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Detection of phosphorus oxyanions in synthetic geothermal water using ion chromatography–mass spectrometry techniques

Michelle M. Ivey, Krishna L. Foster *

Department of Chemistry and Biochemistry, California State University, 5151 State University Drive, Los Angeles, CA 90032-8282, USA

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Abstract

Recent developments in microbiology suggest that reduced inorganic phosphorus oxyanions, including hypophosphite and phosphite, may be present in nature. These studies have inspired the development of specific and sensitive methods that detect phosphorus oxyanions in natural water. This paper will discuss a new technique that couples suppressed conductivity ion chromatography (Dionex AS17 analytical column and potassium hydroxide eluent) with electrospray mass spectrometry (IC/MS) with limits of detection nearly 200 times lower than those reported using suppressed conductivity detection. The technique was optimized for the detection of hypophosphite, phosphite, and phosphate in a synthetic geothermal water matrix. Samples were pre-treated with silver and sulfonic acid cartridges, and injection loop sizes as large as 800 μl were employed to enhance instrument sensitivity. All peaks were clearly resolved, and calibrations were linear with estimated 3σ limits of detection of 0.011, 0.0020, and 0.029 μM for hypophosphite, phosphite, and phosphate, respectively.

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

1. Introduction

Recent studies show microorganisms contain enzymes that metabolize reduced trivalent (III) phosphite and univalent (I) hypophosphite [1], suggesting that microorganisms can utilize phosphorus oyxanions in oxidation states other than pentavalent (V) phosphate, the most common form of bioavailable phosphorus. Moreover, highly reduced forms of phosphorus, namely phophine (III) have been detected in nature in reducing environments such as sewage [2,3], marine sediments [4], and in industrial [5] and agricultural [6] processes. In natural environments, phosphate is the only thermodynamically stable phosphorus oxyanion [7], and consequently the most abundant with measured concentrations ranging from 0.3 to 30 μM, in pristine environments [8]. Non-equilibrium states persist throughout nature, and consequently, hypophosphite and phosphite may be present at concentrations less than the micromolar concentrations of phosphate. The implications of measuring reduced phosphorus oxyanions in nature would be profound. These measurements may help elucidate the mechanism of phosphine gas formation, and inspire the scientific community to reevaluate the cycling of phosphorus in biological systems. The first step towards measuring reduced phosphorus oxyanions is to develop new methods to detect sub-micromolar concentrations of these oxyanions in matrices representative of natural waters.

Geothermal waters are likely sites for the existence of naturally occurring reduced phosphorus oxyanions. Present in hot springs and oceanic vents, these waters are influenced by the reducing environment below the Earth's surface. Anions in this matrix include fluoride, chloride, bromide, nitrate, hydrogen carbonate, and sulfate. Methods for the detection of reduced phosphorus oxyanions include ion chromatography [9–16], ^{31}P NMR with D_2O locking [1], spectrophotometric determination in a flow injection system [17], and gas chromatography coupled with mass spectrometry [18]. Sample preparation for the ^{31}P NMR and flow injection techniques includes non-discriminatory oxidation processes that do not distinguish hypophosphite from phosphite. Ion chromatography is the most specific of the listed techniques. In the McDowell et al. study, hypophosphite, phosphite and phosphate were detected in a synthetic geothermal water matrix with reported 3σ limits of detection of 0.83, 0.39, and 0.35 μM, respectively, using ion chromatography with sup-

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* Corresponding author. Tel.: +1 323 3432309; fax: +1 323 3436490.
E-mail address: kfoster@calstatela.edu (K.L. Foster).

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pressed conductivity detection [14]. These experiments were performed after diluting full strength synthetic geothermal water by a factor of four. Further improvement of the limits of detection could not be achieved with higher samples volumes and concentrations, because this was hindered by the inability to resolve the fluoride/hypophosphite, and phosphite/hydrogen carbonate peak pairs. Suppressed conductivity detection is a non-specific technique that detects anything that affects the resistivity of the solution through the conductivity cell. If background ions were removed from the matrix, or if ion chromatography were coupled with a detector that responds only to a specific characteristic of the analyte, then larger volumes and concentrations of sample could be delivered to the column, and consequently, limits of detection could be lowered.

Ion chromatography coupled with detectors with higher specificity, such as mass spectrometers or inductively coupled plasma (ICP) elemental detectors [9,11,16], provides the specificity necessary to improve the limits of detection for phosphorous oxyanions in complex matrices. Inductively coupled plasma detectors have been used to measure hypophosphite, phosphite and phosphate as ^{31}P in complex matrices with limits of detection near 10 μg l^{-1}. While ICP techniques are noted for their specificity in identification of elemental phosphourus, the sensitivity of these techniques is limited by the high ionization potential of elemental phosphorus [19]. Other specific detectors such as electrospray mass spectrometry coupled with ion chromatography have the potential to lower the limits of detection even further. This paper introduces a two-dimensional analytical technique that couples the resolution capabilities of ion chromatography with the specificity of electrospray mass spectrometry. The contributions of matrix simplification prior to analysis and variations in the concentrations and volumes of injected sample to the optimization of this technique will be discussed.

2. Methods

2.1. Standards

A 1 mM stock solution of three phosphorus oxyanions was prepared from the following individual stock solutions. Phosphate was used directly from a commercial potassium dihydrogen phosphate (KH_2PO_4) standard containing 1000 ppm of phosphate (LabChem Inc.). Hypophosphite and phosphite solutions were prepared by dissolving 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) in 18.2 MΩ cm Nanopure (Barnstead) water.

All studies were performed with equimolar concentrations of the three phosphorus oxyanions in synthetic geothermal water prepared from sodium salts. The phosphorus oxyanion concentrations varied from 0 to 10 μM, and the anion concentrations were determined from literature values for Hot Creek, a geothermal system near Mammoth Lakes, CA [20]. The 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. Synthetic geothermal water stock solution was prepared from sodium hydrogen carbonate (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), sodium fluoride solution (standardized 0.100 M, Fisher Scientific) and sodium nitrate solution (1000 ppm, 98%, SPEX CertiPrep). All solutions prepared from salts were filtered with 0.2 μm cellulose acetate filters, stored in HDPE bottles at 4 °C, and used within 30 days of preparation.

2.2. Sample preparation

The results of simplifying the geothermal matrix by removing superfluous anions including chloride, bromide and hydrogen carbonate were examined in this study. Samples were filtered through two OnGuard II cartridges (silver, Dionex p/n 057089 and sulfonic acid, Dionex p/n 057085) connected in series [21]. The first cartridge contained silver ions, which removed chloride and bromide by the precipitation of insoluble silver chloride and silver bromide salts. The second cartridge contained a styrene-based, strong resin in the H^+ form, which has a strong affinity for multivalent cations, and would also trap any silver ion breakthrough from the first cartridge. The second cartridge also converted carbonate to carbonic acid, which was then removed by sparging with nitrogen (UHP, Gilmore Liquid-Air) for 5 min to accelerate the removal of carbon dioxide gas from the system [21].

2.3. Ion chromatography system with conductivity detector

Two Dionex DX 600 suppressed conductivity ion chromatography (IC) systems were employed in this study. The first utilized a GS50 gradient pump, an AS50 autosampler, and a CD25 conductivity detector. The second system was equipped with a LC20 chromatography compartment, an IP25 isocratic pump and a CD20 conductivity detector. Both systems employed hydrophilic quaternary ammonium functionalized 4 mm analytical columns (Dionex IonPac AS17), preceded by guard columns (Dionex IonPac AG17) for anion separation; EG40 electrolytic eluent generators; and ASRS Ultra II suppressors. All samples were refrigerated prior to injection.

Potassium hydroxide eluent was generated electrolytically. Degassed 18.2 MΩ cm Nanopure water was stored in a 4 l plastic bottle under 40 kPa UHP helium. An ATC-3 trap column was used to strip trace anion contaminants from the water before the water was pumped at 1.5 ml min^{-1} into a Dionex EG40 eluent generator where the potassium hydroxide eluent was generated electrolytically. The gradient was as follows: 0.5 mM from 0 to 5 min; 0.5–9 mM from 5 to 13 min; 9.0 mM between 13 and 16 min; 9.0–35.0 from 16 to 18 min, and 35 mM from 18 to 20 min. This gradient was adjusted throughout the study to compensate for retention time shifts attributed to column aging [22].

After separation, the eluent stream was directed through a suppressor (Dionex ASRS Ultra II, current 300 mA), which converted the hydroxide eluent to water, resulting in anions in neutral pH water, which could then be monitored via a conductivity detector. The suppressor was regenerated with an external