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Detection of hypophosphite, phosphite, and orthophosphate in natural geothermal water by ion chromatography

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Abstract

Current doctrine states that phosphorus is incorporated into cells in the pentavalent(V) oxidation state as orthophosphate. However, recent studies show that microorganisms contain enzymes used to metabolize reduced forms of phosphorous, including phosphite(III) and hypophosphite(I), which suggests that there is a natural source for these chemical species. This paper will discuss suppressed conductivity ion chromatography methods developed to detect hypophosphite, phosphite, and orthophosphate in a geothermal water matrix containing fluoride, chloride, bromide, nitrate, hydrogen carbonate and sulfate. All peaks were clearly resolved, and calibrations were linear with estimated 3σ detection limits of 0.83, 0.39, and 0.35 μM for hypophosphite, phosphite, and orthophosphate, respectively.

Keywords: Water analysis; Geochemistry; Phosphorus compounds; Hypophosphite; Phosphite; Orthophosphate

1. Introduction

Methods for the detection of environmental phosphorus are continuously being refined to detect lower concentrations and provide better speciation of its many physio-chemical forms. The three primary types of environmental phosphorus are organic phosphorus assimilated into living organisms, particulate phosphorus found in rocks and sediments, and various forms of inorganic dissolved reactive phosphorus (DRP) [1,2]. Of these species, DRP is of greatest importance to environmental studies because these reactive species are typically the limiting nutrient for algae, bacteria, and plant growth in fresh water and soil [3]. The overabundance of DRP in lakes, streams and coastal regions due to human activity causes eutrophication—the excess plant growth that destroys the ecological balance in these systems. Typically, DRP is primarily believed to be fully oxidized (pentavalent, oxidation state of V) orthophosphate, primarily $H_2PO_4^-$ and HPO_4^{2-} [4]. Reduced forms of phosphorus, including trivalent(III) phosphite (phosphorous acid oxyanions $H_2PO_3^-$ and HPO_3^{2-}), and univalent (I) hypophosphite (hypophosphorous acid oxyanion HPO_2^-), are both potential contributors to measured DRP concentrations. Recent developments in the field of microbiology suggest that reduced forms of phosphorus may, like orthophosphate, provide living organisms with nutrients [5–10]. Consequently, it is probable that the DRP in natural water and soil contains hypophosphite and phosphite. The ion chromatography (IC) methods discussed in this work will help elucidate the contribution of reduced inorganic phosphorus compounds to the total DRP in natural water systems, specifically geothermal waters.

Adams and Conrad [5] were the first to report on the biological oxidation of phosphite, an oxyanion of phosphorous acid. Since then, other researchers have also studied bacterial oxidation of phosphite and hypophosphite [6–10]. In all these cases, the organisms were able to utilize either one or both of the reduced phosphorus oxyanions as their sole phosphorus source, oxidizing these compounds under both aerobic and anaerobic conditions. In 2000, German researchers made the next key finding in the developing story of bacterial utilization of reduced phosphorus. A novel organism, *Desulfotignum phosphitoxidans*, was isolated from marine sediments that grew by the anaerobic oxidation of

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phosphite to orthophosphate coupled with the reduction of sulfate to hydrogen sulfide [8,10]. While previous reports had provided evidence for the assimilatory metabolism of reduced phosphorus compounds, this paper provided evidence for phosphite oxidation for energy metabolism. Thus, the microbiological importance of reduced phosphorus oxyanions has been unequivocally established.

Phosphite and phosphine (PH_3, a fully reduced highly reactive toxic phosphorus gas) have been detected in reducing environments including sewage [11,12], marine sediments [13], and in agricultural processes [14]. For example, while conducting a mass balance for phosphorus in an open-air sewage treatment plant, Devai et al. [11] could not account for 30–45% of the total phosphorus. Using GC-MS analytical techniques, they were able to measure an average of 112 mg m^{-3} of phosphine gas released from sewage plant sediments, and determined that ~20–50% of the phosphorus deficit can be attributed to the formation of phosphine gas [11]. The mechanism of phosphine(-III) formation from orthophosphate(V) is likely to include the intermediates phosphite(III) and hypophosphite(I) [15].

Previous observations of reduced phosphorus in the environment have been limited to sites highly perturbed by human activity [11–14]. However, naturally occurring reducing environments such as geothermal waters in hot springs and oceanic vents may present the best environment for the detection of reduced phosphorus species. The first step towards measuring reduced phosphorus in such systems is to develop appropriate analytical techniques that are sensitive, selective, fast, reproducible, and economical.

In the detection of phosphorus, several types of DRP may be found in natural and waste water systems [4]. Time-tested techniques for the separation of these compounds include normal-pressure ion-exchange, paper, thin-layer and gel chromatography [16–18]. Recently, alternative rapid high-performance separation techniques including capillary electrophoresis [19,20] and ion chromatography [21–24] have been introduced. These separation techniques are coupled with a variety of detectors including UV–Vis spectroscopy [24], conductivity [21,22], nuclear magnetic resonance spectroscopy [25], and inductively coupled plasma mass spectrometry [26]. Fast-flow injection studies have been conducted as well [27]. Few systems provide the selectivity, sensitivity, speed and affordability of suppressed conductivity ion chromatography that is required for the detection of total reactive phosphorus in natural water, with concentrations typically in the μM to mM range [4,28]. Therefore, the techniques selected must be very sensitive with sub-micromolar limits of detection. Recent developments in the field of ion chromatography, such as commercially available electrolytic eluent generation sources, have enhanced the sensitivity of suppressed conductivity detection, making this technique even more attractive for environmental applications.

This paper will discuss selective and sensitive ion chromatography analytical techniques used to measure hypophosphite, phosphite and orthophosphate in a matrix representative of natural geothermal waters. The results include peak assignment verification by mass spectrometry techniques. It will also address the challenges associated with resolving hypophosphite from fluoride, and phosphite from hydrogen carbonate, while minimizing broadening of orthophosphate, within a run time less than 25 min.

2. Methods

2.1. Standards and eluents

The orthophosphate standard was prepared by diluting a commercial potassium dihydrogen phosphate (KH_2PO_4) standard that contained 1000 ppm of phosphate (LabChem Inc.) in 18 MΩ Nanopure water. Sodium-hypophosphite monohydrate ($NaH_2PO_2{\cdot}H_2O$, Sigma) and sodium phosphite-5-hydrate [$Na_2(PHO_3){\cdot}5H_2O$, Riedel-de Haën] salts were used to prepare hypophosphite, and phosphite samples, respectively. A stock solution including all three standards at a concentration of 1 mM each was prepared by diluting individual 100 mM standards in 18 MΩ Nanopure (Barnsted) water, and stored at 4 °C for up to 3 days.

The stability of the standards (data not shown) under various storage conditions was studied using ion chromatography and mass spectrometry. Variables included container material, storage temperature, and exposure to ambient light. Direct inject mass spectra of 100 μM samples stored under different conditions for periods up to 3 days were compared to samples analyzed immediately after dilution. The ion chromatography results also show that 100 μM standards of individual orthophosphate, phosphite and hypophosphite standards refrigerated at 4 °C in amber HDPE bottles were stable for periods up to 15 days. More detailed investigations of filterable reactive phosphorus solutions gave similar results [29]. Based on these results, fresh samples were prepared whenever possible for our experiment.

Phosphorus standards were calibrated in synthetic geothermal water made from sodium salts. Target anion concentrations and pH were determined from literature values for Hot Creek, a geothermal system in the eastern Sierra Nevada [30]. The final anion concentrations were 8.1 mM hydrogen carbonate, 5.5 mM chloride, 0.91 mM sulfate, 0.81 mM bromide, 0.43 mM fluoride, and 0.01 mM nitrate. The reagents used to make the synthetic geothermal water were sodium bicarbonate (Certified ACS grade, Fisher Scientific), sodium chloride (Certified ACS grade, Fisher Scientific), anhydrous sodium sulfate (Certified ACS grade, Fisher Scientific), sodium bromide (Certified ACS grade, Fisher Scientific), a standardized sodium fluoride solution (0.100 M, Fisher Scientific), and a sodium nitrate solution (1000 ppm, 98%, SPEX CertiPrep). The final, measured pH of the synthetic geothermal water was 8.66 and close to measured pH value of 8.3 at Hot Creek [31]. All standards

were filtered with 0.2 μm cellulose acetate filters and stored at 4 °C for up to 1 week in amber HDPE bottles prior to analysis.

Potassium hydroxide eluent was generated electrolytically using a Dionex EG40 eluent generator. To form the eluent, 18 MΩ Barnsted Nanopure water was initially degassed using ultra high purity (UHP) helium gas (Gilmore-Liquid Air) and stored in 4 l plastic bottles pressurized at 40 kPa using UHP helium. The water was introduced into a Dionex EGCII potassium hydroxide eluent cartridge to prepare the eluent. The eluent exiting the cartridge was passed through a second degassing unit in the ion chromatography apparatus before it was introduced to the analytical column.

2.2. Ion chromatography system with conductivity detector

Our studies employed a suppressed ion chromatography system (Dionex DX 500) configured with a CD25 suppressed conductivity detector. The system is equipped with a GS50 gradient pump and an EG40 electrolytic eluent generator configured with a potassium hydroxide eluent cartridge. Multi-step gradient concentrations ranging from 0.5 to 35 mM allow for the complete separation of phosphorus anions in aqueous media in ∼18 min at a constant flow rate of 1.5 ml min^{-1}. The gradient was as follows: 0.5 mM from 0 to 4.1 min; 0.5 to 1.0 mM from 4.1 to 4.5 min; 1.0 mM between 4.5 and 7.0 min; 1.0 to 10.0 mM from 7.0 to 14 min; and 10.0 to 35.0 mM from 14 to 18 min. The total run time was 22 min. One ATC-3 trap column was located before the analytical column to strip trace anion contaminants from the eluent before it reached the guard and analytical columns. The system employed a Dionex IonPac AS17 4 mm analytical column coupled with an AG17 guard column with hydrophilic quaternary ammonium functional groups for anion separation.

The suppressor (ASRS Ultra) was regenerated with an external water source (stored in pressurized 4 l plastic containers) that was pumped through the suppressor at a rate of 10–15 ml min^{-1}. Not only does the external regeneration decrease the background noise, which is critical for the analysis of trace compounds, but it also allows the suppressor effluent to stay neutral after exiting the suppressor. In this system where the eluent is potassium hydroxide, the suppressor waste composition is simply water with dissolved analytes. This provides a unique opportunity to interface the IC system with secondary detectors including mass spectrometers.

All separations were conducted at room temperature. Samples were transferred using micropipettes (Finnpipette) to single-use 10 ml autosampler polyvials (Dionex) used without additional treatment. These samples were stored in a refrigerated sample compartment at 4 °C, and injected by the AS50 autosampler using a 15 μl injection loop unless otherwise stated.

2.3. Ion chromatography with mass spectrometry detector

The IC effluent was analyzed by mass spectrometry in select experiments to verify the peak assignments for orthophosphate, phosphite and hypophosphite. The IC effluent produced at a flow rate of 1.5 ml min^{-1} was collected in sterile 8 ml polystyrene Falcon tubes using a Spectra/Chrom CF-1 automated fraction collector advanced every 60 s. These fractions were then analyzed individually by either atmospheric pressure chemical ionization (APCI) or inductively coupled plasma (ICP) mass spectrometry. For the APCI-MS experiments, the fractions were syringe injected at a flow rate of 25 μl min^{-1} into a stream of water–methanol (90:10) pumped at 1 ml min^{-1}, and directed into a ThermoFinnigan LCQ-Deca multiple mass spectrometry (MS^n) instrument. The liquid was vaporized at 500 °C, and ionized by a corona discharge needle at 5 kV before being mass filtered using ion-trap mass spectrometry. Specifications of the ICP-MS experiments include acidification of the samples with trace-pure concentrated nitric acid (1% final concentration acid) and phosphorus detection on a Hewlett-Packard 4500 instrument.

3. Results and discussion

3.1. Peak assignments in 18 MΩ water solutions

The peaks in Fig. 1 show the order of elution of an 18 MΩ water solution that contained hypophosphite, phosphite and orthophosphate each at a concentration of 100 μM. A larger injection loop (500 μl) and sharper gradient were used for this experiment as compared to the methods designed for phosphorus oxyanion detection in synthetic geothermal water. The initial assignment of these peaks was determined

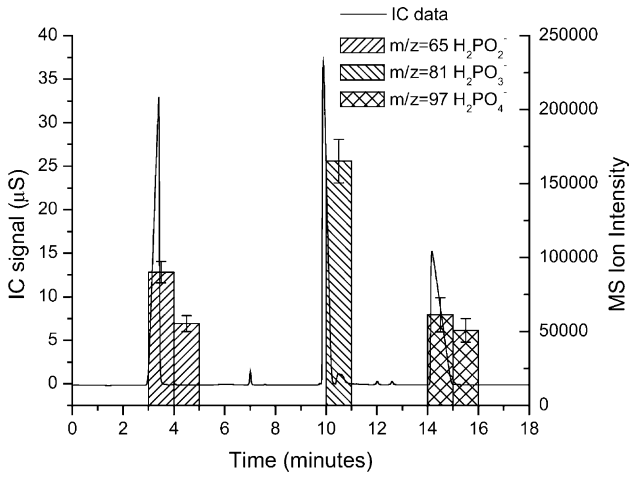

Fig. 1. Five hundred microliter injection of hypophosphite, phosphite, and phosphate, each at a concentration of 100 μM in 18 MΩ water. The line represents IC signal intensity as a function of retention time. The columns symbolize 1 min ion chromatography effluent fractions analyzed by APCI-MS.

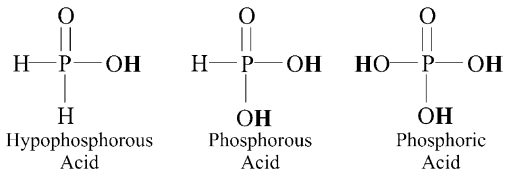

Fig. 2. Chemical structures of hypophosphorous (H_3PO_2), phosphorous (H_3PO_3), and phosphoric (H_3PO_4) acid. Acidic protons are illustrated with bold lettering.

by injecting single compound solutions into the ion chromatograph (data not shown). The observed order of elution was hypophosphite, followed by phosphite, and finally orthophosphate, which is consistent with the order observed in synthetic geothermal waters discussed below.

Previous ion chromatography studies report different elution orders for hypophosphite, phosphite, and orthophosphate [21–24]. A careful comparison of these studies shows that the fluctuating order of phosphorus oxyanion elution results from the different pH values of the eluents, and the pH sensitive nature of phosphorus oxyanion equilibria. The previous studies all use either acidic [21–23] or near neutral eluents [24], which are more sensitive to phosphorus oxyanion pH equilibria than alkaline solutions such as the potassium hydroxide eluent used in the current study. The theoretical equilibrium concentrations of phosphorus oxyanions in solution can be calculated using acid deprotonization equilibrium constants (pK values) [4]. Fig. 2 shows the structures of hypophosphorous acid, phosphorous acid, and phosphoric acid, with acidic protons highlighted using bold lettering. Hypophosphorous acid (H_3PO_2), has a pK_1 of 1.1. For phosphorous acid (H_3PO_3), the pK_1 value is 1.3, and the pK_2 value is 6.7. Phosphoric acid (H_3PO_4) has pK values of 2.1, 7.2, and 12.7 for the first, second, and third proton removals, respectively. Note that both phosphorous (H_3PO_3) and phosphoric (H_3PO_4) acids have pK_2 values close to 7, which predicts competition between single and double charged species for these compounds in near neutral solutions.

In this work, the eluent was alkaline with pH ranging from 10.0 to 12.0. Consequently, at equilibrium, the dominant species in solution are singly charged hypophosphite ($H_2PO_2^-$), doubly charged phosphite (HPO_3^{2-}), and doubly charged orthophosphate (HPO_4^{2-}). The highly alkaline character of the eluent in this system ensures reproducible chromatographs that are immune to peak switching caused by minor pH changes because of contaminations such as dissolved carbon dioxide inherent in near neutral eluents throughout day-to-day operations [32]. In the Mehra and Pelletier study, the eluent of choice was 4-amino-2-hydroxybenzoic acid which had a near neutral 6.3–6.5 [24] pH range, and consequently, the Mehra and Pelletier method is susceptible to phosphite and orthophosphate peak switching. Assuming that the eluent pH remained in the 6.3–6.5 range as reported, the dominant equilibrium concentrations of the three oxyanions in the Mehra and Pelletier study are all singly charged ($H_2PO_2^-$, $H_2PO_3^-$, and $H_2PO_4^-$). Based on charge and molecular size, one would predict the order of elution to be $H_2PO_2^-$ followed by $H_2PO_3^-$. Orthophosphate would be last. This order of elution does not match the reported order, which supports the need to use either extremely acidic or alkaline eluent solutions for reliable measurements of hypophosphite, phosphite and orthophosphate in aqueous solution.

3.2. Peak assignment verification by mass spectrometry

Peak assignments have been verified with mass spectrometry techniques. In these experiments, a standard solution comprised of hypophosphite, phosphite, and orthophosphate each at a concentration of 100 μM, was injected into the IC using a 500 μl injection loop. The effluent of this sample was collected by an automated fraction collector advanced every 60 s. It is important to note that although the eluent in this work is alkaline, after passing through the suppressor the effluent is pure water and the pH is neutral. In this system, alkaline eluent is neutralized by the suppressor to form water. The neutrality of the effluent is preserved in the waste because suppressor regeneration is conducted using an external water source. Having the phosphorus oxyanions in a neutral water solution was critical to the success of the IC effluent analysis by APCI-MS because excess ions in the ionization vapor add excessive noise to the spectra [33]. Configuring an ion chromatography system with neutral effluent as we have done here can be useful for different forms of secondary detection as well, including ICP-MS [34]. This is yet another advantage of using the method presented in this work for monitoring phosphorus oxyanions over different eluent selections [21–24].

The APCI mass spectrometer cannot analyze mass-to-charge (m/z) ratios below 50, therefore only singly charged hypophosphite, phosphite and orthophosphate anions can be detected by this instrument. An examination of the acid dissociation equilibrium constants [4] show that a significant portion of the hypophosphite, phosphite and orthophosphate ions are singly charged species at the neutral pH characteristic of the water–methanol (90:10) solution in the APCI-MS experiments, and the instrument's inability to detect doubly charged ions is of little concern.

The filled columns in Fig. 1 represent the ion intensity of 1 min ion chromatography effluent fractions that contained m/z ratios of 65, 81 or 97, monitored using APCI in the negative ion mode. Peak observations ±0.5 amu of these values are not unusual because ion-trap mass spectrometers are not high-resolution instruments. Mass-to-charge ratios 65, 81, and 97 have been assigned to $H_2PO_2^-$, $H_2PO_3^-$ and $H_2PO_4^-$, respectively.

The solid lines in Fig. 1 trace the ion chromatography data for the same injection of mixed hypophosphite, phosphite, and orthophosphate each at concentrations of 100 μM. The MS signal is delayed ~30 s behind the peaks seen by IC detection, due to the additional time it takes for the compounds to travel from the IC detector to the fraction collector. As

seen by IC, the peaks for these concentrated samples are 0.4–0.9 min in width, which results in peaks corresponding to hypophosphite and phosphate being collected in two adjacent fractions. The data presented in Fig. 1 confirm that the three major peaks seen in the ion chromatograph are indeed due to hypophosphite, phosphite, and orthophosphate and that these species elute in this order.

To further verify the peaks assigned to be phosphorus oxyanions, IC fractions were acidified using 1% nitric acid and analyzed for total phosphorus by inductively coupled plasma mass spectrometry (ICP-MS). These data (not shown) confirm the presence of phosphorus within peaks with retention times identified as phosphorus containing compounds by the ion chromatograph.

3.3. Detection of reduced phosphorus in synthetic geothermal water

Fig. 3 illustrates the detection of hypophosphite, phosphite and phosphate in a 1:4 dilution of synthetic geothermal water [30] in 18 MΩ water. This dilution was selected to simultaneously minimize peak width and maximize sensitivity, which is critical to the successful detection of hypophosphite and phosphite in this matrix. A detailed discussion of $H_2PO_2^-$ and HPO_3^{2-} resolution is presented below. The order of elution for the common anions in this study agrees with literature values [35]. The three halogens, fluoride, chloride, and bromide, elute in this order, followed by nitrate, hydrogen carbonate and sulfate. The observed retention times (min) for the common anions are 3.5 for fluoride, 9.8 for chloride, 12.9 for bromide, 13.4 for nitrate, 15.7 for hydrogen carbonate, and 17.0 for sulfate within an error of ±0.2 min.

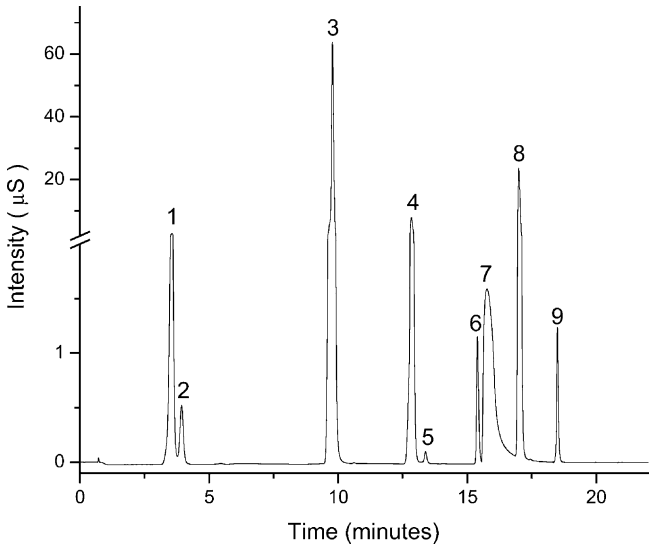

Fig. 3. The chromatograph for a 1:4 dilution of synthetic geothermal water spiked with hypophosphite, phosphite and orthophosphate at concentrations of 20 μM each. The peak assignments are: (1) fluoride; (2) hypophosphite; (3) chloride; (4) bromide; (5) nitrate; (6) phosphite; (7) hydrogen carbonate; (8) sulfate; (9) phosphate.

The detection of reduced phosphorus oxyanions in a matrix representative of geothermal water is complicated by the presence of fluoride and hydrogen carbonate. Attempts were made to accelerate the elution of phosphite and orthophosphate by increasing the slope of the gradient between 4.1 min and 14.0 min. These experiments resulted in reduced resolution between the phosphite and hydrogen carbonate peaks. Even under optimized conditions the baseline resolution between fluoride and hypophosphite is 1.52, and the resolution between phosphite and hydrogen carbonate is 0.92 [36]. Standard techniques for increasing sensitivity for trace-level detection [32] including increasing the size of the injection loop or pre-concentrating the sample were not used in this work because the larger peak widths would interfere with the resolution of the fluoride/hypophosphite and phosphite/hydrogen carbonate pairs. Despite the challenges of fluoride/hypophosphite and phosphite/hydrogen carbonate resolution, the chromatography techniques reported here are well-suited for detecting hypophosphite and phosphite in natural geothermal water, especially if curve fitting is used to analyze the peaks. The resolution was achieved by performing calibrations in a 1:4 dilution of the synthetic geothermal water rather than in fully concentrated solutions. Additionally, this study employed eluent with an unusually small KOH concentration (0.50 mM), for the first 4.1 min of each run to resolve fluoride and hypophosphite.

Fig. 4 shows close-ups of the hypophosphite (a), phosphite (b), and orthophosphate (c) calibration data. In these experiments, fluoride, hydrogen carbonate and the other anions of synthetic geothermal water are held constant at concentrations representative of a 1:4 dilution of geothermal water. All injections were performed using a 15 μl injection loop. The calibrations are linear in the reported range of 1.25–20.0 μM. Table 1 reports the linear regression coefficient, slopes, intercepts, and standard error that support our claims of linearity. Table 1 also reports the detection limit defined as 3σ of a 15 μl 18 MΩ water injection, and the precision of 20 separate injections of 1:4 synthetic geothermal water that contains hypophosphite, phosphite and orthophosphate, each at a concentration of 5 μM. The dead time measured as the first peak in the chromatograph was 0.73 min [32]. The observed retention times were 3.9, 15.4, and 18.5 min with a ±0.2 min error for hypophosphite, phosphite, and orthophosphate, respectively. These data were used to calculate retention factors of 4.3 for hypophosphite, 14.4 for phosphite, and 17.5 for orthophosphate [32]. Note that the fluoride/hypophosphite and phosphite/hydrogen carbonate pairs are clearly resolved, even at the lowest concentrations.

Previous studies by Mehra and Pelletier have reported trace level detection of hypophosphite, phosphite, and orthophosphate ions in a synthetic aqueous solution that contained chloride, nitrate and sulfate [24]. These experiments employed a low-capacity polymeric analytical column, 4-amino-2-hyroxybenzoic acid eluent solutions with concentrations ranging from 2 to 6 mM, and indirect ultraviolet

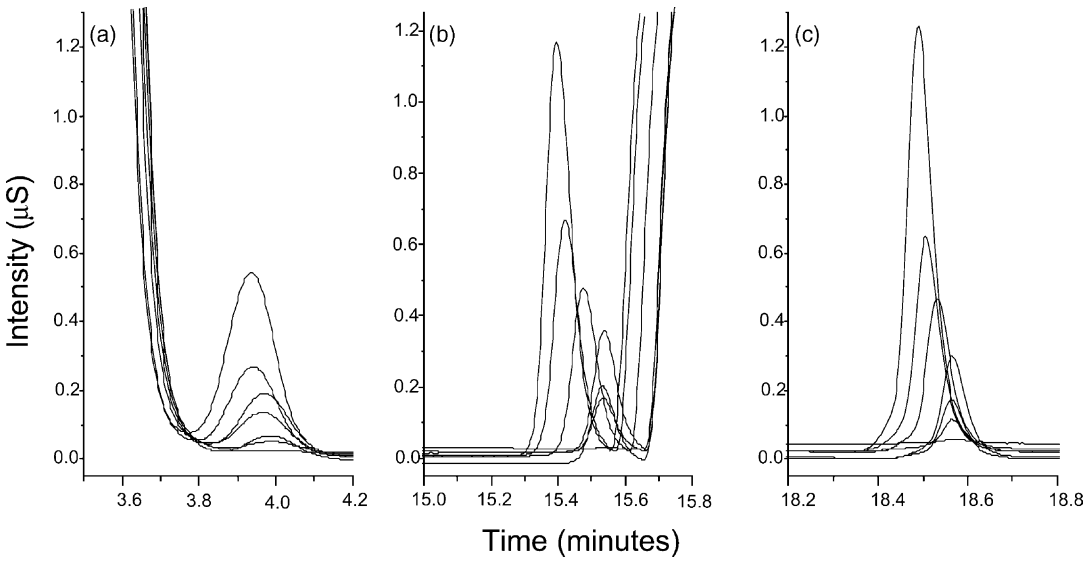

Fig. 4. Fifteen microliter injections of hypophosphite (a), phosphite (b), and orthophosphate (c) dissolved in 18 MΩ synthetic geothermal water. Concentrations are 1.25, 2.50, 5.00, 7.50, 10.0, and 20.0 μM for each of the three phosphorous oxyanions.

Table 1
Calibration parameters, sensitivity, and detection limits for the phosphorous anion methods

Ion	Hypophosphite	Phosphite	Phosphate
Range (μM)	1.25–20.00	1.25–20.00	1.25–20.00
Linear regression coefficient, r^2	0.99	0.99	0.99
Number of data points	7	6	6
Slope (μS min μM^{-1})	0.0038	0.0055	0.0046
Intercept (μS min)	0.005	0.028	0.020
Relative standard error	18.0%	7.52%	5.26%
Detection limit (DL) (μM)	0.83	0.39	0.35
Precision (p) (μS min)	0.08	0.07	0.07

The relative standard error for the calibration is reported as 1σ. DL: detection limit (μM) is three times the standard deviation of the noise for 18 MΩ water injections. p: precision is the 3σ error for 20 repeats of 1:4 synthetic geothermal water spiked with hypophosphite, phosphite, and phosphate each at concentrations of 5 μM.

detection. In this study, the detection limits defined as the peak height response that is three times the average background levels were 7.7 μM for hypophosphite, and 15 μM for both phosphite and orthophosphate, while the detection limits reported here are 0.83, 0.39, and 0.35 μM for hypophosphite, phosphite, and orthophosphate, respectively. The results of this study show a marked improvement in detection limit, due in large part to the use of suppressed conductivity detection over ultraviolet detection methods [24]. Sensitivity was further enhanced by employing an external water source to regenerate the suppressor, and by using an electrolytic eluent generation apparatus.

It is important to note that fluoride and hydrogen carbonate are absent from the Mehra and Pelletier matrix [24]. In analysis of geothermal water, Eccles found that hydrogen carbonate was the most concentrated anion with a concentration of 8.1 mM [30]. Although fluoride has a relatively low concentration (0.43 mM in the Eccles study), the resolution of fluoride and hypophosphite is one of the greatest challenges in the detection of hypophosphite in geothermal water. In fact, Mehra and Pelletier report retention factors of 0.8 and 1.1 for fluoride and hypophosphite in their work; however, there is no discussion of the resolution between these peaks in their article. The improvements in ion chromatography methods for trace level detection of phosphorus oxyanions in a matrix representative of geothermal water discussed here is critical for the ion chromatographic detection of reduced phosphorus oxyanions in real world applications.

4. Conclusions

The suppressed conductivity ion chromatography methods reported here are well-suited for the detection of reduced phosphorus oxyanions in geothermal waters. This study reports a significant improvement in detection limits for these species and the applicability of these techniques to the measurement of reduced phosphorus in waters with the same general composition as our synthetic geothermal water. The 0.35 μM detection limit for orthophosphate is adequate for the monitoring of the estimate 0.3–30 μM concentration

[28]. Hypophosphite and phosphite are clearly resolved from neighboring fluoride and hydrogen carbonate peaks, and detectable at concentrations as low as 0.83 and 0.39 μM, respectively. Because the predominant common anions found in other water bodies are the same, it is likely that this method can be used to detect phosphorus oxyanions in other systems. Our method was successful in this task in large part because suppressed conductivity was selected as the detector. Other factors that contribute to the peak resolution and exceptional detection limits reported here include careful stationary and mobile phase selection, use of a low-noise electrolytic eluent generator, customized eluent gradient implementation, and use of external water to regenerate the suppressor. Peak assignments in this study are based not only on retention times, but are verified by mass spectrometry as well. The success of the mass spectrometry experiments is due to the neutral pH of the effluent produced by the ion chromatography method developed here, which can also be useful for the employment of other secondary detectors. The methods developed here can be used in future studies to determine if reduced phosphorus oxyanions are present in nature and confirm their role as integral components of biological systems.

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