Ion Chromatographic Determination of Hypophosphite, Phosphite and Orthophosphate Ions along with Chloride, Nitrate and Sulfate Ions Using Indirect Ultraviolet Detection

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The anions H_2PO_2^- , H_2PO_3^- and H_2PO_4^- along with Cl^- , NO_3^- and SO_4^{2-} have been simultaneously determined by single-column ion chromatography in an indirect ultraviolet detection mode. The analytical approach employs 4-amino-2-hydroxybenzoic acid as an eluent at 4 mM in the pH range 5.5–6.5 and at a 2 ml/min flow rate. Clean separations occur on a polymeric analytical column within 10 min, and the anions can be quantified in the sub-ppm range.

Keywords Ion chromatography, indirect ultraviolet detection, hypophosphite, phosphite, orthophosphate, chloride, nitrate, sulfate, 4-amino-2-hydroxybenzoic acid

The aqueous equilibria of the phosphate species is complex and fascinating due to the formation of mono and polymeric species in the system.¹ The oxoanions of the monomeric oxoacids of phosphorus are particularly of environmental concern due to their pronounced fertilizing effect on algal growth in natural water.

Amongst the analytical methods for the quantitation of such oxoanionic species of phosphorus in low concentrations, colorimetric procedures have so far remained popular.² However, the emergence of ion chromatography as a versatile analytical technique (since its introduction by Small *et al.*³) has simplified the analysis of a mixture of anions. The principles and applications of ion chromatography have been reviewed by various authors.^{4,9,15} At present, a single-column approach is gaining wider popularity over the dual-column technique initially suggested, due to operational simplicity. The orthophosphate anion has been analyzed in a variety of samples: water^{5–8}, soil and plant^{10,15}, and food.¹¹ Very little information, however, is available on the quantification of other oxoanions derived from monomeric oxoacids of phosphorus.

There is interest in the quantification of hypophosphite (H_2PO_2^-), phosphite (H_2PO_3^-) and orthophosphate ($\text{H}_2\text{PO}_4^{3-}$) anions in natural aqueous systems. These may generate either through bottom sediment phosphorus exchanges or by interactions of phosphate bearing industrial discharges into bodies of natural water.¹⁴ Tanaka *et al.*¹² attempted to separate oxoanions of phosphorus along with other common anions on a cation exchange column, but obtained poor resolutions. Hirai *et al.*¹³ proposed an elaborous flow

injection scheme incorporating a postcolumn reactor before the detection of such anions. Ryder¹⁴ suggested a single-column ion chromatography procedure for the above-mentioned oxoanions of phosphorus using 20 mM succinic acid as an eluent in the conductivity detection mode. More recently, ultraviolet detection in ion chromatography is gaining wider acceptance due to increased sensitivity and selectivity.^{4,9} The species of interest have been determined in both direct and indirect UV detection modes.

The present communication presents a single-column ion chromatographic procedure for the simultaneous analysis of monomeric oxoanions of phosphorus (H_2PO_2^- , H_2PO_3^- , H_2PO_4^-) along with Cl^- , NO_3^- and SO_4^{2-} anions. The eluent, 4-amino-2-hydroxybenzoic acid, in an indirect ultraviolet mode offers sensitive signal changes in the presence of these anions.

Experimental

The ion chromatographic system comprised a Perkin Elmer LC-10 pump with a Rheodyne-7125 injector valve and a variable-wavelength detector (Perkin Elmer LC-90) sensitive to 0.005 a.u. The chromatographic data were recorded graphically utilizing a 10-mV output pulse from the detector. The signal from the detector was interchanged regarding polarity on the recorder, so as to invert chromatograms which appeared in an easily interpretable form. Separations were performed on a low-capacity polymeric analytical column, Hamilton PRP×100 (150×4.6 mm i.d.), fitted with a 50-μl sample

loop. A precolumn of the same material served as a guard column.

Reagents and eluents

Standard solutions of anions (1000 ppm) were prepared by dissolving appropriate amounts of analytical-grade sodium or potassium salts in water previously purified by a Millipore Milli-Q System.

Working standards were prepared daily by dilution of the stock.

Eluent solutions of 4-amino-2-hydroxybenzoic acid (2–6 mM) were prepared by directly weighing the requisite amounts in 2 l of purified water. The pH of the eluents was adjusted by a dropwise addition of 2 M NaOH. The eluent solutions in all cases were filtered through a 0.45 μm filter (Millipore HAW 804700) before setting the final volume in 2-l standard flasks. Each eluent was degassed for at least 20 min with helium before introduction into the liquid chromatograph. The separations were essentially carried out at a flow rate of 2 ml/min in order to avoid any excessive pressure build up on the analytical column. Each sample, in turn, was filtered through a 0.22- μm filter before injection in order to avoid column contamination.

The experimental approach involved an initial optimization of the pH, flow rate and eluent concentration. At a flow rate of 2 ml/min the eluent at 4 mM offered convenient separations of the ions of interest in the pH range 5.0–7.0. These parameters were thereafter maintained in the quantitation studies.

Results and Discussion

Separation of hypophosphite, phosphite and phosphate ions using a 4-mM eluent in the pH range 5.5–6.5 occurs smoothly within a relatively short time period. A typical chromatogram is shown in Fig. 1. All three ions elute with sharp peaks and maintain the following order: hypophosphite (H_2PO_2^-), phosphate (H_2PO_4^-) and phosphite (H_2PO_3^-). Under more acidic conditions ($\text{pH} < 5$) chromatographic peaks are somewhat distorted and the resolution is drastically reduced. This behavior apparently suggests that the eluent efficacy increases along with its dissociation. The first ionization constant of the eluent ($\text{p}K_1=4.31$) determined in this study suggests that it is fully dissociated around pH 6.30; further, in this pH range it should function most effectively. This is, in fact, supported experimentally since not only the oxoanions of phosphorus, but other monovalent and divalent anions, can be chromatographed and detected successively using an indirect ultraviolet detection mode. The adjusted retention times of the separated ions are shown in Table 1. Clean separation of phosphorus oxoanions in the presence of commonly occurring Cl^- , NO_3^- and SO_4^{2-} ions is of environmental interest. A typical chromatogram shown in Fig. 2 depicts the utility of this

Fig. 1 Separation of hypophosphite, phosphite and orthophosphate anions. Eluent: 4 mM, pH 6.5, 2 ml/min. 1, H_2PO_2^- , 5 ppm; 2, H_2PO_4^- , 5 ppm; 3, H_2PO_3^- , 5 ppm.

Table 1 Adjusted retention times of hypophosphite, phosphite, orthophosphate and other common anions

Ion	Adjusted retention time/min
H_2PO_2^-	1.1
H_2PO_4^-	2.7
H_2PO_3^-	4.6
F^-	0.8
Cl^-	1.5
NO_3^-	3.2
SO_4^{2-}	7.8
$\text{S}_2\text{O}_3^{2-}$	13.2

Eluent: 4 mM, pH 5.8, 2 ml/min.

procedure.

Since the oxoanions of phosphorus elute early, it is inferred that all of these behave as monovalent species in the system. The first and second ionization constants of the parent compounds (H_3PO_2 : $\text{p}K_1=1.12$; H_3PO_3 : $\text{p}K_1=1.20$, $\text{p}K_2=6.70$; H_3PO_4 : $\text{p}K_1=2.21$, $\text{p}K_2=7.18$) also predict that in the pH range 6.3–6.5 the species H_2PO_2^- , H_2PO_3^- and H_2PO_4^- , respectively predominate the equilibria. This monovalent behavior, in turn, justifies their early elution like other monovalent anions (e.g. halides), though the elution of H_2PO_4^- before H_2PO_3^- is somewhat surprising. The analytical linear range (cf. Fig. 3) and instrumental quantification limits for the analyzed anionic species are shown in Table 2. In order to avoid any column overloading, the linearity was tested only up to 20 ppm in all but one case. The instrumental quantification limit, defined as a peak height response that is three times the average background level, occurs at 0.5, 1.25 and 1.5 ppm for

Fig. 2 Simultaneous separation of hypophosphite, phosphite, phosphite and orthophosphate anions along with chloride, nitrate and sulfate ions, with 4 mM eluent at pH 5.8 and 2 ml/min. 1, H_2PO_2^- , 10 ppm; 2, Cl^- , 10 ppm; 3, H_2PO_3^- , 10 ppm; 4, NO_3^- , 10 ppm; 5, H_2PO_4^- , 10 ppm; 6, SO_4^{2-} , 20 ppm.

Fig. 3 Analytical response curves for hypophosphite, phosphite and orthophosphate under optimized experimental conditions.

Table 2 Quantification limits for anions

Anion	Linear range, ppm	Quantification limit, ppm
H_2PO_2^-	0.5 - 20	0.5
H_2PO_3^-	1.25 - 20	1.25
H_2PO_4^-	2.50 - 20	1.5
Cl^-	0.5 - 10	0.3
NO_3^-	1.25 - 20	1.25
SO_4^{2-}	2.0 - 60	2.0

Eluent: 4 mM, pH 6.0 at 2 ml/min.

H_2PO_2^- , H_2PO_3^- and H_2PO_4^- ions, respectively. Based on a sample loop of 50 μl used in the experiment, the absolute weight amounted to 0.025, 0.0625 and 0.125 μg , respectively, per injection for these ions. The lowest value in the linear analytical range data in Table 2 represents the lowest concentration where graphical linearity is maintained, which may not coincide with the instrumental quantification limit.

The anions Cl^- , NO_3^- and SO_4^{2-} are also quantifiable in both the ppm and sub ppm range. As can be seen for other ion chromatographic eluents, the sensitivity decreases as $\text{Cl}^- > \text{NO}_3^- > \text{SO}_4^{2-}$ with 4-amino-2-hydroxybenzoic acid.

However, the quantification limits can be further improved by using a larger sample loop (e.g. 500 μl) or making use of preconcentration chemistry before column injection.

Samples of both local drinking water and natural surface water were analyzed for anionic contaminants, including phosphate ions, by this method. Only Cl^- , NO_3^- and SO_4^{2-} were detected. In view of the environmental importance of the phosphorus species, a quantitative recovery of each phosphorus oxoanions was ascertained by spiking natural water with a known concentration. A 10-ppm phosphorus oxoanion spiked solution in Millipore Milli-Q purified water was in each case adjusted to pH $\approx$ 6.0 and chromatographed repeatedly ($n=6$); the average peak height observed for a species served as the standard. Individually spiked lake water samples were initially filtered through a C-18 Sep Pak cartridge in order to eliminate any organics; they were thereafter chromatographed according to the above-described procedure. The comparative data for the average peak heights (standard and sample) show a 95% or better recovery with a relative standard deviation of less than 2.9% for H_2PO_2^- and H_2PO_4^- anions; it is close to 90% for H_2PO_3^- , with a relative standard deviation of about 4%. Thus, routine monitoring of phosphorus oxoanions contamination in natural water can be envisaged using this approach. This procedure can, and will, be adapted for other sample types associated with similar phosphorus species.

The fact that the eluent absorbs strongly at 322 nm is useful in the indirect UV detection mode. However, variations up to ± 3 nm are observed whenever the pH or concentration is modified. Therefore, the best wavelength for analytical observations was selected every time the experimental conditions were modified. The 4-amino-2-hydroxybenzoic acid ($pK=4.31$) is somewhat stronger than 4-aminobenzoic acid ($pK=4.92$) and 4-hydroxybenzoic acid ($pK=4.48$). The last species has been recommended as an eluent in many ion chromatographic separations using conductivity detection.⁸ In this respect this study presents a novel use of 4-amino-2-hydroxybenzoic acid as an efficient eluent for the ion chromatographic analysis of monomeric oxoanions of phosphorus along with Cl^- , NO_3^- , and SO_4^{2-} in an indirect UV detection mode. In fact, the analytical sensitivity compares favorably with other eluents used

for phosphorus oxoanions separations by ion chromatography. The single column ion chromatographic conductivity detection approach¹⁴ required 20-mM succinic acid strength to elute H_2PO_2^- , H_2PO_4^- and H_2PO_3^- ions within about 18 min. The proposed eluent (in this context) is more sensitive, since a much lower concentration (4 mM) is required for separating the described phosphorus oxoanions along with the Cl^- , NO_3^- and SO_4^{2-} which occur within 10 min.

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