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# The development of iodide-based methods for batch and on-line determinations of phosphite in aqueous samples

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**Abstract**

Recent developments in the field of microbiology and research on the origin of life have suggested a possible significant role for reduced, inorganic forms of phosphorus (P) such as phosphite [$HPO_3^{2-}$, P(+III)] and hypophosphite [$H_2PO_2^-$, P(+I)] in the biogeochemical cycling of P. New, robust methods are required for the detection of reduced P compounds in order to confirm the importance of these species in the overall cycling of P in the environment. To this end, we have developed new batch and flow injection (FI) methods for the determination of P(+III) in aqueous solutions. The batch method is based on the reaction of P(+III) with a mixed-iodide solution containing tri-iodide ($I_3^-$) and penta-iodide ($I_5^-$). The oxidation of P(+III) consumes free $I_3^-$ and $I_5^-$ in solution. The remaining $I_3^-$ and $I_5^-$ subunits are then allowed to react with the amylose content in starch to form a blue complex, which has a $\lambda_{max}$ of 580 nm. The measurement of this blue complex is directly correlated with the concentration of P(+III). The on-line FI method employs the same reaction between P(+III) and mixed-iodide producing phosphate [P(+V)] that is determined spectrophotometrically by the molybdenum blue method employing ascorbic acid at a $\lambda_{max}$ of 710 nm. The linear range for both the batch and FI determination of P(+III) was 1.0–50 μM with detection limits of 0.70 and 0.36 μM, respectively. Interference studies for the batch method show that arsenite [As(+III)] and sulfite [S(+IV)] can also be determined by this technique; however, these interferences can be circumvented by oxidizing As(+III) and S(+IV) using $KMnO_4$ which is an ineffective oxidant for P(+III). Both methods were applied to P(+III) determinations in ultra-pure water and simulated creek water. Results and analytical figures of merit are reported and future work is considered.


*Keywords:* Phosphite; Reduced phosphorus; Spectrophotometric detection; Batch method; Flow injection (FI)

## 1. Introduction

Although phosphorus (P) is believed to occur almost exclusively in the environment as the fully oxidized phosphate [$PO_4^{3-}$, P(+V)], recent developments in the field of microbiology and research on the origin of life, have made a compelling case for the role and significance of reduced P species [1–15]. One of these reduced P species is phosphite [$HPO_3^{2-}$, P(+III)], a chemical analogue to environmentally relevant species such as arsenite [As(+III)] and nitrite [N(+III)]. The traditional molybdate-reactive method of P determination is excellent for the detection of fully oxidized P(+V) in aqueous solutions [13,16–22] but reduced forms of P such as phosphite, hypophosphite [$H_2PO_2^-$, P(+I)] and various forms of phosphides [P(−III)] are indistinguishable from P(+V) in the traditional molybdate-reactive method of detection. There is thus a need to develop fast and sensitive methods for the detection of these compounds in environmental samples. To this end, a new method has been developed based on the oxidation of P(+III) to P(+V) by a mixed-iodide solution containing tri-iodide ($I_3^-$) and penta-iodide ($I_5^-$) subunits. These iodide subunits, produced by

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the reaction between iodine ($I_2$) and iodide ($I^-$), are known to form polyiodide species (i.e. $I_9^{3-}$, $I_{11}^{3-}$, $I_{13}^{3-}$ and $I_{15}^{3-}$) which react with the amylose content in starch [23–27] to form a blue complex with a $\lambda_{max}$ of 580 nm. The key role of $I_3^-$ and $I_5^-$ in the amylose-iodide chain has been proven by Raman spectral data and is consistent with the equilibrium model proposed by Yu et al. [25]:

$$(I_5^-)\text{-}(I_5^-)_n\text{-}(I_5^-) \overset{I_2}{\longleftrightarrow} (I_5^-)\text{-}(I_5^-)_n\text{-}(I_3^-) \overset{I_2}{\longleftrightarrow} (I_3^-)\text{-}(I_5^-)_n\text{-}(I_3^-) \overset{I_2}{\longleftrightarrow} (I_3^-)\text{-}(I_3^-)_n\text{-}(I_3^-)$$

In this model, it is assumed that the amylose helix stabilizes the formed polyiodide chains. P(+III) can react with excess $I_3^-$ and $I_5^-$, reducing the amount of free $I_3^-$ and $I_5^-$ available for complex formation, resulting in a quantifiable decrease in absorbance.

A variation of the aforementioned reactions has been used for the determination of thallium in environmental samples [28], although the method is quite different than the batch and on-line methods presented in this paper. To date, the amylose-iodide method presented above has not been developed for the determination of P(+III) in aqueous samples. Given the significant role for phosphite and reduced P in microbiology and their possibly significant roles in the environment, sensitive techniques are necessary for the determination of P(+III) and other reduced P compounds.

This paper describes the development of novel iodide-based methods for the determination of phosphite in aqueous samples. Both batch and flow injection (FI) on-line methods are presented and applied to P(+III) determination in ultra-pure water and simulated creek water samples. The latter matrix was chosen to closely represent geothermal waters (e.g. hot springs). Such waters represent naturally occurring reducing environments that are minimally perturbed by human activity and may be ideal locations for the detection of reduced P species [29]. The batch method is relatively easy to implement both in the laboratory and the field, making it a robust and real-time detection tool. FI has many unique features including limited sample/reagent consumption, short analysis time, on-line separation, and pre-concentration and physicochemical conversion of analytes into detectable species [21,30]. Given the generic and adaptive nature of both methods, it is believed that other analytes [e.g. P(+I), As(+III)] can be detected. Future studies will explore such chemistries.

## 2. Experimental

### 2.1. Reagents and solutions

All chemicals used were of analytical reagent grade and prepared with ultra-pure water (Millipore, 18.2 MΩ). All containers, bottles and glassware used during this study were soaked overnight in a solution of HCl (10%, v/v) and rinsed three times with ultra-pure water and dried at room temperature.

#### 2.1.1. Batch method

The iodide solution was prepared by dissolving 0.2212 g of KI (Sigma) in ultra-pure water and diluting to 250 mL. The iodate solution was prepared by dissolving 0.0357 g of $KIO_3$ (Mallinckrodt) with ultra-pure water and diluting to 250 mL. The 0.5% starch solution was prepared by dissolving 0.25 g of soluble starch (Matheson Coleman & Bell) in a total volume of 50 mL and microwaving the solution to a boil. The solution was left sealed for 1 day at room temperature to let the amylose solubilize. The 4.0 mM HCl solution was prepared by diluting 83.3 mL of concentrated (12 M) trace metal grade HCl (Fisher) into a final volume of 250 mL. P(+III) working standards in the range of 0–100 μM were prepared by serial dilutions from a 50 mM $Na_2(PHO_3)\cdot 5H_2O$ (Riedel-deHaën) stock solution which was prepared daily. A 10N NaOH solution was prepared by dissolving 10 g of NaOH pellets (Fisher) in 25 mL of ultra-pure water. A 0.1N NaOH solution was prepared by dilution of the 10N solution. The $NaNO_2$ (Matheson Coleman & Bell) solution was prepared by adding 1.725 g of $NaNO_2$ to 250 mL of ultra-pure water. Potassium permanganate ($KMnO_4$, Fisher) at a concentration of 0.01 M was used as an oxidizing agent for pretreatment of interfering species such as As(+III) and S(+IV) in samples.

Simulated creek water was prepared by dissolving salts or diluting stock solutions of salts into 1 L of ultra-pure water. Anion concentrations and pH were determined from the literature for Hot Creek in the eastern Sierra Nevada [31]. The final concentrations of the constituents are as follows—$NO_3^-$: 10 μM as $NaNO_3$ (Fisher); $HCO_3^-$: 8 mM as $NaHCO_3$ (EM Science); $Cl^-$: 2 mM as $MgCl_2\cdot 6H_2O$ (Sigma) and 1 mM as $CaCl_2$ (Fisher); $Br^-$: 0.8 mM as KBr (Fisher); $F^-$: 0.4 mM as $CaF_2$ (Mallinckrodt); $SO_4^{2-}$: 0.6 mM as $Na_2SO_4$ (EM Science); $Ca^{2+}$: 0.2 mM from the $CaF_2$ and 0.5 mM from the $CaCl_2$; $Na^+$: 9.2 mM from the $NaNO_3$, $NaHCO_3$ and $Na_2SO_4$; $Mg^{2+}$: 1.0 mM from the $MgCl_2\cdot 6H_2O$; $K^+$: 0.8 mM from KBr. The pH was measured at 8.3.

The interference of the following individual species on the batch method was also tested: arsenic as arsenite [$H_3AsO_3$, As(+III)] from $As_2O_3$ (Sigma) or arsenate [$H_3AsO_4$, As(+V)] from $Na_2HAsO_4$ (Fisher), nitrogen as nitrate [$NO_3^-$, N(+V)] from $NaNO_3$ (Fisher) or nitrite [$NO_2^-$, N(+III)] from $NaNO_2$ (Matheson Coleman & Bell), phosphate [$PO_4^{3-}$, P(+V)] from $NaH_2PO_4$ (Fisher) and sulfur as sulfate [$SO_4^{2-}$, S(+VI)] from $K_2SO_4$ (Fisher) or sulfite [$SO_3^{2-}$, S(+IV)] from $Na_2SO_3$ (Matheson Coleman & Bell). Interference by each of these species was tested in the presence of P(+III). Both the P(+III) and the competing ion were at equal concentration of 5 or 25 μM.

#### 2.1.2. FI method

The iodide solution was prepared by dissolving 0.3453 g of KI (Sigma) in ultra-pure water and diluting to 250 mL. The iodate solution was prepared by dissolving 0.0797 g of $KIO_3$ (Mallinckrodt) in ultra-pure water and diluting to 250 mL. The ammonium molybdate solution was prepared by dissolving 10 g of ammonium molybdate (Fisher) and 28 mL of $H_2SO_4$ into 1 L of ultra-pure water. The ascorbic acid (Fisher) solution was prepared by dissolving 5 g into 100 mL of ultra-pure water. P(+III)

working standards in the range of 1.0–50 μM were prepared from a stock 500 μM $Na_2(PHO_3)\cdot 5H_2O$ solution which was prepared daily.

### 2.2. Instrumentation

#### 2.2.1. Spectrophotometry

Batch detection of the blue colored amylose-iodide complex was performed on a Spectronic® Genesys™ 2 Spectrophotometer with an 8-position multi-cell holder. Samples were prepared in 50 mL polypropylene tubes (BD Falcon™) and transferred into 1 cm polystyrene disposable cuvettes for measurement at 580 nm. A FIAlab Sequential Injection/Flow Injection Analysis unit (FIAlab Instruments, Model 3500) was used as a platform for all on-line determinations and adapted accordingly.

#### 2.2.2. Ion chromatography

The oxidation of P(+III) to P(+V) by the mixed-iodide solution was confirmed by detecting these species using an ion chromatography (IC) system. Our studies employed a suppressed IC system (Dionex ICS-3000) equipped with an electrolytic eluent generator configured with a KOH eluent cartridge. The 25 mM KOH eluent was generated electrolytically and used for all analyses. A 25 μL injection loop was employed. The system also employed a Dionex IonPac® AS18 column coupled with an IonPac® AG18 guard column for the separation of anion aqueous species. The total run time was 25 min.

### 2.3. Methods

#### 2.3.1. Batch

A schematic of the overall batch method procedure is shown in Fig. 1. All samples were prepared and processed at room temperature. Thirty milliliters of mixed-iodide solution (color reagent) was prepared by adding in sequence: 10 mL of iodide stock solution, 10 mL of HCl stock solution, 5 mL of iodate stock solution and 5 mL of ultra-pure water. This solution was set aside for exactly 10 min. A 10 mL volume of a P(+III) sample was transferred to a 50 mL polypropylene tube (BD Falcon™). At the end of the 10 min period, 2 mL of the color reagent was added to the P(+III) sample and the mixture vortexed briefly. 20 μL of each of 0.1 M NaOH and 0.1 M $NaNO_2$ was added to all samples. The samples were then vortexed briefly and allowed to react for 30 min. Soluble starch (500 μL) was added to the phosphite sample at the end of the 30 min reaction. To ensure that the proper volume of soluble starch was being delivered to each sample, new pipette tips were used for each addition of the viscous solution. The sample was vortexed and the absorbance measured at 580 nm.

#### 2.3.2. FI

A schematic of the FI instrument (FIAlab Instruments, Model 3500) manifold used for on-line P(+III) measurements is shown in Fig. 2. This manifold consisted of a miniature fiber-optic spectrometer (Ocean Optics Inc., Dunedin, FL) with two 1024 element linear CCD arrays, a miniature halogen lamp (LS-1 Ocean Optics Inc.) and a Lab-on-Valve microchannel multipurpose flow cell. PTFE tubing (Upchurch Scientific, Oak Harbor, WA, 0.8 mm i.d.) was used in all fluidic connections. The FIAlab analyzer was adapted to allow an external peristaltic pump to be used for reagent introduction. The on-line method employs the same reaction between P(+III) and mixed-iodide producing phosphate [P(+V)] with a slightly modified procedure. The P(+V) generated is determined spectrophotometrically by the molybdenum blue method employing ascorbic acid at 710 nm. A heated reaction coil (50 °C) was incorporated to help eliminate black precipitate present upon mixing the sample with the mixed-iodide species. See detailed discussion on optimum experimental conditions in Section 3.2.1.

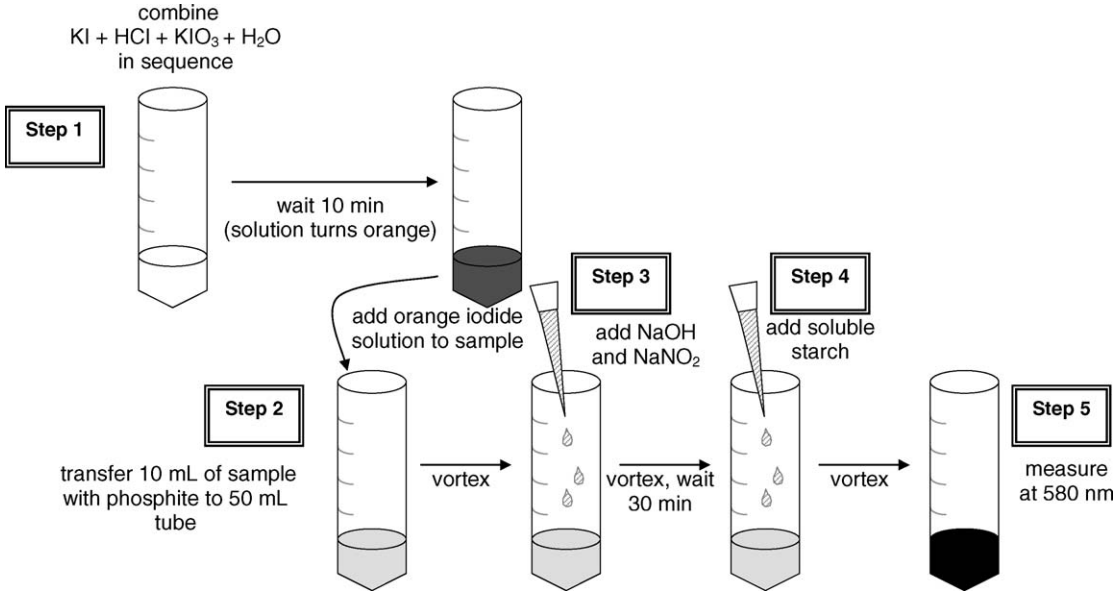


Fig. 1. Schematic of the batch method for the determination of phosphite in aqueous samples.

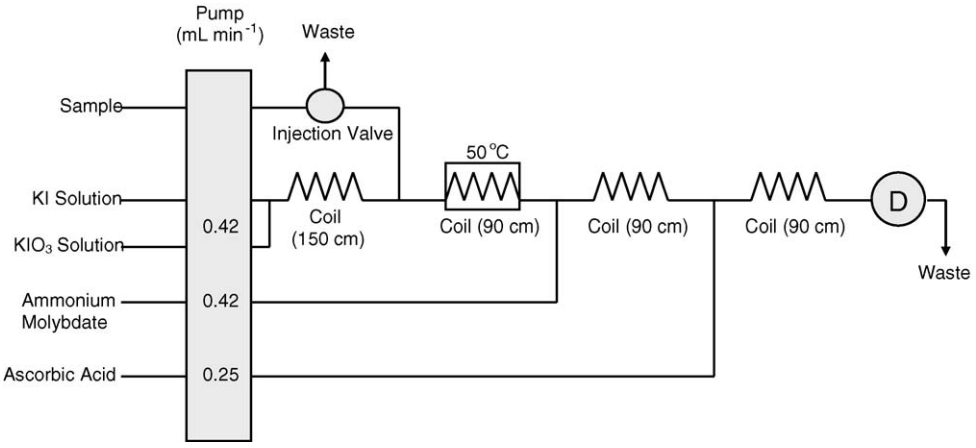


Fig. 2. Schematic of the FI manifold for the on-line determination of phosphite in aqueous samples.

## 3. Results and discussion

### 3.1. Batch method

#### 3.1.1. Calibration

The batch method for the determination of P(+III) based on the chemistry between mixed iodide and starch was developed and the optimized conditions are reported here. In the absence of P(+III), mixed iodide and starch will produce a blue colored complex that will absorb strongly at 580 nm. When P(+III) is present in solution it will react with the mixed iodide, making it unavailable for complex formation with starch. Thus, the sample without P(+III) will develop the deepest blue color resulting in the highest absorbance reading. With increasing concentrations of P(+III), the absorbance readings will decrease proportionally. Fig. 3a is a representative calibration curve for P(+III) in ultra-pure water in the concentration range of 0–25 μM. A comparison of the data for three batch method calibrations in ultra-pure water is given in Table 1. Reproducibility for triplicate measurements of P(+III) standards were <3.0% R.S.D. The pooled data also showed good reproducibility and a linear correlation of $r^2 = 0.9993$. The limit of detection (LOD) for the batch method (0.70 μM) was determined as the analyte concentration giving a signal equal to the blank signal plus three times the standard deviations of the blank.

Results obtained using this method are highly sensitive to the procedures outlined in Fig. 1. Care should be taken in reproducing the procedures exactly as described in Fig. 1. In particular, the allotted reaction time is critical to the formation of the mixed-iodide species and must be carefully controlled. Moreover, the production of mixed-iodide species in the color reagent shown in step 1 of Fig. 1 occurs under acidic conditions. The oxidative capacity of the color reagent is also optimal under acidic conditions. Adding the NaOH directly to the color reagent compromises the oxidative capacity of this solution in step 2. Addition of NaOH in step 3 quenches the oxidation reaction by terminating the further production of mixed-iodide species. The described procedure allows 30 min for the reaction to stabilize (step 3). Longer (45 min–1 h) stabilization times were also tested; however, no significant differences in results were observed (data not shown). The $NaNO_2$ was added to the samples to establish comparable ionic strengths for all samples (including blanks) being analyzed. Samples with very low initial concentrations of $Na^+$ (i.e. ultra-pure water) yielded lower absorbance readings when the ionic strength was not adjusted. The results presented above were obtained using a calibration curve of 0–25 μM P(+III);

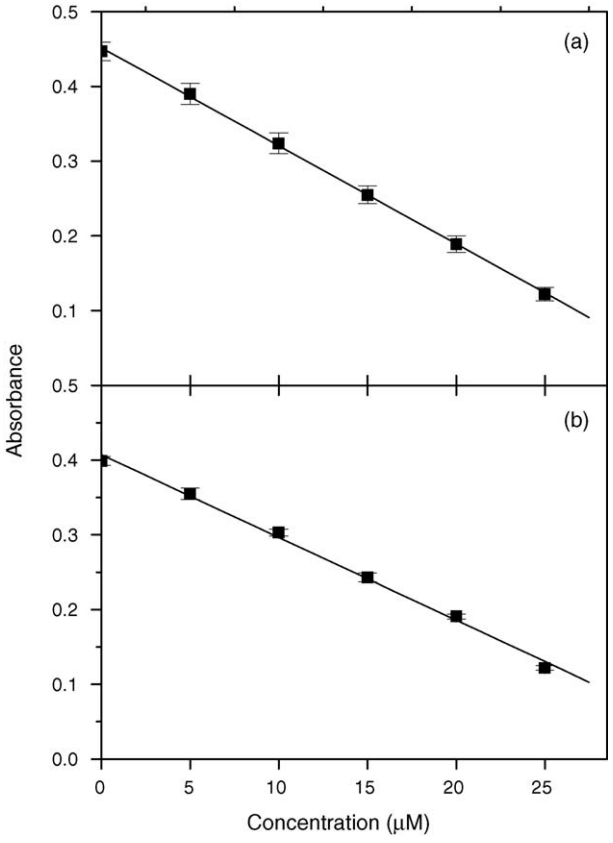


Fig. 3. Representative calibration curves for batch mixed-iodide method for the determination of P(+III) in: (a) ultra-pure water and (b) synthetic creek water. The blanks, containing no P(+III) result in the greatest complex formation and thus highest absorbance. With increasing concentrations of P(+III), complex formation is reduced, lowering absorbance and resulting in decreasing linear trends as shown. Error bars = 3σ.

Table 1
Batch calibration data for the determination of phosphite in ultra-pure water samples

| [P(III)] (μM) | Calibration 1 | | Calibration 2 | | Calibration 3 | | Pooled data | |
|---|---|---|---|---|---|---|---|---|
| | Mean absorbance | R.S.D. (%) ($n=3$) | Mean absorbance | R.S.D. (%) ($n=3$) | Mean absorbance | R.S.D. (%) ($n=3$) | Mean absorbance | R.S.D. (%) ($n=3$) |
| 0 | 0.447 | 0.9 | 0.457 | 0.8 | 0.469 | 0.3 | 0.458 | 2.4 |
| 5 | 0.390 | 1.2 | 0.404 | 0.7 | 0.418 | 0.6 | 0.404 | 3.5 |
| 10 | 0.324 | 1.4 | 0.330 | 1.1 | 0.345 | 0.9 | 0.333 | 3.3 |
| 15 | 0.255 | 1.6 | 0.266 | 1.3 | 0.274 | 1.1 | 0.265 | 3.6 |
| 20 | 0.189 | 1.9 | 0.207 | 1.4 | 0.211 | 1.4 | 0.202 | 5.9 |
| 25 | 0.122 | 2.5 | 0.141 | 1.8 | 0.142 | 1.5 | 0.135 | 8.5 |
| $r^2$ | 0.9994 | | 0.9988 | | 0.9983 | | 0.9993 | |
| Slope | −0.0131 | | −0.0128 | | −0.0133 | | −0.0131 | |
| Intercept (absorbance) | 0.4517 | | 0.4606 | | 0.4757 | | 0.4627 | |

however, the $KIO_3$ concentration can be manipulated to allow for a higher detection range. Doubling the concentration of $KIO_3$ in the protocol, for example, will produce more iodide subunits in the solution that would accommodate a calibration of P(+III) in the 0–50 μM range.

### 3.1.2. Simulated creek water studies

Ultimately, we plan to further develop the methods described here for the analysis of natural water samples. Here we demonstrate the effectiveness of these methods for measuring P(+III) in simulated creek water. Fig. 3b is a representative batch calibration curve for P(+III) in simulated freshwater in the concentration range between 0 and 25 μM. Reproducibility for triplicate measurements of P(+III) standards were typically <1.0% R.S.D. with good linear correlation ($r^2 = 0.9979$). The batch method experimental mean was compared to the known spiked value [6 μM P(+III)]. The observed value of $|t|$ was 1.90, less than the critical value of $t_4 = 2.78$ ($P = 0.05$), thus confirming no evidence of systemic error in the method.

### 3.1.3. Interference studies

We anticipated that multiple reduced species could react with mixed iodide, resulting in a concomitant decrease in complex formation with soluble starch and absorbance. To test this hypothesis, we prepared solutions of the following reduced species relevant to natural waters: As(+III), N(+III) and S(+IV). The effect of each of these species was tested at concentrations mimicking the concentration of P(+III) in solution (5 and 25 μM). Additionally, oxidized forms of these elements [As(+V), N(+V), S(+VI) and P(+V)] were also tested under similar conditions. As(+III) and S(+IV) presented significant interferences by behaving similarly to P(+III) in solution (data not shown). However, these interferences were removed by pre-treating 10 mL of the sample with 40 μL of 0.01 M $KMnO_4$ which effectively and simultaneously oxidizes As(+III) and S(+IV) (in the range of 0–50 μM) and does not effect P(+III) over this concentration range. In this paper, we have reported the data for As(+III). Standard solutions (each solution containing same concentrations of P(+III), P(+V), As(+V) and As(+III) in the range of 2–50 μM and a total volume of 10 mL) were pre-treated with differing amounts of potassium permanganate ($KMnO_4$), and subsequently analyzed by IC. The concentrations of P(+III), P(+V) and As(+V) before and after the addition of $KMnO_4$ are shown in Table 2. Concentrations of As(+III) can be calculated as the difference in As(+V) concentrations before and after oxidation. As can be seen, 20 μL of 0.001 M $KMnO_4$ is able to oxidize 3.10–3.72 μM As(+III) in 10 mL of solutions, regardless of the concentration range. The oxidation capacity seems to correspond directly to the amount of $KMnO_4$ added, with 37 μM As(+III) oxidized by 20 μL of 0.01 M $KMnO_4$. Therefore, by adding 40 μL of 0.01 M $KMnO_4$, a complete oxidation of up to 50 μM As(+III) can be achieved. The data also indicate that this amount of $KMnO_4$ added to 10 mL solutions has no effect on P(+III) over the 2–50 μM concentration range. Similar results were obtained for sulfite (data not shown), with 40 μL of $KMnO_4$ completely oxidizing 50 μM of sulfite and showing no effect on 50 μM of P(+III). N(+III) is always present in the system as it is added to fix the ionic strength; thus, it is difficult to fully assess its interference behavior. However, N(+III) levels are generally negligible in natural waters and are seldom present in concentrations large enough to cause interference with this method [32].

### 3.1.4. Ion chromatography validation

The oxidation of P(+III) to P(+V) by the mixed-iodide solution was confirmed by detecting these species using the Dionex ICS-3000 IC system. Solutions of P(+III) in ultra-pure water were prepared at concentrations of 5, 10 and 20 μM and injected onto the IC. These same samples where then oxidized by the mixed-iodide solution following the procedures detailed here and injected onto the IC. Fig. 4 confirms the complete oxidation of P(+III), which appears as a distinct peak with a retention time of 6.3 min (Fig. 4a), to P(+V), which appears as a distinct peak with a retention time of 21.0 min (Fig. 4b). Only the data set for the 20 μM of P(+III) are shown here for simplicity, but all concentrations tested confirmed our results. It should be noted from Fig. 4 that there are a few peaks which are believed to be from the mixed-iodide solution, eluted before and after the P(+III) peak in the oxidized sample. Although they cannot be identified by the IC method, they have retention times noticeably different from the peaks of P(+III) and P(+V). Thus, identification and quantification of P(+III) and P(+V) would not be interfered.

Table 2
Effect of $KMnO_4$ on inorganic species as measured by IC

| $KMnO_4$ concentration (M) | Volume of $KMnO_4$ (μL) | Volume of sample (mL) | P(+III) concentration (μM) | | P(+V) concentration (μM) | | Arsenate concentration (μM) | | Arsenite concentration (μM) [total As − As(+V)] |
|---|---|---|---|---|---|---|---|---|---|
| | | | Untreated solution | Treated solution | Untreated solution | Treated solution | Untreated solution [As(+V)] | Treated solution [As(+V) + As(+III)] | |
| 0.001 | 20 | 10 | 1.65 | 1.66 | 1.87 | 1.60 | 1.67 | 3.62 | 1.95 |
| 0.001 | 20 | 10 | 4.37 | 4.55 | 6.07 | 6.42 | 5.30 | 8.46 | 3.16 |
| 0.001 | 20 | 10 | 9.15 | 9.09 | 9.16 | 8.69 | 9.09 | 12.81 | 3.72 |
| 0.001 | 20 | 10 | 30.35 | 30.24 | 29.24 | 28.79 | 29.8 | 32.90 | 3.10 |
| 0.001 | 20 | 10 | 50.01 | 48.62 | 50.53 | 49.35 | 50.28 | 53.80 | 3.52 |
| 0.01 | 20 | 10 | 50.06 | 51.42 | 50.8 | 49.39 | 50.34 | 87.66 | 37.38 |
| 0.01 | 40 | 10 | 1.69 | 1.76 | 1.81 | 1.79 | 1.64 | 3.43 | 1.79 |
| 0.01 | 40 | 10 | 4.41 | 4.57 | 4.77 | 4.78 | 4.74 | 9.41 | 4.67 |
| 0.01 | 40 | 10 | 8.99 | 8.85 | 9.37 | 9.14 | 9.13 | 18.04 | 8.91 |
| 0.01 | 40 | 10 | 29.38 | 29.37 | 28.96 | 29.62 | 29.8 | 59.90 | 30.10 |
| 0.01 | 40 | 10 | 50.65 | 49.72 | 50.78 | 51.25 | 50.27 | 104.62 | 54.35 |

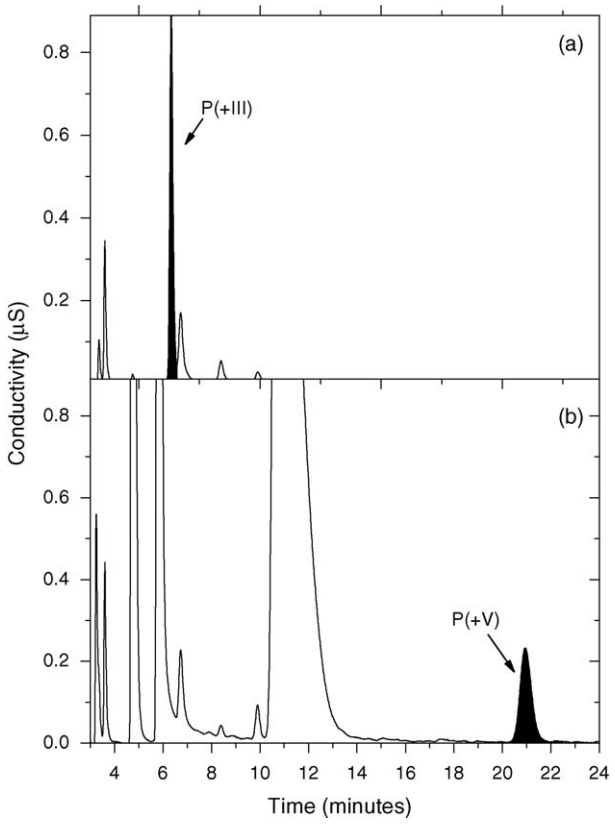


Fig. 4. Validation of oxidation of P(+III) by IC: (a) injection of a 20 μM P(+III) solution shown before oxidation in panel and (b) after the addition of mixed-iodide solution to oxidize P(+III) to P(+V).

### 3.2. FI method

#### 3.2.1. Calibration

Conditions for the calibration studies were optimized using a univariate approach. Here, one variable (e.g. flow rate) was modified while maintaining the other variables at their constant levels. Optimized flow conditions for reagent lines were as follows: $KI/KIO_3$—0.42 mL $min^{-1}$, ammonium molybdate—0.42 mL $min^{-1}$ and ascorbic acid—0.25 mL $min^{-1}$. The flow rate for the sample was not important and was used only to fill the sample loop (125 μL) in the injection valve.

A comparison of the data for three FI method calibrations is given in Table 3. Reproducibility for replicate injections of P(+III) standards (0–25 μM) was typically <3.9% R.S.D. ($n = 3$). The pooled data also showed good linear correlation ($r^2 = 0.9921$). An example FI calibration curve is shown in Fig. 5. The LOD for the FI method was calculated to be 0.36 μM with a linear range of 1.0–50 μM. Sample throughput was approximately 45 samples $h^{-1}$.

#### 3.2.2. Interference studies

As with the batch method, As(+III) proved to be an interfering ion, but at a higher level of 50 μM. An adopted on-line sample preparation procedure [33] incorporating $1.0 \times 10^{-2}$ mol $L^{-1}$

Table 3
FI calibration data for the determination of phosphite in ultra-pure water samples

| [P(III)] (μM) | Calibration 1 | | Calibration 2 | | Calibration 3 | | Pooled data | |
|---|---|---|---|---|---|---|---|---|
| | Mean absorbance | R.S.D. (%) ($n=3$) | Mean absorbance | R.S.D. (%) ($n=3$) | Mean absorbance | R.S.D. (%) ($n=3$) | Mean absorbance | R.S.D. (%) ($n=3$) |
| 0 | 0.0004 | 0.8 | 0.0005 | 1.4 | 0.0005 | 1.2 | 0.0005 | 1.3 |
| 5 | 0.0110 | 5.9 | 0.0125 | 3.9 | 0.0155 | 3.4 | 0.0131 | 3.2 |
| 10 | 0.0182 | 1.3 | 0.0181 | 0.9 | 0.0179 | 6.2 | 0.0181 | 0.8 |
| 15 | 0.0295 | 2.2 | 0.0302 | 1.8 | 0.0305 | 1.8 | 0.0301 | 1.7 |
| 25 | 0.0532 | 3.4 | 0.0529 | 3.1 | 0.0532 | 2.2 | 0.0530 | 0.4 |
| $r^2$ | 0.9935 | | 0.9914 | | 0.9924 | | 0.9921 | |
| Slope | 0.0021 | | 0.0021 | | 0.0021 | | 0.0021 | |
| Intercept (absorbance) | −0.0006 | | 0.0002 | | −0.0006 | | 0.0002 | |

$KMnO_4$ + 1.0 mol $L^{-1}$ $H_2SO_4$ in a heated reactor (50 °C) was used to assess the elimination of said interference. No significant removal of As(+III) interference was noticed upon incorporation of this method. In addition, higher limits of detection (1.1 μM) and R.S.D. (typically >7%) were achieved using this method. We thus chose to use the method reported in this paper for all subsequent experiments.

### *3.2.3. Simulated creek water intercomparison and significance studies*

An intercomparison study between the batch method and FI instrument on spiked [6 μM P(+III)] simulated creek water samples was undertaken. A paired *t*-test showed no significant differences at $P=0.05$ (the critical value of $|t|$ was 2.57 and the calculated value of $|t|$ was 1.89). This means that the data acquired from the FI instrument can be directly compared to the batch method. The FI method experimental mean was compared to the known spiked value [6 μM P(+III)]. The observed value of $|t|$ was 2.19, less than the critical value of $t_4=2.78$ ($P=0.05$), thus confirming no evidence of systemic error in the method. All statistical methods were performed as described by Miller and Miller [34].

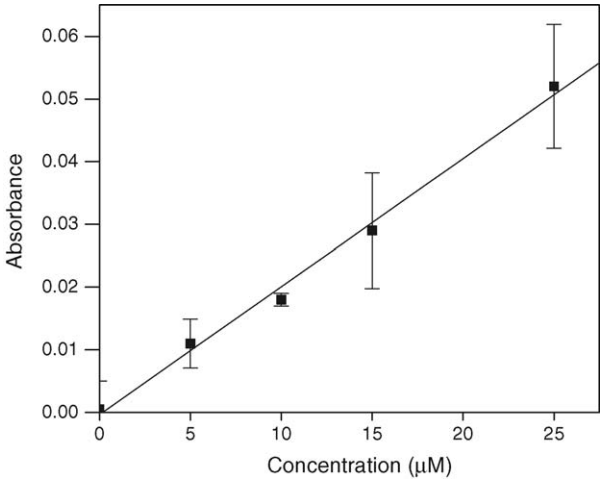


Fig. 5. Representative FI method calibration curve for P(+III) in ultra-pure water. Error bars = 3σ.

## 4. Conclusions

To confirm the significance of reduced P species in nature, it is important to have the analytical capabilities to detect these species in natural samples. Here we present two novel methods for the rapid determination of reduced P as P(+III) by spectrophotometric analysis. Calibration results showed good reproducibility and linearity with detection limits 0.70 and 0.36 μM for the batch and FI methods, respectively. These methods have proven to be effective in determining low levels of P(+III) in simulated creek water. To this end, we hope to further develop these for use with natural water samples. Future work will explore the use of mixed iodide and $KMnO_4$ in the batch and on-line oxidation and ultimate detection of P(+I) and As(+III) in aqueous samples.

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