was replaced with a new 3-D system (Fig. 1(b)) in which the molybdate/antimony and ascorbic acid reagents were located in spatially separated pairs of 3 mm (Zone 1) and 7 mm (Zone 2) circular hydrophilic zones, respectively. After applying 0.5 μL of molybdate/antimony reagent and 1.5 μL ascorbic acid solution into hydrophilic Zones 1 and 2, respectively, the paper was folded so that the two reagent zones coincided in adjacent layers. The folded paper device was then laminated (GBC HeatSeal™ H65) to maintain the alignment of the sandwiched reagent zones and to prevent evaporation and contamination of the reagent solutions deposited in these zones. A tissue biopsy punch was used to punch a 2 mm diameter hole in the plastic cover centred over the ascorbic acid reagent zones (Zone 2) to allow sample introduction. To prevent further drying of the reagent zones after punching the sample introduction hole, the aperture was covered with a masking tape until such time as a sample was added. This approach gave much improved sensitivity and repeatability, and reagent stability for up to one day was achieved under ambient conditions. Paper-based devices that had up to 15 separate molybdate/antimony reagent and ascorbic acid zone pairs in a single card with dimensions similar to that of a credit card (ca. 78 mm × 58 mm) were prepared using this approach, as shown in Fig. 1(c).

A further improvement to this design was made to extend the storage life of the device. In the spectrophotometric method for phosphate determination, the useful lifetime of combined molybdate/antimony/ascorbic acid reagent is 24 h or less [6,8], presumably because of the gradual reduction of Mo(VI) by ascorbic acid, and similar reagent stability was noted in the folded-paper-based devices. In order to avoid contact between the two reagent

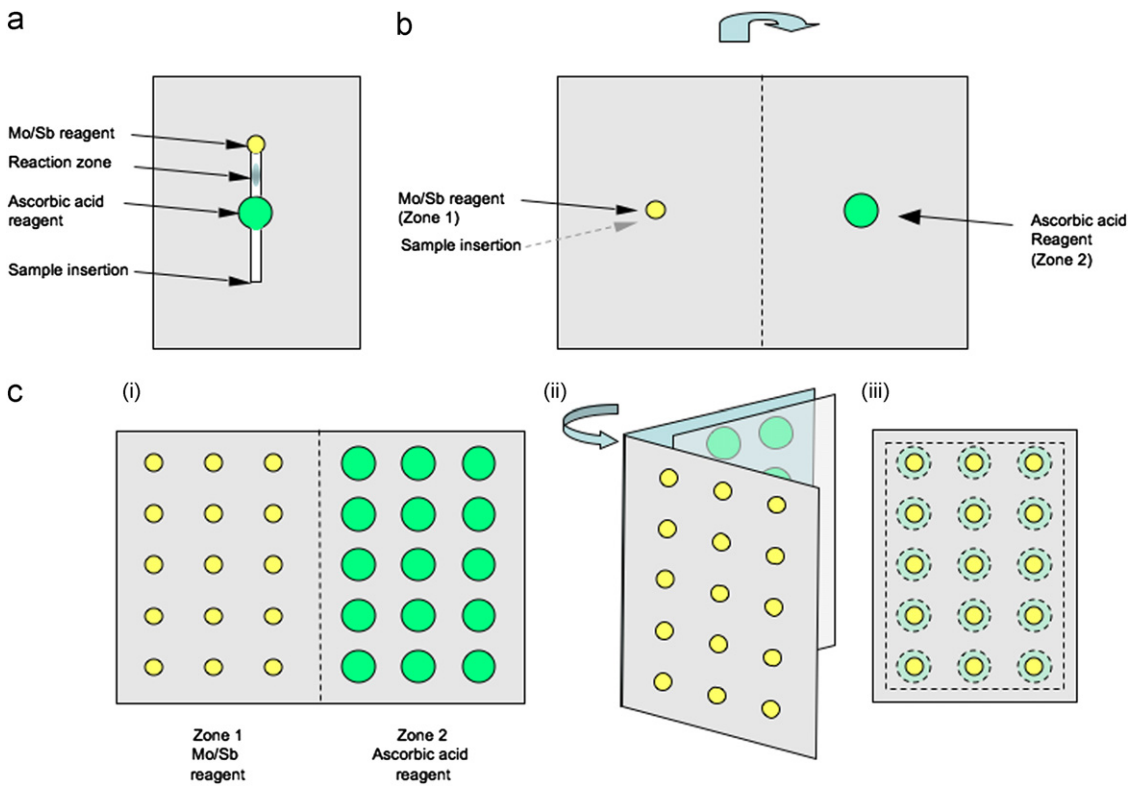

Fig. 1. Development of paper based devices for the determination of reactive phosphorus: (a) A channel design, where sample migrates longitudinally through the ascorbic acid and molybdate zones; (b) A folded paper design, where sample migrates laterally from the molybdate zone to the ascorbic acid zone on the adjacent sheet: (c) Summary of the sequence of operations involved in preparing a 5 × 3 folded paper-based device: (i) the paper is printed and reagents added to each of the hydrophilic zones; (ii) a removable Teflon® or cellulose acetate sheet is inserted between the folded leaves of the printed device to separate the two zones until sample application is required; (iii) the device is sealed by lamination in a plastic pouch and holes punched in the Zone 1 side to facilitate sample addition.

zones pending sample application, a thin sheet of PTFE or cellulose acetate was interleaved between the two paper layers (Fig. 1(c)(ii)) prior to lamination. Immediately before adding a sample to the paper-based device, the interleaving sheet was removed by snipping off one end of the laminated card and simply drawing it out. The storage stability of the paper-based device was markedly improved by this modification as discussed in Section 3.2.3.

2.3. Reactive phosphate measurement procedure

After applying 10 μL of a standard or sample to each sample hole of the laminated card using an autopipette, the holes were covered with strips of adhesive tape to prevent evaporation while colour formation proceeded at ambient temperature (21–25 °C). After a predetermined and constant period for colour development (typically 10 min), the Zone 1 side of the device was scanned by means of a flatbed scanner (Canoscan™ Lide 700 f) and the image was processed by Image J software (National Institutes of Health, USA, http://imagej.nih.gov./ij). For each of the PAMB coloured spots on the card, an RGB (red, green, blue) colour intensity profile plot was obtained for a chord passing through the centre of the spot. The highest sensitivity was obtained when the intensity of the red colour was used for calibrating the paper-based device. A calibration plot was prepared using red colour intensity values from 10 spots for each of three concentrations and a blank, and 10 spot intensity values were used to compute the concentration for samples. Dixon's Q-test [24] was used to exclude outliers from calibration and sample data.

The proposed paper-based method was validated by determining reactive phosphate in a certified reference material and a number of water and soil water samples The results were compared with those obtained using either the APHA batch method [8] with a single-beam spectrophotometer (Libra S12, Biochrom), equipped with a 1 cm cuvette and an independent laboratory which used a validated flow injection analysis method [8,25]. Soil waters were separated from 400 g soil using a centrifuge (GT20, Spintron Pty Ltd, Dandenong, Australia) operated at 1500 rpm (500 g) for 15 min.

3. Results and discussion

3.1. Optimisation of reagent conditions

The reagent conditions described by Drummond and Maher [26] were used as an approximate starting point to determine the optimal reagent concentrations for the proposed paper-based device. The colour intensity of the PAMB complex was examined as a function of acidity, molybdate concentration, the volumes of the molybdate/antimony and ascorbic acid reagents applied to the paper, and the colour development time. The optimal values of these parameters and the working ranges within which they were studied are summarised in Table 1. The parameters are listed in the order in which the optimisation was conducted. The selectivity for orthophosphate with respect to the most common interferent, silicate, was also evaluated.

3.1.1. Acidity

The formation reaction of the PAMB complex is complicated in that the acid concentration affects not only the amount of the PAMB complex formed, but also the rate of its formation [27]. Auto-reduction of Mo(Vl) to Mo(V) in the absence of orthophosphate can occur if the final reaction conditions have pH of $>$ ca. 0.9 [5,26]. The reaction pH and the Mo(VI) concentration are key factors for optimal formation of the PAMB complex. A pH range of

Table 1
Paper-based device parameters optimised in the present study.

Parameter	Range	Optimal value
Sulphuric acid in the molybdate/antimony reagent (mol L^{-1})	5.0–8.0	6.6
Volume of the molybdate/antimony reagent added to Zone 1 (μL)	0.5–1.5	0.5
Volume of the ascorbic acid reagent added to Zone 2 (μL)	0.5–2.5	1.5
Molybdate concentration in the molybdate/antimony reagent (mmol L^{-1})	42–210	126
Antimony(III) concentration in the molybdate/antimony reagent (mmol L^{-1})	6–42	6
Reaction time (min)	5–90	40

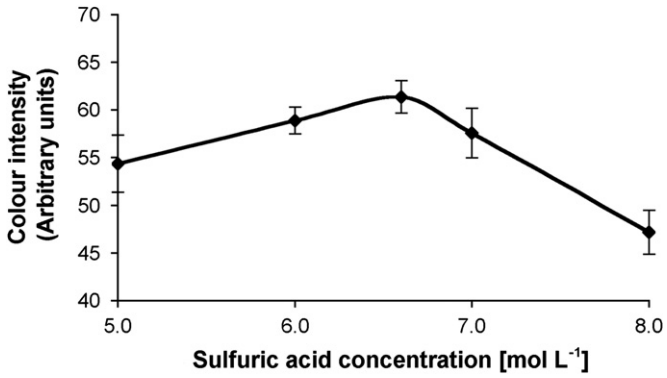

Fig. 2. Effect of acidity on colour intensity. Error bars are $\pm 1\sigma_{n-1}$ for $n=10$.

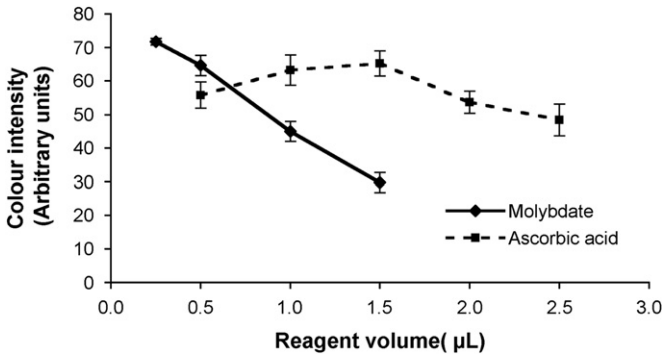

Fig. 3. The effect of varying the volumes of molybdate/antimony (■) and ascorbic acid (▲) reagents in Zones 1 and 2, respectively, on the colour intensity. Error bars are $\pm 1_{n-1}$ for $n=10$.

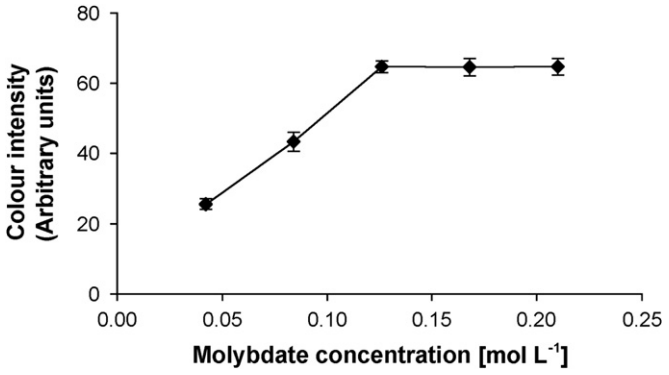

Fig. 4. Effect of molybdate concentration in the molybdate/antimony reagent on colour intensity. Error bars are $\pm 1_{n-1}$ for $n=10$.

0.3–0.9 favours the formation of the molybdenum dimer [5,28], and the best sensitivity for orthophosphate detection occurs at the upper end of this range [26]. The optimum acidity of the molybdate/antimony reagent was determined by measuring the colour intensity produced by a 5 mg L^{-1} P standard at five different sulphuric acid concentrations (Fig. 2).

The maximum sensitivity was achieved at 6.6 M sulphuric acid in the molybdate/antimony reagent, which corresponded to a final sulphuric acid concentration in the paper-based device of ca. 0.33 mol L^{-1}. This value is midway between that reported by Murphy and Riley [6] (0.4 mol L^{-1}) and Drummond and Maher [26] (0.13 mol L^{-1}).

3.1.2. Reagent volumes

The volumes of the molybdate/antimony and ascorbic acid reagents applied to Zones 1 and , respectively, were varied to determine the values that gave the maximum colour intensity. Fig. 3 shows that for a 10 μL sample addition, an increasing volume of the molybdate/antimony reagent applied to Zone 1 caused a decrease in colour intensity. This effect is probably due to over acidification of the final reaction mixture, which is known to cause a decrease in sensitivity [26].

Fig. 3 also shows the effect of increasing ascorbic acid volume. A reagent volume of 1.5 μL gave maximum colour intensity, and hence this volume was used in all subsequent applications of the paper-based device.

3.1.3. Reagent concentrations

Previous studies by Going and Eisenreich [29] and Drummond and Maher [26] have shown that a $[H^+]$:$[MoO_4^{2-}]$ ratio of 45–80 is required in order to achieve complete formation of the PAMB complex while avoiding self reduction of molybdate. To determine whether this range applied in the paper-based device, the effect of varying the molybdate concentration, $[MoO_4^{2-}]$, in the molybdate/antimony reagent on colour intensity was studied. A constant $[MoO_4^{2-}]$/[Sb] ratio of 21 was maintained throughout these experiments. Fig. 4 shows that the colour intensity initially increased with increasing the molybdate concentration and reached a plateau at 0.126 mol L^{-1} molybdate. This value was assumed to be the optimal molybdate concentration. It corresponded to a $[H^+]$:$[MoO_4^{2-}]$ ratio of 52 taking into account that the H_2SO_4 concentration in the molybdate/antimony reagent was 6.6 mol L^{-1}. This ratio is within the optimal range of 45–80 previously reported [26,29].

Antimony(III) is involved in the formation of the PAMB complex, in addition to having a possible catalytic role [28]. To explore the possibilities of further improving sensitivity, the concentration of antimony(III) was increased incrementally from 0.006 mol L^{-1}, which corresponded to the optimal molybdate concentration of 0.126 mol L^{-1} in the molybdate/antimony solution to 0.042 mol L^{-1}. The sensitivity remained constant for antimony(III) concentrations up to 0.021 mol L^{-1} and decreased marginally thereafter. Therefore, 0.006 mol L^{-1} was selected as the optimal antimony(III) concentration.

Going and Eisenreich [29] proposed that the concentration of ascorbic acid in the final reaction mixture should be at least 20 times greater than that of orthophosphate, i.e. for 10 mg L^{-1} *P*

this translates to ca. 6.7 mM. The final concentration of ascorbic acid in the paper-based device (75 mM) is considerably in excess of that.

3.1.4. Reaction time and colour development

The rate of formation of the PAMB complex at room temperature (21–25 °C) is shown in Fig. 5. The maximum and most reproducible colour intensity occurred at 40 min after sample addition. Thereafter there was a gradual decrease in the colour intensity which is consistent with the observations of other authors who recommend that measurements should be made within a time window of 10–30 min before the complex starts degrading [8]. After 10 min development time there was sufficient colour development to permit reliable quantification to be performed, and this time was adopted for the analysis protocol as it gave acceptable sensitivity while minimising the sample processing time. To test the reliability of the measurement within a time window of 9–11 min after sample addition, calibration data were obtained 9, 10 and 11 min after the addition of the standards. In all these cases very similar calibration equations were obtained:

$$At\ 9\ \text{min}: \text{Colour intensity} = (18.0 \pm 0.30)C_P + (20.44 \pm 0.17)$$

$$R^2 = 0.9989$$

$$\text{At } 10\ \text{min}: \text{Colour intensity} = (18.06 \pm 0.33)C_P + (20.62 \pm 0.19)$$

$$R^2 = 0.9986$$

$$\text{At } 11\ \text{min}: \text{Colour intensity} = (17.92 \pm 0.30)C_P + (20.95 \pm 0.19)$$

$$R^2 = 0.9989$$

where C_P is the phosphate concentration in mg L^{-1} P.

These results suggest that measurement of colour intensity within a 1 min window 10 min after the addition of a sample should give repeatable results, and it is this protocol that has been adopted for all real sample analysis reported here.

It was also observed that the ambient humidity could also have a pronounced effect on the colour development and repeatability following sample addition. This is presumably because of varying rates of sample evaporation, and in part this effect was overcome by laminating the folded paper device. However, the humidity effect was not entirely eliminated until the sample hole over Zone 2 was covered with a tape to completely encapsulate the reagent zones. The effectiveness of a number of different tapes (common adhesive tape, invisible tape, Parafilm® and masking tape) were tested at a relative humidity of 33%, and of these, the masking tape gave the maximum recovery for added orthophosphate.

An interesting phenomenon was observed when the paper-based devices were used in the field, viz; that when exposed to strong sunlight the molybdate/antimony zone underwent autoreduction to PAMB. This behaviour was not observed under laboratory lighting conditions, and is attributed to the effects of the UV component of sunlight, and is consistent with other reports on the photochemical behaviour of polyoxometalates such as molybdates [30]. The practical solution to this potential problem is to keep the paper-based devices enclosed in paper envelopes until such time as they are used, and to ensure they are not used in direct sunlight. Further investigations of this behaviour are in progress.

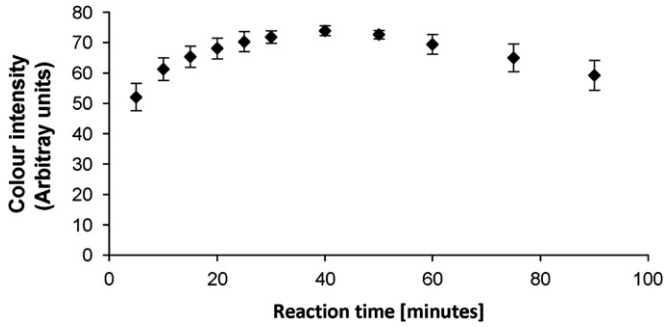

Fig. 5. Effect of reaction time on colour intensity. Error bars are $\pm 1_{n-1}$ for $n=6$.

3.2. Analytical performance

3.2.1. Analytical figures of merit

The linearity of the response of the paper-based device was tested over three orders of magnitude of orthophosphate concentration using a set of 12 orthophosphate standard solutions and deionised water as the blank. All the tests were performed at room temperature and repeated more than ten times.

The results showed that there are two linear concentration ranges, an observation also noted by Lennox for solution measurements [31]. The lower range (0.1–1.0 mg L^{-1} P), which is consistent with the usable linear range recommended for the PAMB method [8], was described by Eq. (1).

$$\text{Colour intensity} = (18.48 \pm 0.37)C_P + (20.62 \pm 0.21), \quad R^2 = 0.9956 \tag{1}$$

The higher concentration range (1.0–10 mg L^{-1} P) was fitted by Eq. (2).

$$\text{Colour intensity} = (4.85 \pm 0.05)C_P + (33.30 \pm 0.31), \quad R^2 = 0.9997 \tag{2}$$

The regression coefficients are shown with $\pm$ standard errors. The daily calibration reproducibility over a period of five days for slope and intercept was 2.1% and 1.3% RSD respectively.

Under the optimal experimental conditions described above, the repeatability within and between two paper-based devices was tested using a 5 mg L^{-1} P standard. The repeatability within each of the two devices, expressed as RSD, was 1.6% and 1.3%, respectively, for eight replicate measurements. Between devices, there was no statistically significant difference for the colour intensity: $t_{stat}=0.050$, $p=0.961$, $t_{crit}=2.201$, $df=7$ for a two-tail t-test.

Under optimal conditions, the limit of detection (concentration equivalent to $3\sigma_{n-1}$ of the mean blank signal using the regression method of Miller and Miller [24]) was determined to be 0.05 mg L^{-1} P ($n=10$), and the corresponding limit of determination was 0.16 mg L^{-1} P ($n=10$).

3.2.2. Selectivity

Silicate is the main interferent for the molybdenum blue method in the analysis of surface and wastewaters [8]. To minimise Si interference, tartrate is included in the combined reagent (as potassium antimonyl tartrate), and its role is to prevent the formation of interfering silicomolybdenum blue [6].

The potential effect of silicate was evaluated by spiking 1 mg L^{-1} P standard with different silicate concentrations (0–10 mg L^{-1} Si). The results presented in Table 2 show that there is no interference from silicate in the orthophosphate detection in the concentration range studied. This accords with the findings of Murphy and Riley

Table 2
Effect of added silicate on the determination of a 1 mg L^{-1} P standard.

P standard [mg L^{-1} P]	Added Si (mg L^{-1} Si)	P found (mg L^{-1} P), (σ_{n-1} for $n=10$)
1.0	0	0.98 (0.08)
1.0	1.0	0.98 (0.09)
1.0	2.0	0.98 (0.08)
1.0	5.0	0.99 (0.06)
1.0	10.0	0.98 (0.06)

[6] and Lennox [31], and confirms the suitability of the detection conditions chosen and the efficacy of tartrate in preventing silicate interference.

3.2.3. Stability

The lifetime of the paper-based devices was evaluated under different storage conditions, viz, room temperature (light and dark), ≤ 4 °C (refrigerator) and ≤ -20 °C (freezer), by measuring the colour intensity before the addition of a 5 mg L^{-1} P standard, and after an elapsed period of 10 min for colour development. At room temperature there was no deterioration in sensitivity of the paper-based device without a polymeric sheet separating the two reagent zones up to 24 h. After this period the ascorbic acid reagent zone became yellow as a result of the formation of dehydroascorbic acid and other degradation products [32]. The stability at room temperature could be extended to 48 h when the 2 mm diameter holes in the plastic cover centred over the ascorbic acid reagent zones (Zone 2, Fig. 1(c)) were punched immediately prior to sample introduction rather than straight after lamination. The un-punched device was stable for more than 20 day when kept at ≤ 4 °C, and for more than 112 day at ≤ -20 °C. Inclusion of the interleaving cellulose acetate or Teflon® sheet (Fig. 1(c)) increased the room temperature storage of punched devices from 24 h to 3 and 15 day, respectively.

3.2.4. Analysis of natural samples

Results obtained using the paper-based device were compared with a standard spectrophotometric batch method [8] or a flow injection method [8] performed by an independent laboratory for a certified reference material, a surface water sample (Royal Botanic Gardens lake, Melbourne, Australia) and various soil water samples collected from the Poowong area, SE Australia (Table 3).

There is no significant difference between the results obtained using the paper-based device and reference methods at the 95% confidence level ($t_{stat}=0.0135$, $t_{critical}$ (2 tail), $df=38$), and the excellent agreement between both is indicated by the linear regression equation for which the slope is close to unity:

Table 3
Comparison of results for reactive phosphate in a certified reference material, natural water samples and soil water samples obtained by the proposed paper-based device method and by a reference method performed by an independent laboratory.

Sample designation	Reference method (mg L^{-1} P)[a]	Paper-based device method (mg L^{-1} P) (σ_{n-1} for $n=10$)
c	8.81	8.74 (0.20)
s	0.24	0.22 (0.10)
s	0.56	0.55 (0.19)
s	0.10	0.08 (0.05)
s	0.12	0.13 (0.07)
s	0.59	0.60 (0.08)
s	0.23	0.23 (0.06)
s	7.70	7.70 (0.31)
s	0.50	0.47 (0.03)
s	2.70	2.58 (0.30)
s	0.32	0.32 (0.05)
s	6.33	6.57 (0.63)
s	0.64	0.67 (0.08)
s	6.97	6.86 (0.29)
s	0.59	0.60 (0.07)
s	1.10	1.08 (0.13)
s	2.40	2.42 (0.22)
s	4.78	4.63 (0.64)
w, b	1.12	1.13 (0.15)

c=certified reference material, s=soil water, w=natural water, b=batch method used.

[a] % RSD for this method is reported as 0.19% at 0.5 mg L^{-1} P [25].

Table 4
% Recovery values for seawater spiked with various concentrations of phosphate.

Sample+Spike	Measured concentration (mg L^{-1} P) (σ_{n-1} for $n=10$)	% Recovery
Seawater[a]	0.010 (0.021)	–
Seawater+0.2 mgL^{-1}	0.298 (0.016)	99.6
Seawater+0.4 mgL^{-1}	0.504 (0.040)	101
Seawater+0.8 mgL^{-1}	0.890 (0.024)	99.7
Seawater+1.0 mgL^{-1}	1.090 (0.026)	100

[a] Port Phillip Bay, S.E. Australia.

$C_{P(paper\text{-}based\ \ device)}=(0.999\pm0.013)C_{P(Reference\ \ method)}-(0.009\pm0.047)$, $R^2=0.999$, ($n=20$), where the errors shown for the regression coefficients are the 95% confidence intervals.

The suitability of the proposed method to waters of higher salinity was also assessed by spiking seawater samples with orthophosphate (Table 4). Percentage recoveries of 99.6–100.3% show that the method is applicable to marine waters, and this is not surprising, given that the method uses similar reagents to those used in the Murphy and Riley batch method [6].

4. Conclusions

The paper-based device described in this paper is a low cost (less than $0.03 per determination), fast and simple portable platform for quantitative determination of reactive phosphate in natural water and wastewater samples. The advantages include high sensitivity, accuracy and repeatability, and ease of use. Each determination requires only the application of a small sample volume, no reagent manipulation is needed, only minute amounts of reagents are used, and quantification can be achieved without the need for a subjective colour comparison by the operator. From a Green Chemistry perspective it should be noted that the proposed device uses 4000 times less molybdate/antimony reagent per determination than the conventional batch method.

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