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Optimizing pretreatment for the detection of phosphorus oxyanions using ion chromatography

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OPTIMIZING PRETREATMENT FOR THE DETECTION OF PHOSPHORUS OXYANIONS USING ION CHROMATOGRAPHY.

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Michelle Ivey, and has been approved by the members of his supervisory committee. It was submitted to the faculty of The Honors College and was accepted in partial fulfillment of the requirements for the degree of Bachelor of Arts in Liberal Arts and Sciences.

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ABSTRACT

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Title: Optimizing pretreatment for the detection of phosphorus oxyanions using Ion Chromatography.

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Thesis Advisor: Dr. Michelle Ivey.

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Phosphorus (P) is required for all living organisms. Fully oxidized pentavalent (+V) is the principal form in organisms, however studies on *Desulfotignum phosphitoxidans* show the enzymatic metabolism of reduced P oxyanions. Thus a natural source of reduced P is expected. Geothermal waters are naturally occurring reducing environments and ion chromatography has been used for the detection of submicromolar concentrations of P, yet the detection of reduced oxyanions is complicated by fluoride and hydrogen carbonate with similar elution times as hypophosphite(+I) and phosphite(+III) respectively. Studies had shown that simplifying the matrix through pretreatment with silver and sulfonic acid cartridges improves IC limits of detection (LODs). The effects of pretreatment are dependant upon the total concentration of ions in solution. The purpose of this study is to determine IC phosphorous oxyanion LODs and to maximize signals by analyzing the relationship between filtering techniques, effective concentration of P oxyanions, and total ions in the matrix.

Table of Contents

List of Tables

List of Illustrations

Introduction

Phosphorus (P) is an essential element for life on Earth, encompassing fundamental organic compounds such as nucleic acids, phospholipids, and proteins [Mcdonald, 2001]. Consequently, P has a profound effect on the environment. For instance, adding P in the form of phosphate to lakes usually results in great increases in algal growth [Froelich, 1988; Barrow, 1983]. In nature, P is frequently a limiting nutrient for many species, including economically significant plants. Accordingly, to supply the agricultural requirements of P, calcium phosphate $Ca_3(PO_4)_2$, is used as a fertilizer.

Inorganic P occurs in six oxidation states: -III (e.g., phosphine, PH_3); -I(e.g., phosphine oxides), 0 (elemental P); +I (e.g., phosphenic acids), +III (e.g., phosphonic acids); and +V (e.g., phosphate esters). In different environments, the concentration of P in these oxidation states varies. Simultaneous pH and reduction potential analysis, shown in figure 1, demonstrates that the quantity of each P specie changes in relation to both [Hanrahan, 2005]. In most environmental conditions, like natural waters with pH values between 4-10, the most stable and common form of P is the fully oxidized pentavalent (+V) species [Hanrahan, 2005].

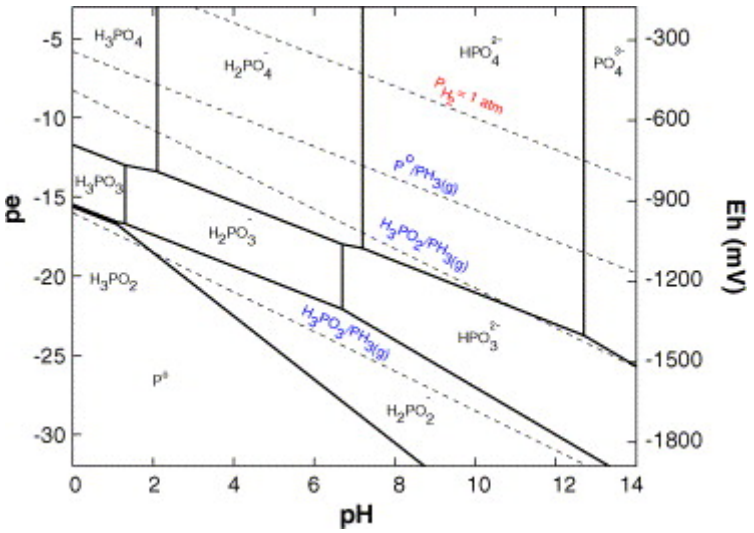

Figure 1 Eh/pe-pH diagram for P species[Hanrahan 2005]

While the pentavalent form is the principal form in the environment, it is also prevalent in organisms. [Garcia Costas, 2001]. Phosphates, inorganic compounds in the fully oxidized state (+V), are considered the main nutritional source of P for algae, bacteria, and plants.

However, organisms such as the bacterium *Desulfotignum phosphitoxidans* can metabolize more reduced forms of P, including phosphite [McDowell, 2004; Schink et al. 2000]. Additionally, the identification of phosphine gas in soil and sediments contradicts the idea that reduced forms of P are not an integrated component of phosphorus' biological cycle [Gassmann, 1993].

Hot springs are naturally occurring reducing environments and P oxyanions are found in geothermal water in submicromolar concentrations [McDowell, 2004]. Thus, studies of reduced P species in the environment are restricted by instruments' limits of detection.

Ion Chromatography (IC) is used for quantitative analysis at submicromolar concentrations. IC is a form of liquid chromatography that uses an ion-exchange column to separate atomic or molecular ions based on the interaction with a resin. In this thesis, I explore ways to improve the techniques for detecting different forms of P in synthetic geothermal water using IC.

Ivey et al (2005) found that the amount of P oxyanions removed from synthetic geothermal water using silver filtering cartridges and sulfonic acid filtering cartridges was dependant upon the total concentration of ions in solution.

The purpose of this study is to determine P oxyanions limits of detection and to analyze the relationship between filtering techniques, effective concentration of P oxyanions, and total ions in the matrix.

Methods:

IC system:

A Dionex ICS 2000 IC system was used for this study. The system was composed of an autosampler, a electrolytic eluent generator, a gradient pump, a guard column, an AS-17 analytical column, an SRS® self-regenerating suppressor, and a DS-6 conductivity cell.

Samples were transferred to single-use 1.5 ml glass autosampler vials (Dionex) and introduced into the IC system by the AS-50 autosampler. The sample followed to a 3.75 µL injection loop.

Potassium hydroxide eluent was produced with an eluent generator supplied with a Dionex EGCII potassium hydroxide eluent cartridge and external degassed 18 MΩ Nanopure water-bubbled with UHP helium for 5 minutes. Multistep gradient concentrations of the eluent were programmed using the following scheme: 0.35 mM from 0 to 5 min; 0.35 mM to 7.00 mM from 5 to 13 min; 7.00 stable from 13 to 16 to min; 7.00 mM to 32.00 mM from 16 to 18 min; and 32.00 mM stable from 18 to 20 min. The eluent exited the cartridge and flowed into the CR-TC, a column to trap anion contaminants, and then to the injection valve into the injection loop. The sample was then pumped into the guard column to trap contaminants and remove particulates and then to the analytical column. The eluent stream followed to the SRS® self-regenerating suppressor. The suppressor converts the eluent, OH^-, to water thus reducing the eluent conductivity and making ions easier to detect. The sample finally reached the DS6 heated conductivity cell to measure its electrical conductance.

Standards preparation:

Synthetic geothermal water was prepared simulating anion concentrations at Hot Creek, a geothermal system in Sierra Nevada, CA. as described by literature and shown on table 1 [Wilkie, 1998].

Component	Concentration (mM)
Bicarbonate	8.1
Chloride	5.5
Sulfate	0.91
Bromide	0.81
Fluoride	0.43
Nitrate	0.01

Table 1: Hot Creek water composition [Wilkie, 1998].

Stock solutions of each component were prepared with 0.1 M anion concentration using 18 MΩ nanopure water. Hydrogen carbonate was prepared from sodium hydrogen carbonate (Certified ACS grade, Fisher Scientific); chloride was prepared from sodium chloride (Certified ACS grade, Fisher Scientific); sulfate was prepared from anhydrous sodium sulfate (Certified ACS grade, Fisher Scientific); bromide was prepared from sodium bromide (Acros), fluoride was prepared from sodium fluoride (Certified ACS grade, Fisher Scientific) and nitrate was prepared from sodium nitrate (Certified ACS grade, Fisher Scientific).

1mM orthophosphate, phosphite and hypophosphate stock solutions were prepared using 18 MΩ nanopure water and dissolving respectively sodium

phosphate monobasic dihydrate (Certified ACS grade, Sigma), sodium phosphite dibasic pentahydrate ($Na_2(PHO_3 \cdot 5H_2O$, Riedel-de Haën), and sodium hypophosphite monohydrate ($NaH_2PO_2 \cdot H_2O$, Sigma).

All solutions where stored in HDPE bottles at 4° C and used within 30 days.

Samples preparation:

Samples used varying concentrations of P oxyanions (0-10μM). All samples were initially filtered using cellulose acetate filters. Additionally, OnGuard II silver cartridges and OnGuard II sulfonic acid cartridges were used to remove anions from the matrix and when both cartridges were used they were connected in series. The OnGuard II silver cartridge removed chloride and bromide from the matrix by producing insoluble silver chloride and silver bromide. The OnGuard II sulfonic acid cartridge removed high concentrations of sulfate from the matrix using a strong H^+ resin. This resin also transformed carbonate ions into carbonic acid. Carbon dioxide gas, in equilibrium with carbonic acid, was removed by bubbling the samples with UHP helium for 1 minute.

Results:

Trial 1:

Hot Creek water Chromatogram

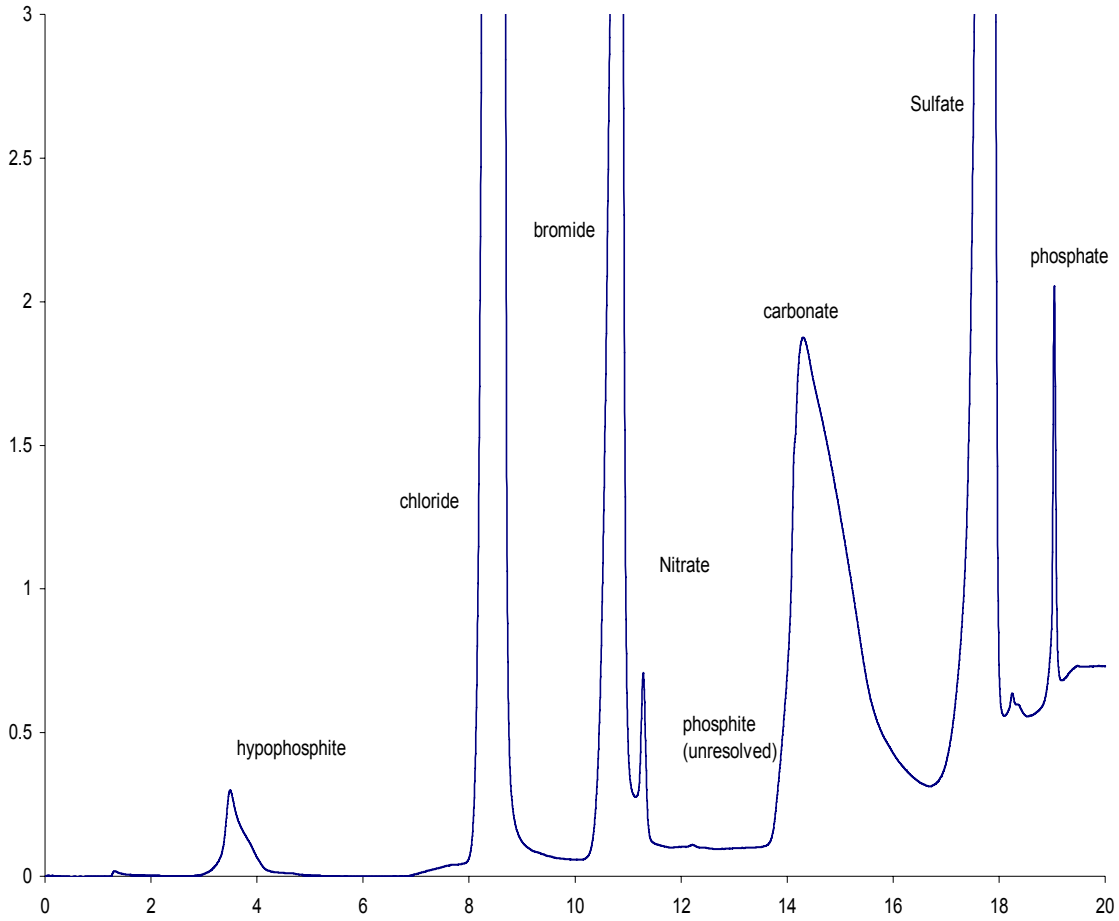

Figure 2: Creek water chromatogram using 10 μM P oxyanions concentration and all other ions following table 1 concentrations. Fluoride was not added to sample.

A solution of synthetic Hot Creek water was prepared with anions following the concentrations shown in table 1 and 10 mM of each of the phosphorus anions. The peaks in the chromatogram of figure 2 show that ions are eluting at expected times as describe by literature [McDowell, 2004; Ivey, 2005] and as shown in table 2.

Component	Approx. RT (Minutes)
Hypophosphite	3.5
Chloride	9
Bromide	10.5
Nitrate	11
Bicarbonate	15
Sulfate	17.5
phosphate	18.5

Table 2: anions elution time for synthetic Hot creek water

Trial 2:

To determine the purity of the stock solutions 1 mM samples of each anion were prepared. A blank with 18 MΩ nanopure water was run.

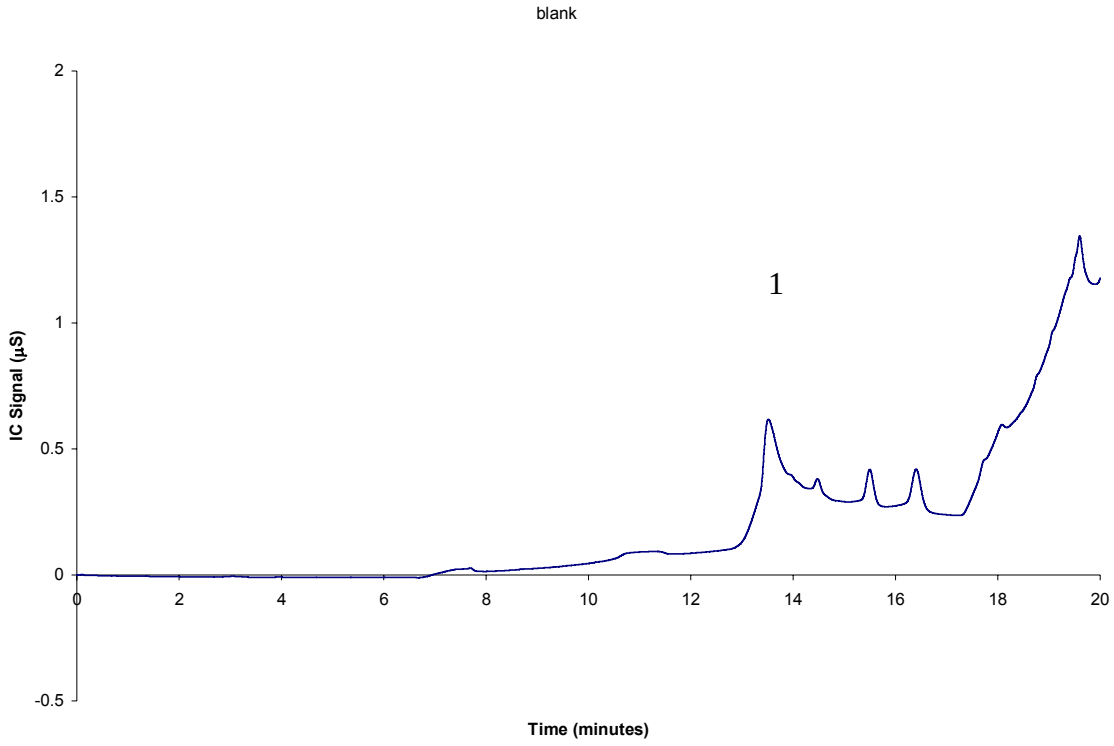

Figure 3: 18 MΩ nanopure water ion chromatogram. Peak assignment is (1) carbonate ion.

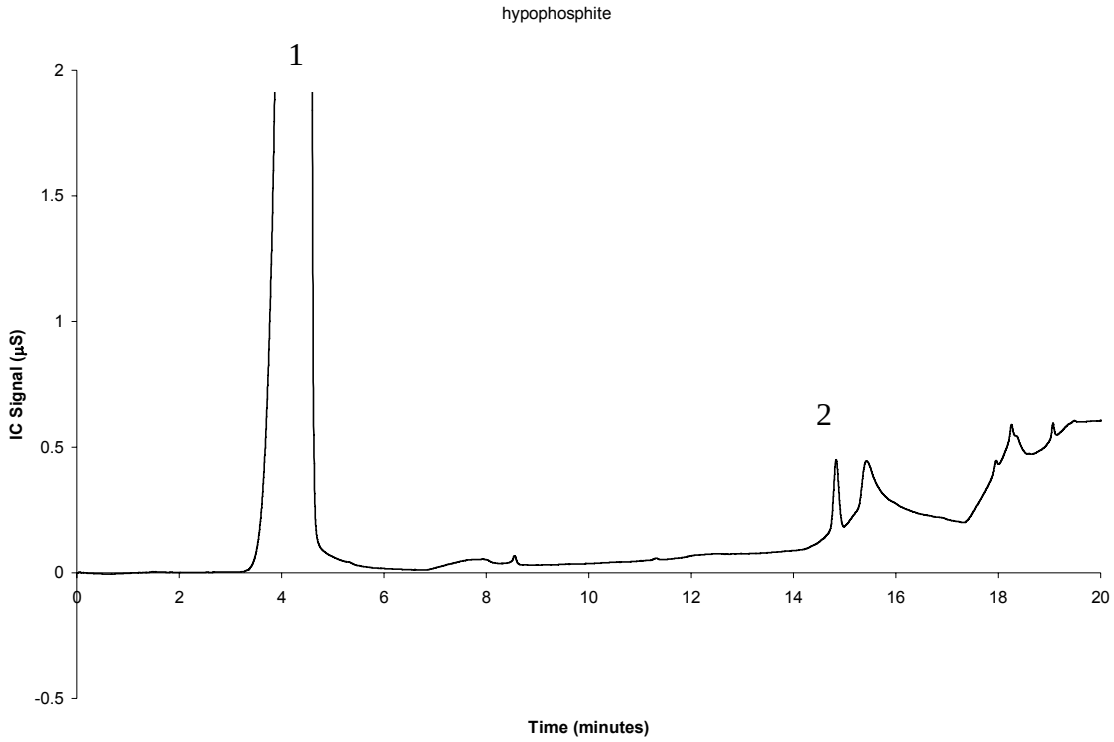

Figure 4: Ion chromatogram for 1mM hypophosphite solution. Peak assignments are (1) hypophosphite; (2) phosphite.

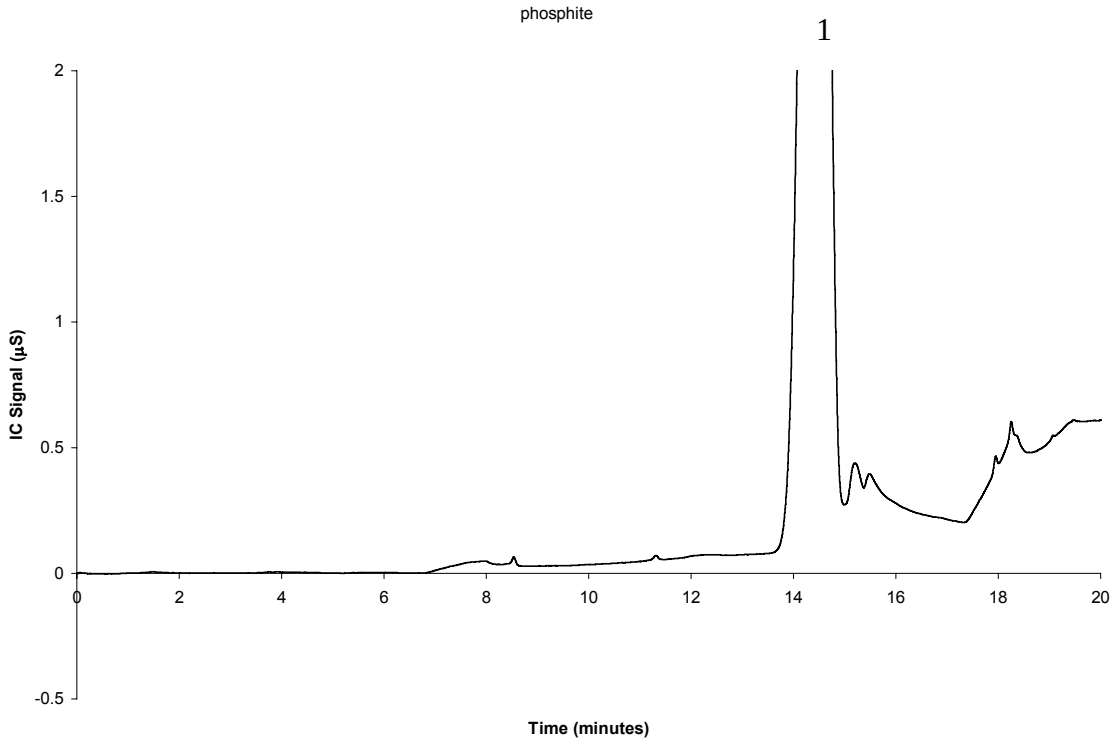

Figure 5: Ion chromatogram for 1mM phosphite solution. Peak assignment is (1) phosphite.

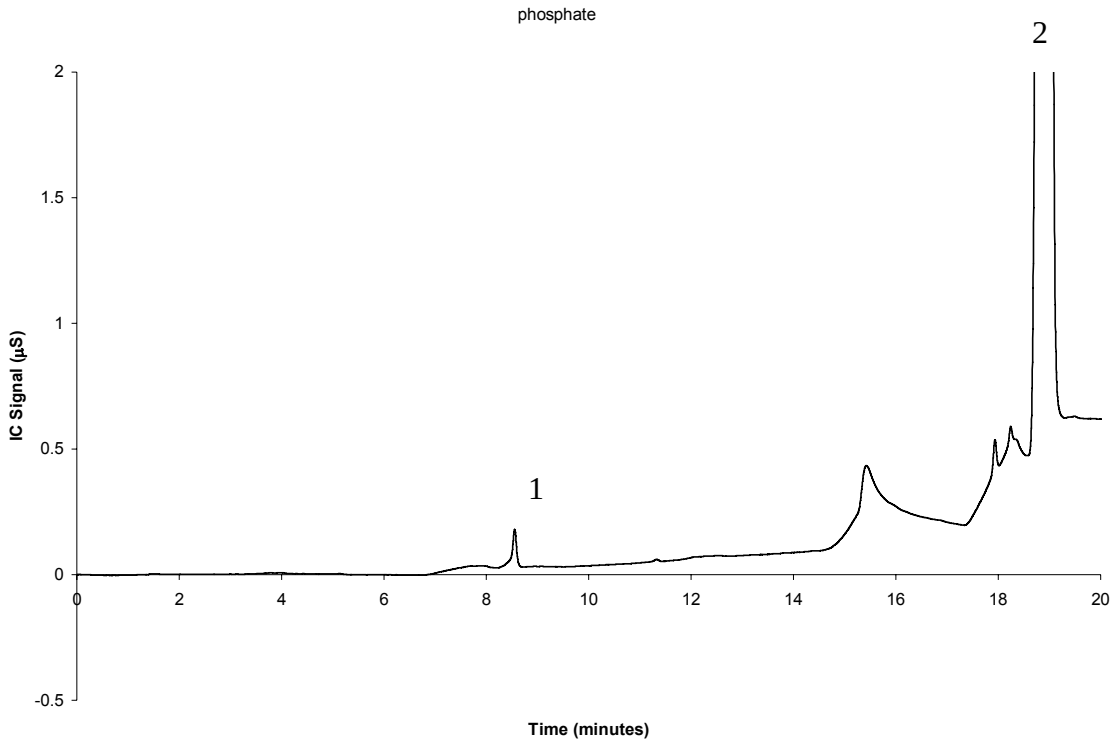

Figure 6: Ion chromatogram for 1mM phosphate solution. Peak assignments are (1) chloride; (2) phosphate.

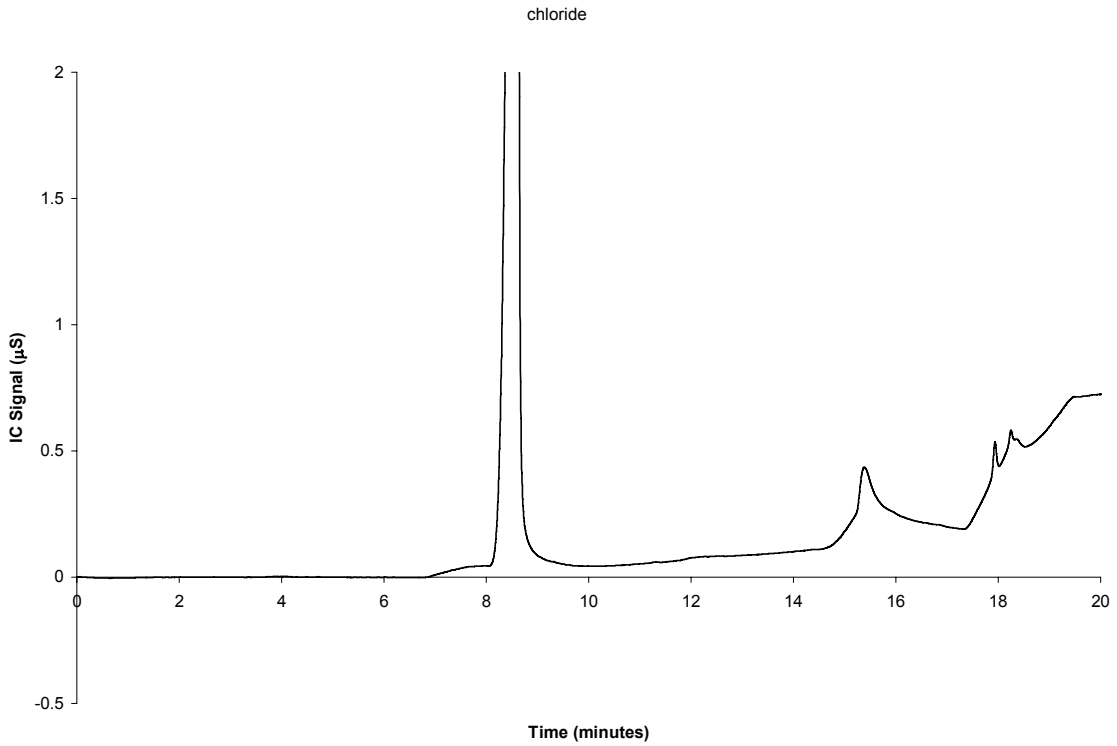

Figure 7: Ion chromatogram for 1mM chloride solution. Peak assignment is (1) chloride.

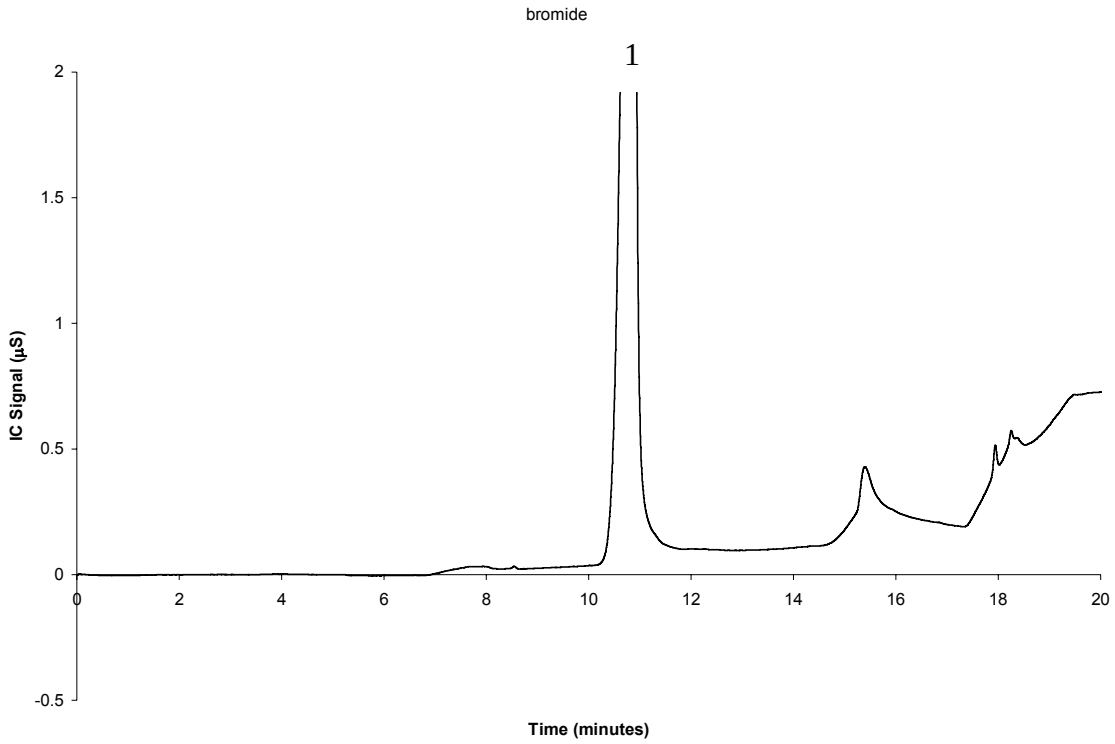

Figure 8: Ion chromatogram for 1mM bromide solution. Peak assignment is (1) bromide.

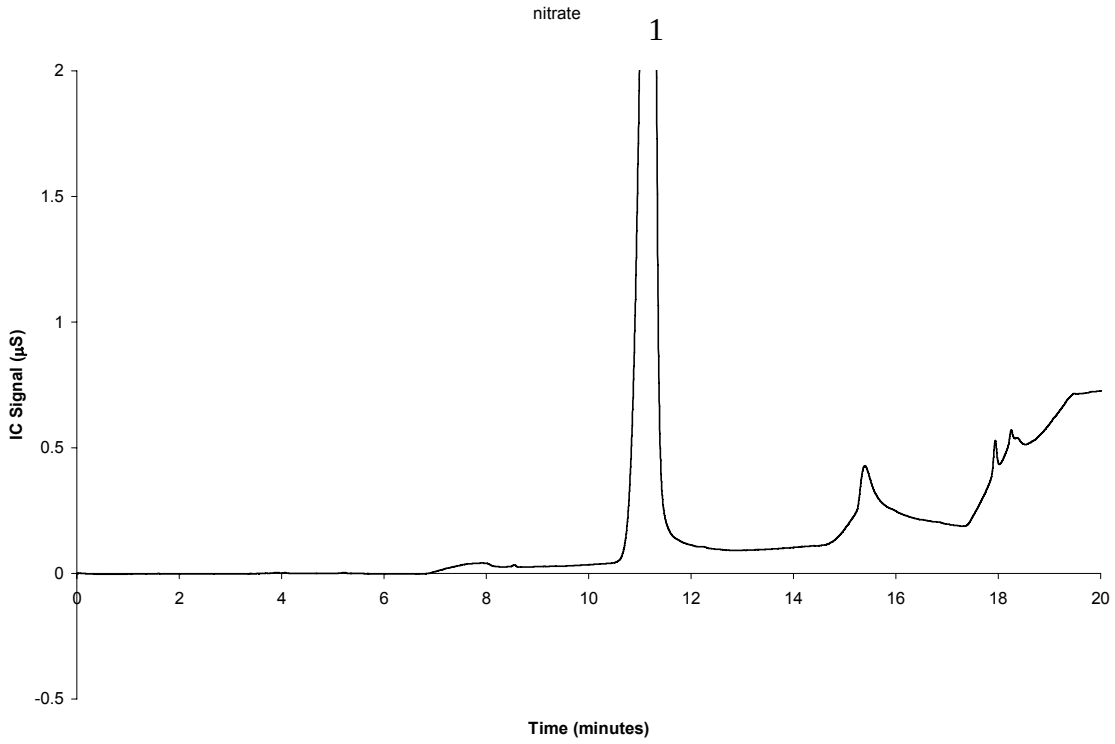

Figure 9: Ion chromatogram for 1mM nitrate solution. Peak assignment is (1) nitrate.

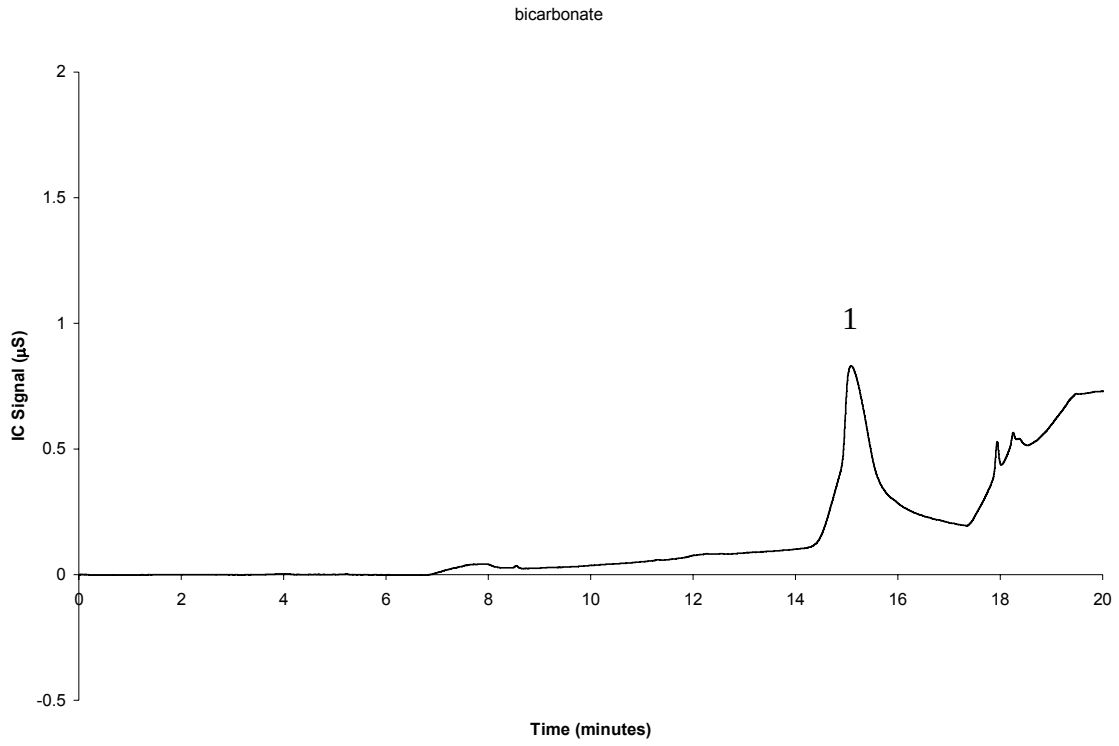

Figure 10: Ion chromatogram for 1mM bicarbonate solution. Peak assignment is (1) bicarbonate.

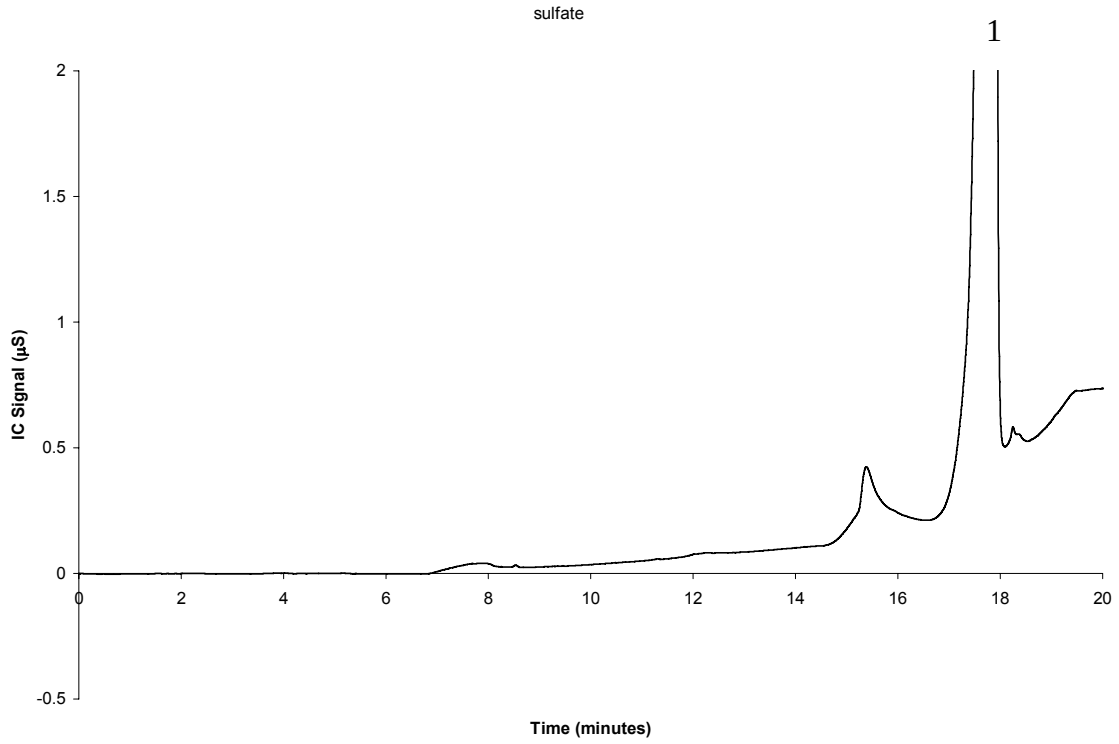

Figure 11: Ion chromatogram for 1mM sulfate solution. Peak assignment is (1) sulfate.

Chromatograms of all samples confirm the purity of all stock solutions and expected elution times. However, presence of trace concentrations of phosphite ions in the hypophosphite solution and chloride ions in the phosphate solution was noticed.

Trial 3:

Samples with constant 20 mM ion concentration (chloride and nitrate) were prepared to determine the effects of increasing chloride concentration on P oxyanions detection using IC. Samples were analyzed before and after filtration using a silver cartridge to remove chloride ions.

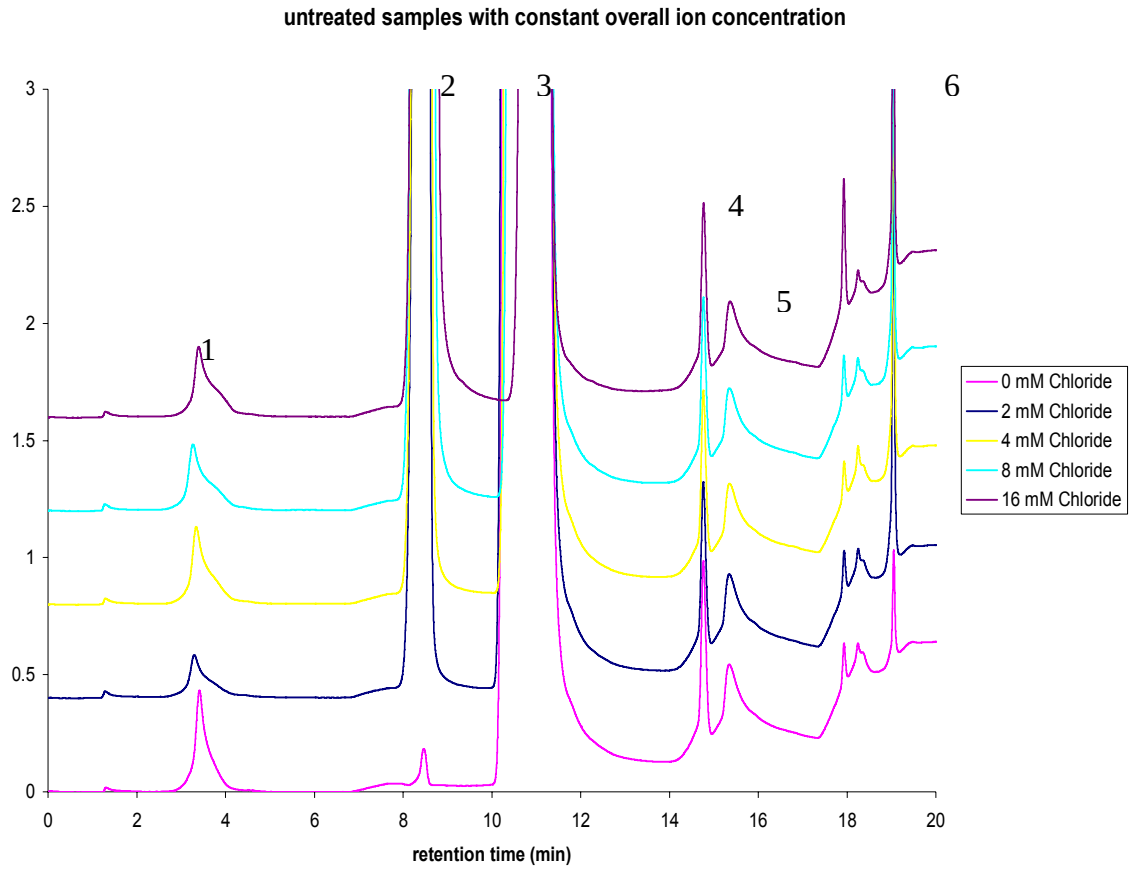

Figure 12: Ion chromatogram for 0-10 mM chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each. Peak assignment is (1) hypophosphite; (2) chloride; (3) nitrate; (4) phosphite; (5) carbonate; (6) phosphate.

Fig. 12 shows the ion chromatogram for increasing chloride concentrations before pretreatment with silver cartridge. The data shows that ions retention time is unchanged with increasing chloride concentration. The observed retention times (min) are 3.4 for hypophosphite, 8.5 for chloride, 10.0 for nitrate, 14.8 for phosphate,15.4 for bicarbonate and 18.3 for sulfate and18.5 for phosphate. The data also depicts broad hypophosphite peaks.

Peak area analysis:

Fig. 13 shows peak area analysis, with hypophosphite average peak area of 0.13 ± 0.03 μS*min; phosphite average peak area of 0.134 ± 0.002 μS*min; phosphate average peak area of 0.10 ± 0.04 μS*min;

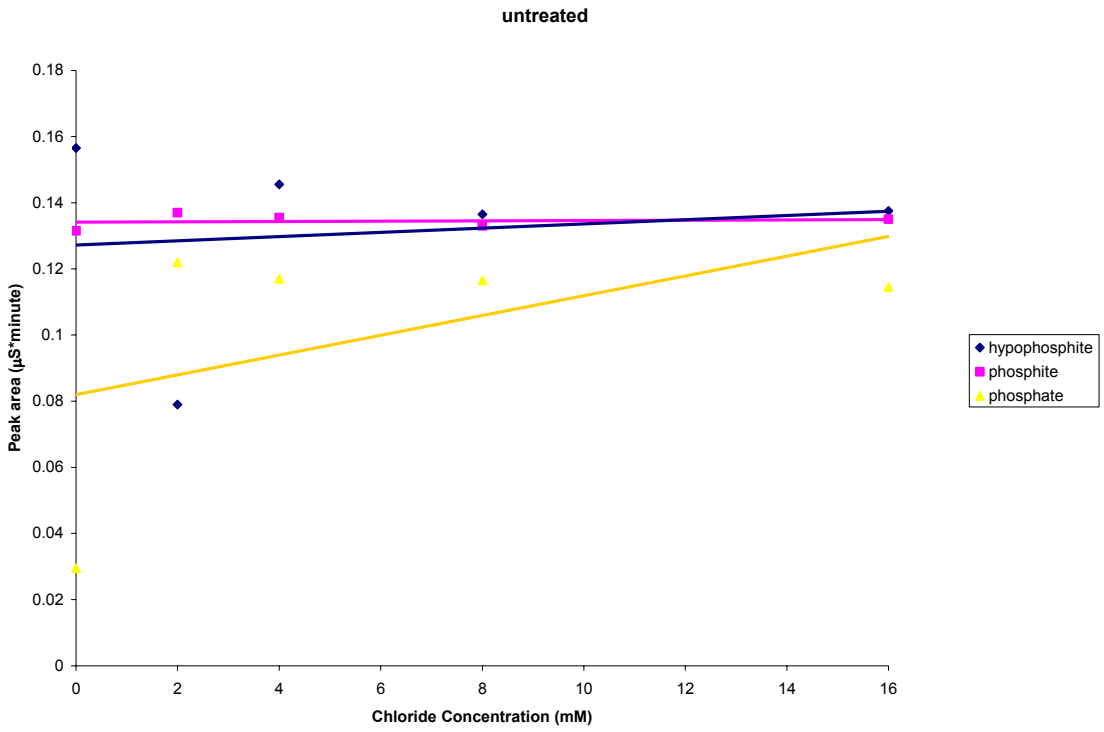

Figure 13: Peak area over chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each.

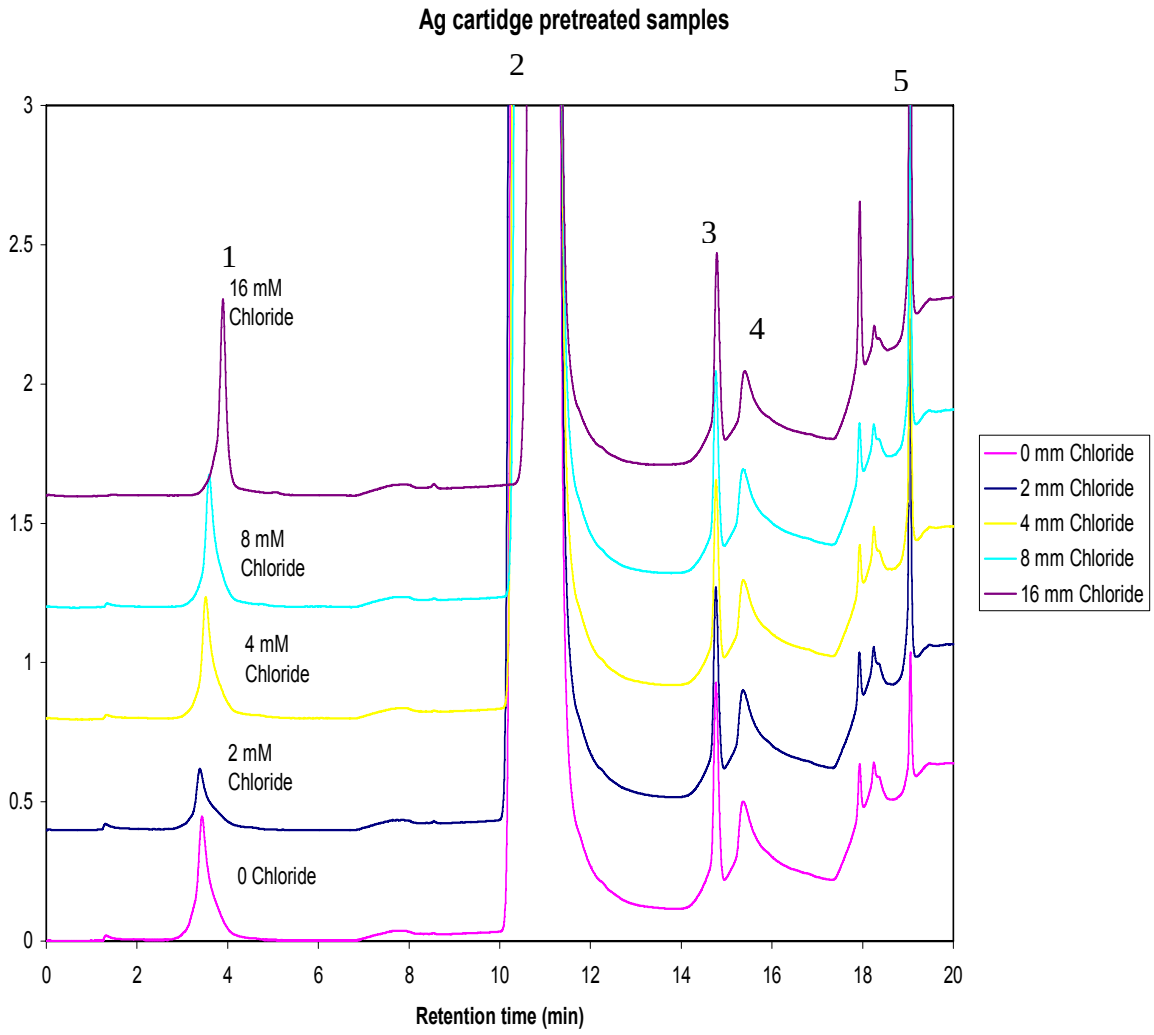

Figure 14: Ion chromatogram for pretreated 0-10 mM chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each. Peak assignment is (1) hypophosphite; (2) nitrate; (3) phosphite; (4) carbonate; (5) phosphate.

Fig. 14 shows the ion chromatograms for samples with increasing chloride concentrations and constant 10 μM concentration for each phosphorus oxyanion after pretreatment with silver cartridge. The data shows that hypophosphite retention time increases, from 3.4 to 3.9 minutes, with increasing chloride concentration. Other ion retention times are unchanged with increasing chloride concentration. The data also depicts hypophosphite peaks narrowing with

increasing pretreatment chloride concentration. Chloride was completely removed after pretreatment.

Peak area analysis:

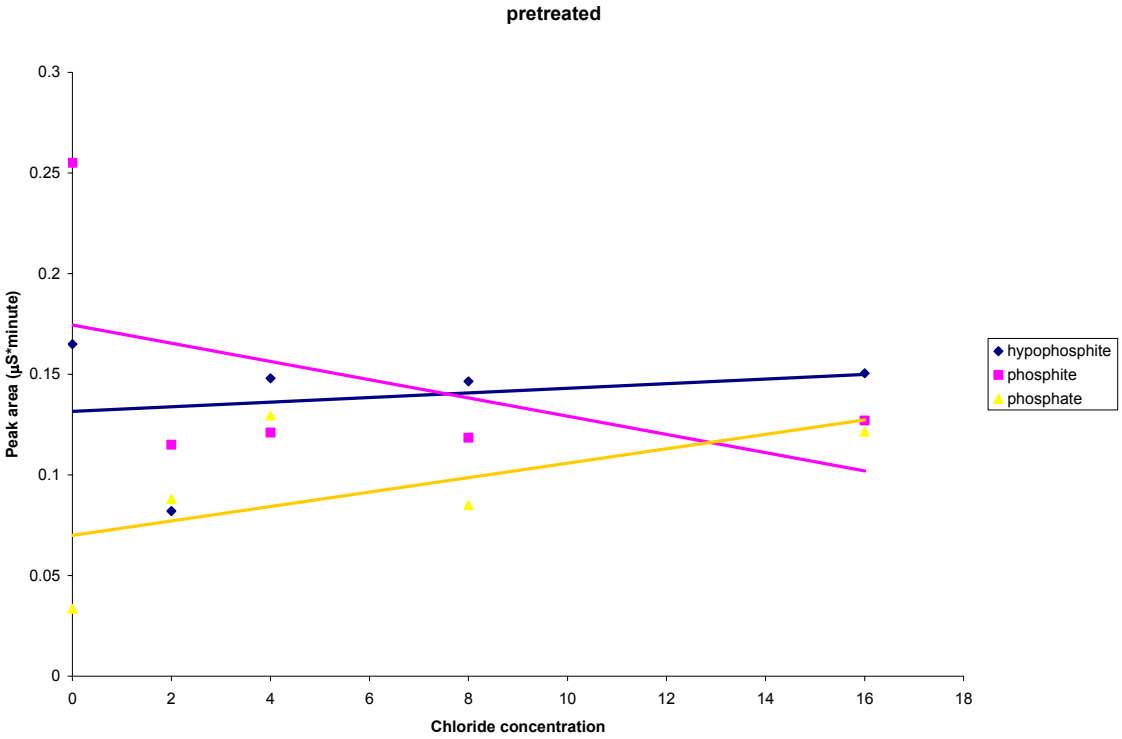

Figure 15: Peak area over chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 µM each, after pretreatment with silver cartridge.

Fig. 15. shows peak area analysis, with hypophosphite average peak area of 0.14 ± 0.03 µS*min; phosphite average peak area of 0.15 ± 0.06 µS*min; phosphate average peak area of 0.09 ± 0.04 µS*min;

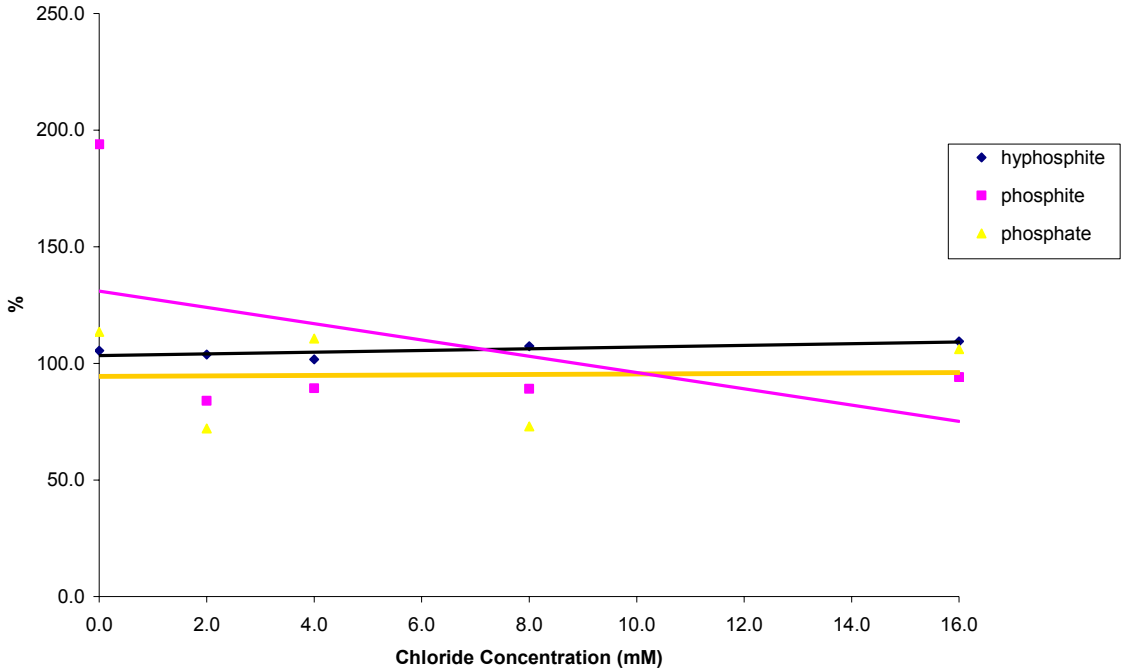

Figure 16: Filtered peak area over unfiltered peak area, with hypophosphite, phosphite and phosphate at concentrations of 10 μM each.

Recovery percentage was analyzed graphically as noted from Fig. 16. Hypophosphite peak area increased by 6 ± 3.0 %; phosphite peak area after treatment increased by 10 ± 47 %; phosphate peak area decreased 4.9 ± 20 %. Due to the high standard error caused by the phosphite peak nearly doubling in size for 0 mM chloride concentration, peak area was also analyzed excluding this point. Phosphite peak area after treatment decreased by 11.1 ± 4.2 %; this experiment needs to be repeated to test whether the apparent increase in phosphate at 0 mM chloride concentration is real, or a result of experimental error.

Trial 4:

Samples with constant overall 20 mM ion concentration (chloride and nitrate) were prepared to determine the effect of increasing chloride concentration on P oxyanions. Additionally, carbonate was added to reach a final concentration of 8.1 mM, simulating Hot Creek water conditions. Samples were analyzed before and after pretreatment using simultaneously a Ag^+ cartridge and a H^+ cartridge connected in series. Additionally, N_2 was bubbled for one minute to remove carbonate ions from samples.

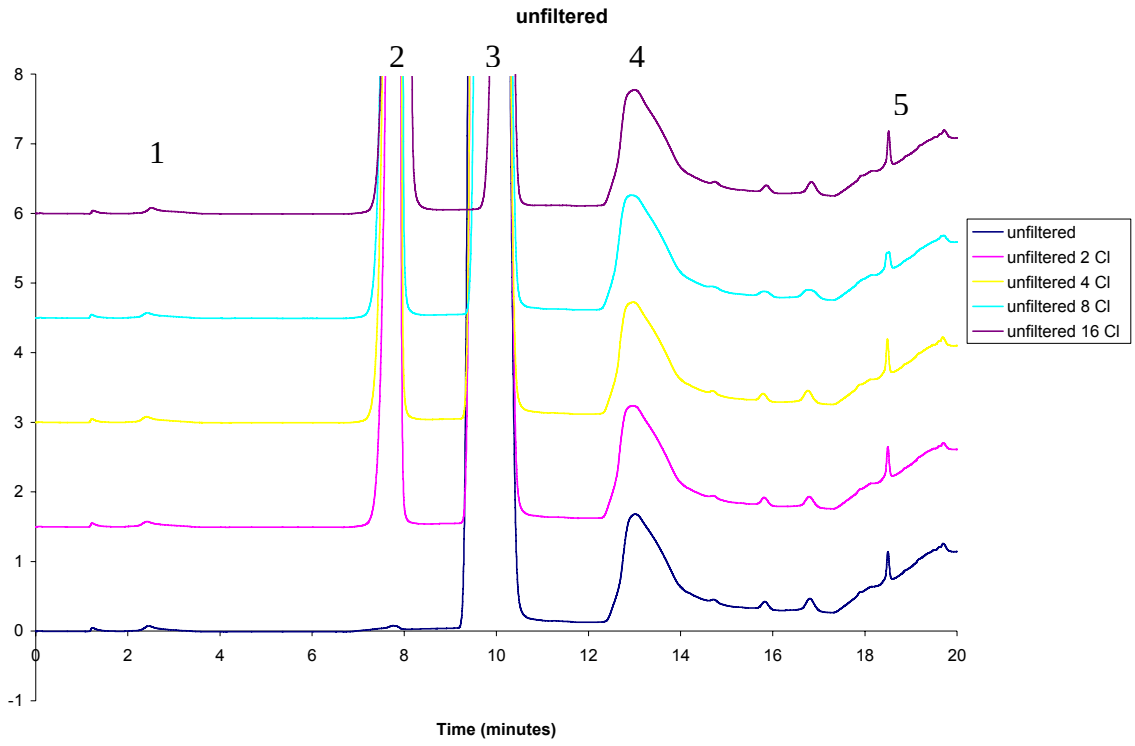

Figure 17: Ion chromatogram for 0-10 mM chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each. Peak assignment is (1) hypophosphite; (2) chloride; (3) nitrate; (4) carbonate with phosphite shoulder on the left side; (5) phosphate.

Fig. 17 illustrates the detection of hypophosphite, phosphite and phosphate at concentrations of 10 μM each in a range of chloride concentrations from 0-16 mM. This concentration range was selected to bracket the Hot Creek

water chloride concentration. Fig. 17 shows that the order of elution of all ions agrees with literature values and table 2. However phosphite is not resolved from carbonate and it appears as a shoulder on left side of carbonate peak.

Peak area analysis:

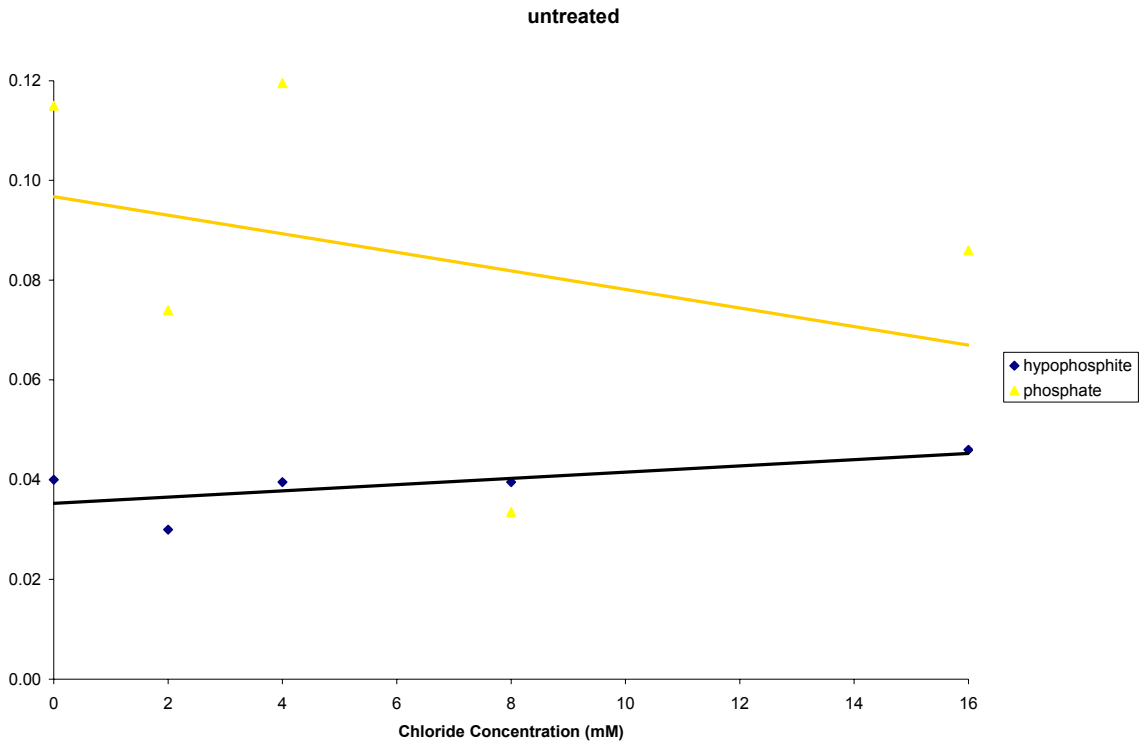

Figure 18: Peak area over chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each. Phosphite is unresolved from carbonate.

Fig. 18 illustrates that hypophosphite peak area average 0.039 ± 0.005 μS*min, three times smaller than 0.131 ± 0.030 μS*min, the peak area average reported during trial 3. On the other hand, phosphate peak area average 0.086 ± 0.035 μS*min. Trial 3 reported an average phosphate peak area of 0.099 ± 0.04 μS*min. Thus, the presence of carbonate ion in solution affects hypophosphite peaks.

The effect of pretreatment with silver and H^+ cartridges is shown by fig. 19. All peaks eluted at expected times. An unknown peak appeared earlier in the chromatogram, but completely resolved from the hypophosphite peak. Trace amounts of chloride were detected. Phosphite peak is resolved from the carbonate peak when pretreated with Ag^+ and H^+ cartridges in series, whereas in untreated samples the hypophosphite peak was unresolved from carbonate. Phosphate decreased significantly from the samples by filtration in contrast to trial 3, when only the Ag^+ cartridge was used and phosphate concentration did not change significantly.

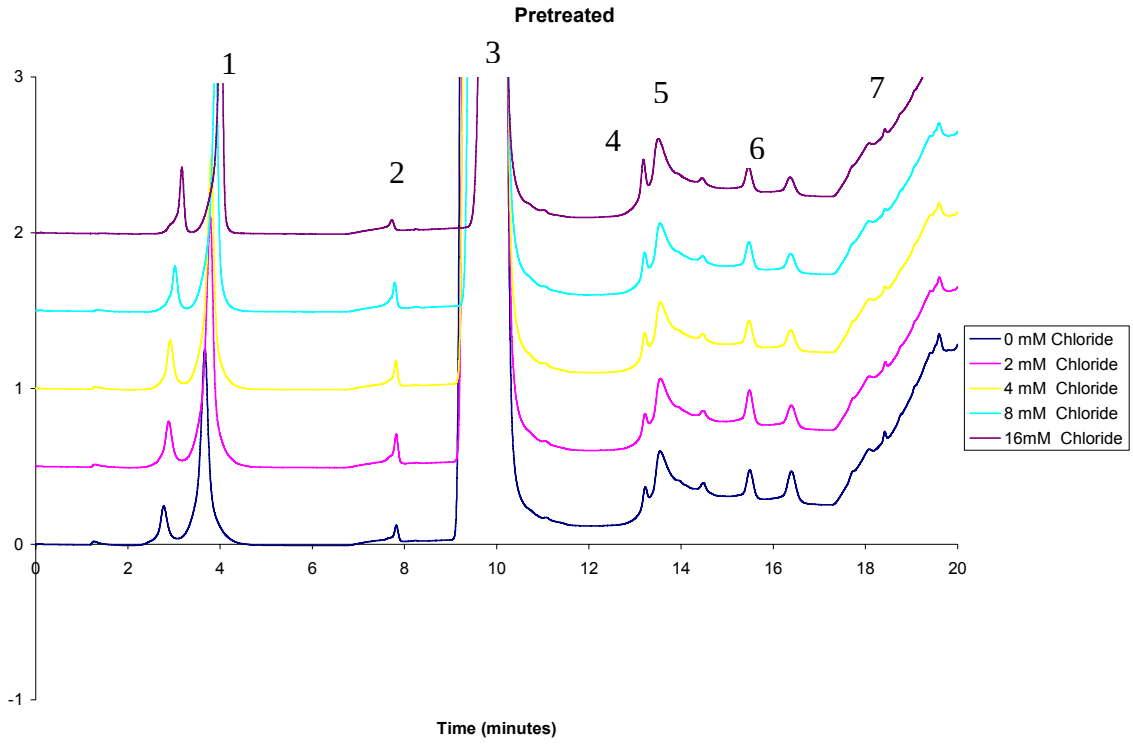

Figure 19: Ion chromatogram for Ag^+ and H^+ pretreated 0-10 mM chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each. Peak assignment are (1) hypophosphite; (2) chloride; (3) nitrate; (4) phosphite; (5) carbonate; (6) sulfate; (7) phosphate

Peak area analysis:

Fig. 20 shows peak area analysis for Ag^+ and H^+ pretreated samples, with hypophosphite average peak area of 0.369 ± 0.061 μS*min; phosphite average peak area of 0.370 ± 0.015 μS*min; phosphate average peak area of 0.033 ± 0.018 μS*min. Hypophosphite average peak area was larger than 0.138 ± 0.032 μS*min, peak area reported during trial 3. Phosphite average peak area was larger than 0.147 ± 0.060 μS*min, peak area registered during trial 3. Phosphate average peak area was smaller than 0.092 ± 0.038 μS*min, peak area from trial 3.

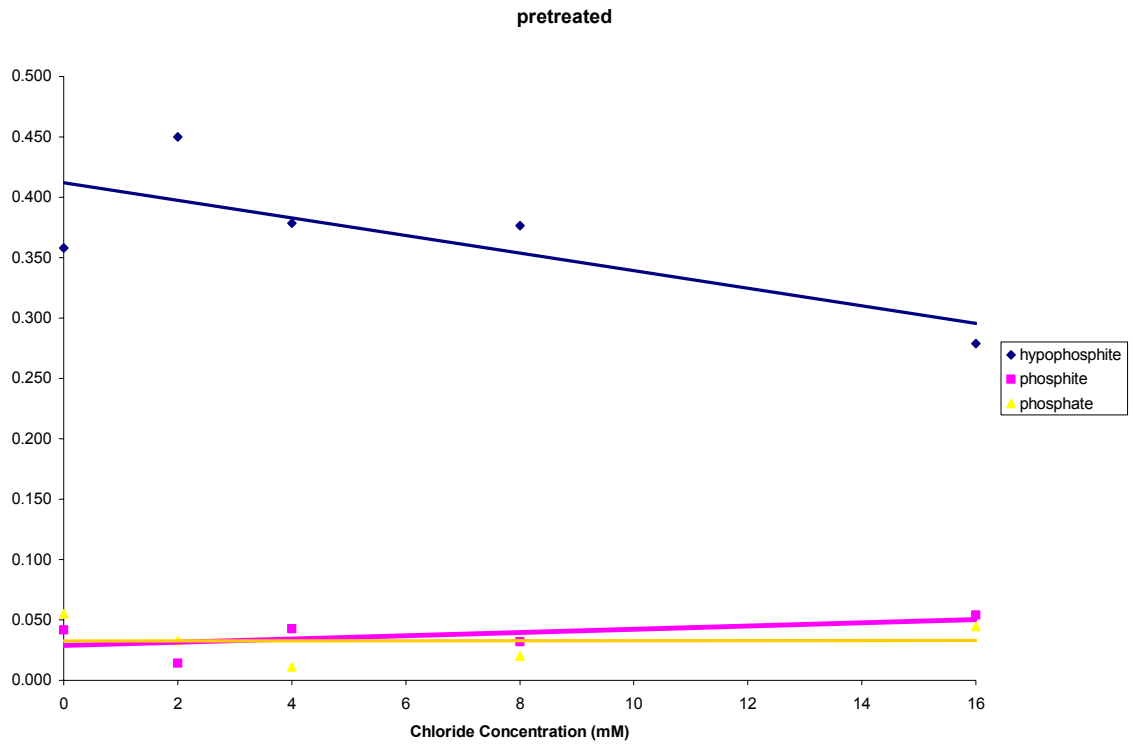

Figure 20: Peak area over chloride concentration with hypophosphite, phosphite and phosphate at concentrations of 10 μM each and 8.1 mM carbonate.

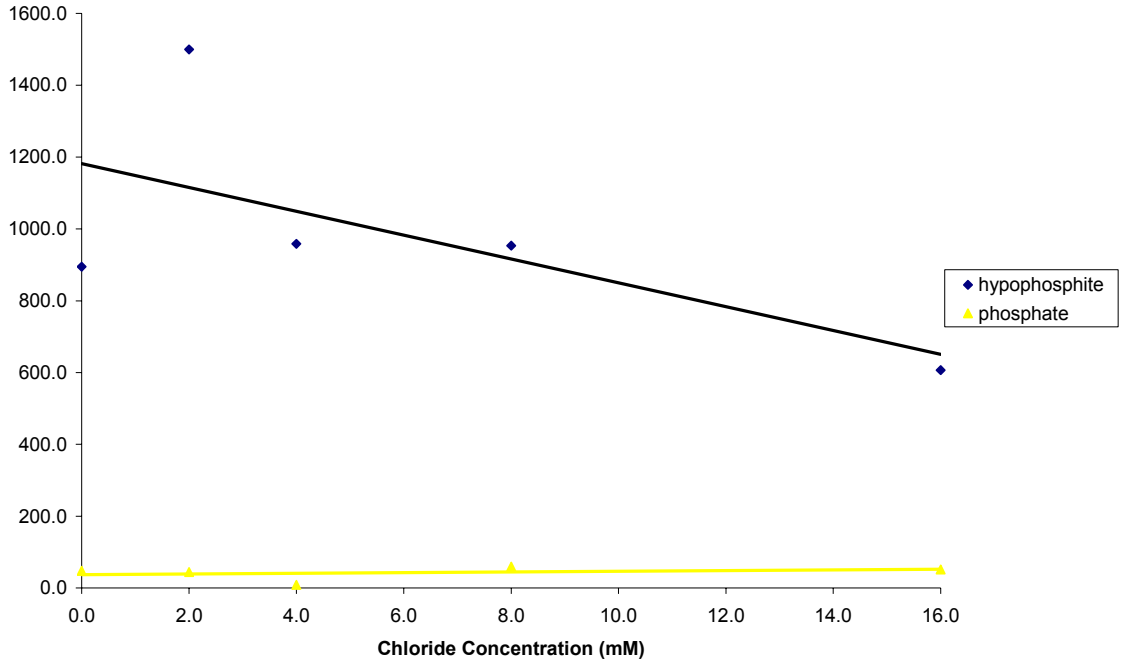

Figure 21: Filtered peak area over unfiltered peak area, with hypophosphite, phosphite and phosphate at concentrations of 10 μM each and 8.1 mM carbonate.

P oxyanions peak area percentages after treatment were analyzed graphically as noted from Fig. 21. Hypophosphite average peak area increased dramatically to 983 ± 323 %. Phosphite peak area after treatment was not analyzed because phosphite peak was unresolved from carbonate peak before treatment. Phosphate peak area decreased to 42.6 ± 19.5 %.

Although hypophosphite peak areas were smaller for untreated trial 4, after treatment the areas for trial 4 are greater than treated trial 3 peak areas.

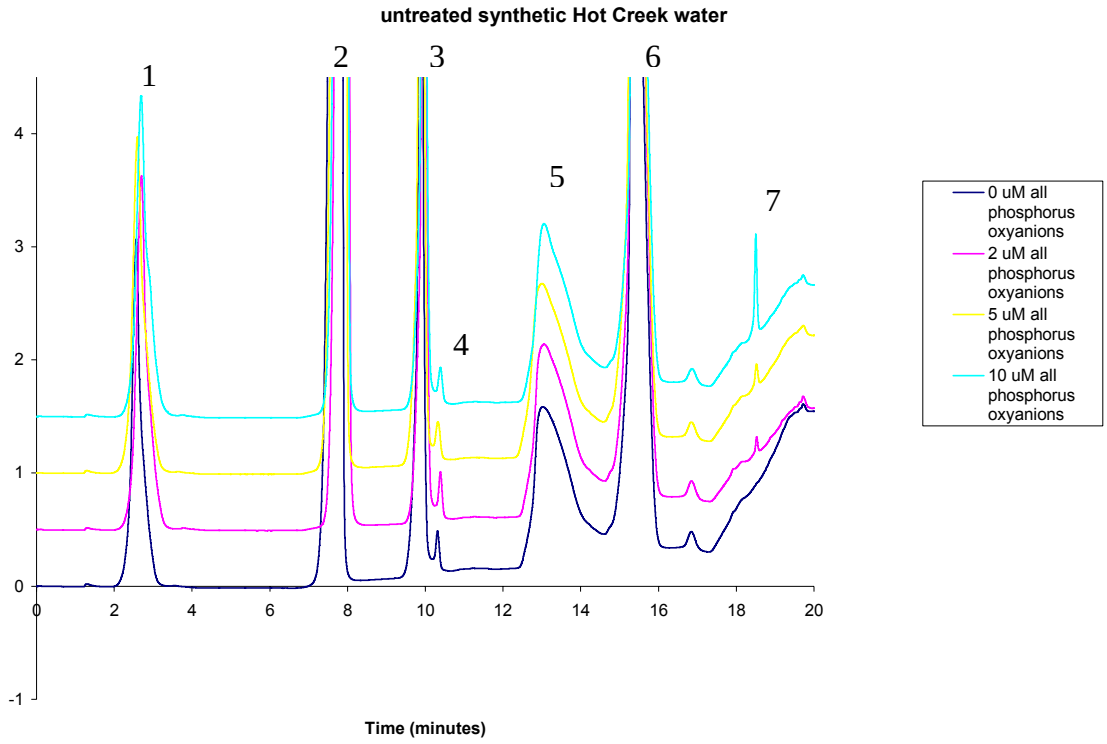

Figure 22: Unfiltered Synthetic Creek water ion chromatogram using 10 μM P oxyanions concentration and all other ions following table 1 concentrations. Peak assignment are (1) fluoride (2) chloride; (3) bromide; (4) nitrate; (5) carbonate; (6) sulfate; (7) phosphate.

Unfiltered Synthetic Creek water ion chromatogram including fluoride is shown in Fig. 22. The solutions were prepared using 0-10 μM P oxyanions concentration and all other ions following table 1 including fluoride. This chromatograms shows that for P submicromolar concentrations as found in Hot Creek water, pretreatment techniques are necessary to separate hypophosphite from fluoride and phosphite from carbonate.

Conclusions:

IC methods described in this study are appropriate for the detection of submicromolar concentrations of P oxyanions. However, hypophosphite and phosphite were only resolved after pretreatment with filtering cartridges when analyzing solutions with increasing chloride concentrations. IC of synthetic geothermal water demonstrated the need for pretreatment to determine the presence of submicromolar concentrations of P oxyanions in solution, since both fluoride and carbonate peaks engulfed hypophosphite and phosphite peaks respectively. Additionally, the use of a smaller injection loop is expected to facilitate peak separation.

Furthermore, pretreatment, with silver cartridges only, caused hypophosphite peak area to increase by 6 $\pm$ 3 %. Additionally, phosphite peak area after treatment increased by 10 $\pm$ 47 %. However phosphate peak area decreased 4.9 $\pm$ 20 %.

Pretreament with a silver cartridge and a sulfonic acid cartridged connected in series caused hypophosphite peak area to increase dramatically by a factor of ten. This technique also caused a decrease in phosphate peak area to 40 % of the area registered before treatment.

Matrix composition prior to treatment with filtering cartridges affected the elution of phosphorus oxyanions during IC. For instance, increasing carbonate to 8.1 mM to simulate Hot Creek water concentration decreased hypophosphite peak area to a third of the peak originated when no additional carbonate was added to solution. Further studies should be carried on to determine the effect of

increasing carbonate concentration on hypophosphite elution while holding the net ion concentration stable, to conclude if the change in areas was due to the increase of carbonate specifically, a net increase of ions in solution or the addition of sulfonic acid cartridge.

Increasing chloride concentration and using silver cartridges demonstrated an increase on hypophosphite retention time. Additionally, area recovery percentage was overall constant with lost of hypophosphite detection as a result of the use of Ag+ filtering cartridge with increasing chloride concentration.

Appendix: Analysis of chromatograms by subtracting blank.

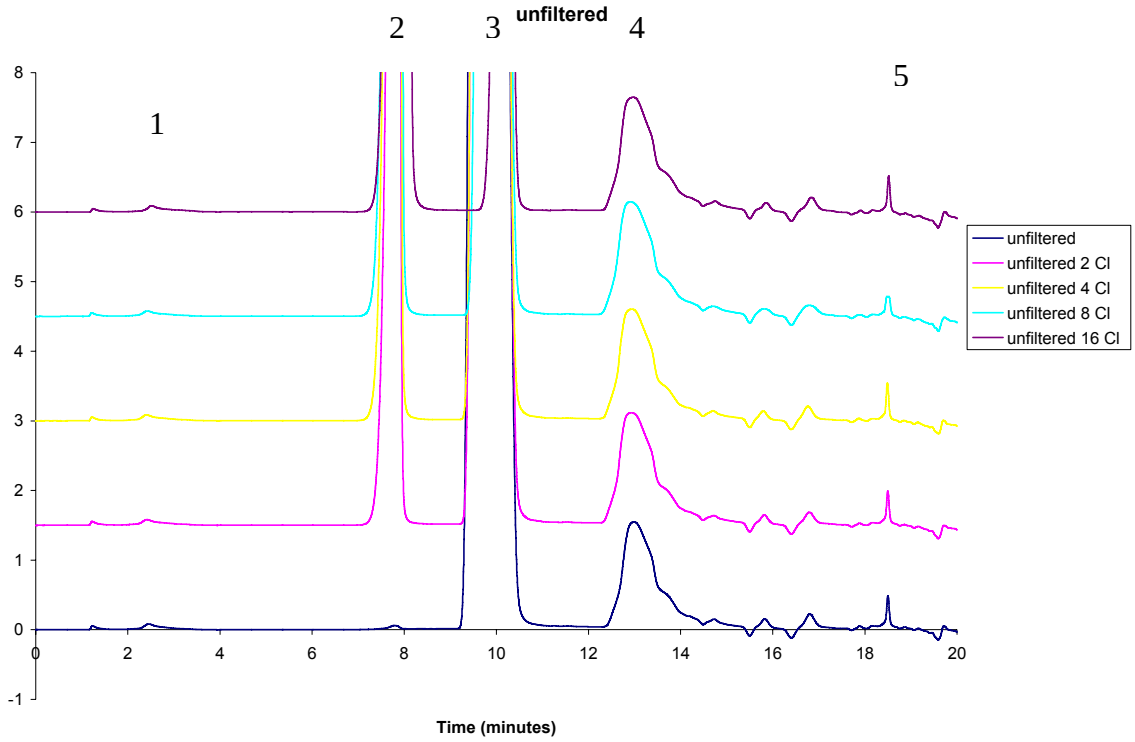

Figure 23: Ion chromatograms for 0-10 mM chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each, adjusted by subtracting blank chromatogram. Peak assignment is (1) hypophosphite; (2) chloride; (3) nitrate; (4) carbonate; (5) phosphate.

To further confirm the validity of peaks and to eliminate redundant data, the blank chromatogram was subtracted from the ion chromatograms for 0-10 mM chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each. Figure 23 illustrates the calculated data and shows a clear phosphate peak eluting at 18.5 min.

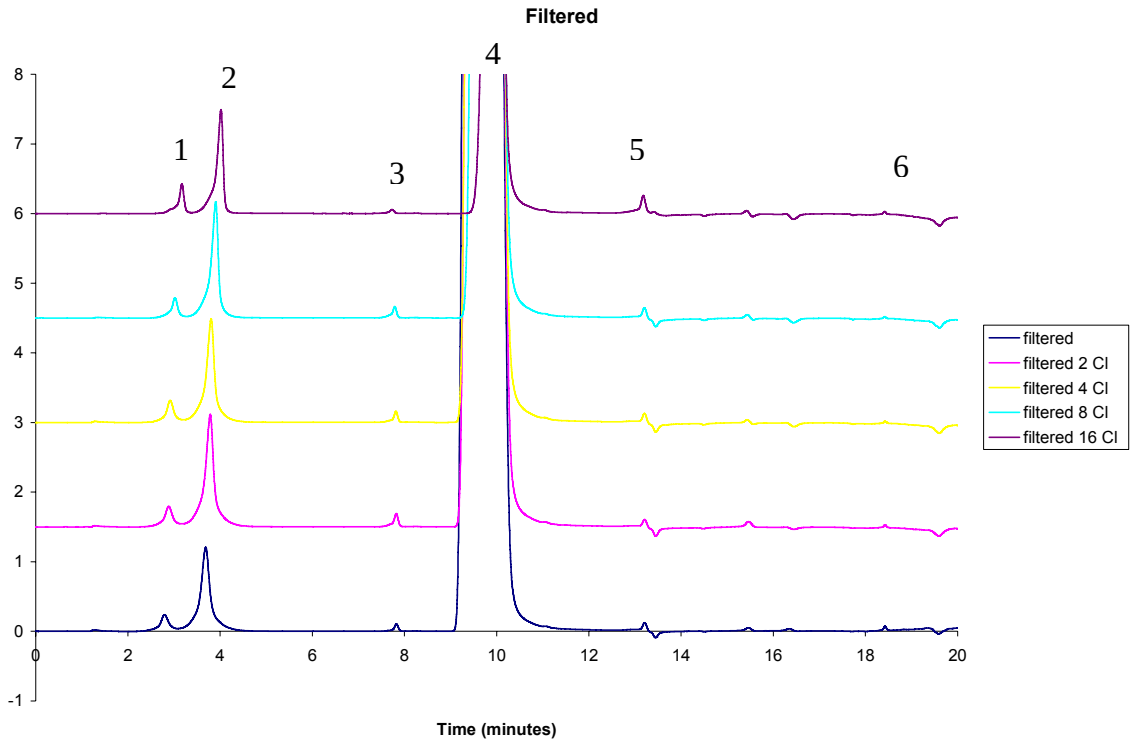

Figure 24: Ion chromatogram for pretreated 0-10 mM chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each, adjusted by subtracting blank chromatogram. Peak assignments are (1) fluoride; (2) hypophosphite; (3) chloride; (4) nitrate; (5) phosphite; (6) phosphate.

To verify sample peaks and to eliminate redundant data, the blanks data was subtracted from the ion chromatograms for 0-10 mM chloride concentrations with hypophosphite, phosphite and phosphate at concentrations of 10 μM each after pretreatment with Ag^+ and H^+ filtering cartridges. Figure 24 illustrates the calculated data. The main advantage of this procedure is the effect over phosphite peaks, by eliminating carbonate peaks from the chromatograms.

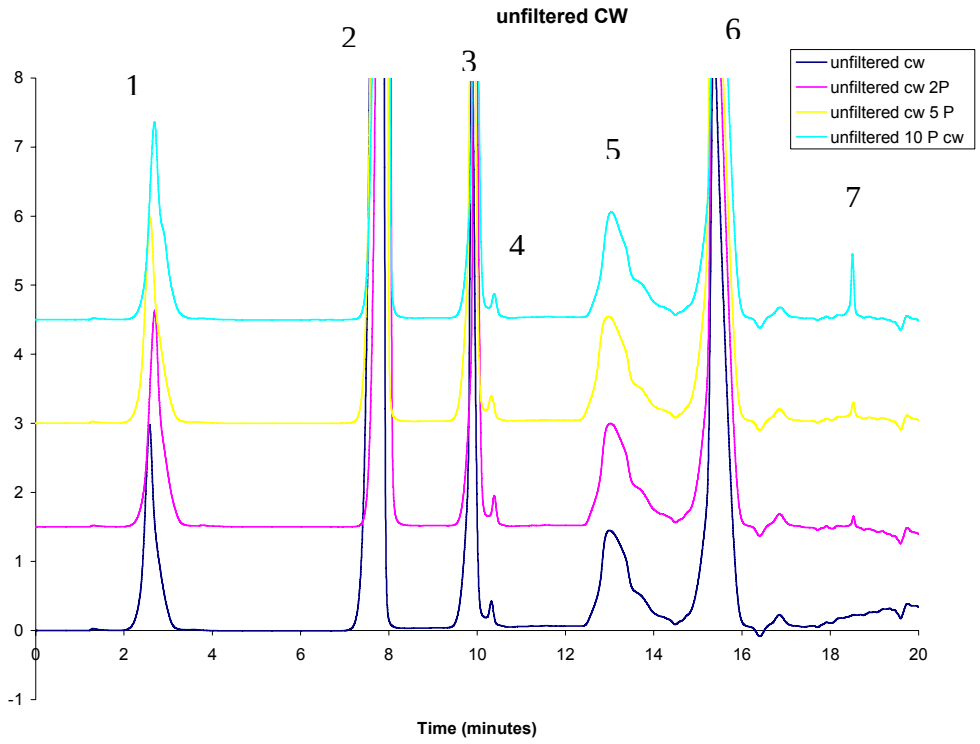

Figure 25: Unfiltered Synthetic Creek water ion chromatogram using 0-10 μM P oxyanions concentration and all other ions following table 1 concentrations, adjusted by subtracting blank chromatogram from data. Peak assignment are (1) hypophosphite; (2) chloride; (3) bromide; (4) nitrate; (5) carbonate; (6) sulfate; (7) phosphate.

Figure 25 illustrates the ion chromatograms of synthetic Hot Creek water detection with increasing concentrations of P oxyanions from 0 to 10 μM. It is important to note that only phosphate was resolved and phosphite and hypophosphite peaks were engulfed by fluoride and carbonate peaks respectively.

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