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DETERMINATION OF SELENATE BY SINGLE-COLUMN ION CHROMATOGRAPHY

U. KARLSON and W. T. FRANKENBERGER, Jr.*
Department of Soil & Environmental Sciences, University of California, Riverside, CA 92521 (U.S.A.)
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SUMMARY

Chromatographic parameters were optimized to determine selenate (SeO_4^{2-}) by single-column ion chromatography with the simultaneous detection of Cl^-, NO_2^-, NO_3^- and SO_4^{2-}. Separation was conducted on a low-capacity anion-exchange column, and anions were quantified by conductimetric detection. The eluent stream consisted of 4 m*M* phthalic acid adjusted to pH 4.6 with sodium borate. Accurate measurement of trace amounts of SeO_4^{2-} was evident in the presence of high background levels of other ions including Cl^-, NO_3^- and up to 40 mg l^{-1} SO_4^{2-}. Resolution of SeO_4^{2-} and SO_4^{2-} was demonstrated up to 80 mg l^{-1} SO_4^{2-}. The detection limit of SeO_4^{2-} was 18 μg l^{-1} with a relative standard deviation of 1.46%.

INTRODUCTION

Recently the interest in the chemistry of selenium in water and soil environments has increased dramatically[1,2]. Selenate (SeO_4^{2-}) is considered to be the major inorganic selenium species in alkaline soils of regions with low rainfall. It is available for plant uptake and is readily leached into groundwater[2,3]. In the Central Valley of California, selenium levels have been reported up to 3800 μg l^{-1} in the groundwater[2].

Methods currently used for determination of selenium in biological and environmental samples include: UV spectrophotometry[4–6], fluorometry[7–10], atomic absorption spectrometry (AAS)[11–14], inductively coupled plasma emission (ICP)[15–18], gas chromatography (GC)[19–22], neutron-activation analysis (NAA)[23,24], X-ray fluorescence spectrometry[25], voltammetry[26], and suppressed ion chromatography (SIC)[27,28]. Many of these methods require organic solvent extraction, a prereduction step and preconcentration to obtain reasonable limits of detection (LODs). Because of their specificity for SeO_3^{2-}, acid reduction is required by most methods. After quantifying total selenium by prereduction, SeO_4^{2-} is estimated by subtraction. In hydride generation–AAS, oxidative digestion ($KMnO_4$) of the sample prevents organic interferences but this treatment destroys the natural distribution of selenium speciation[29]. The hydride generation technique is also subject to interferences between the hydride-forming elements and matrix elements such as iron and copper[16].

Among the chromatographic methods, SIC has been used as a means of determining SeO_4^{2-}, but interfering ions can easily mask its signal response. High concentrations of sulfate (SO_4^{2-}) readily interfere with detection of SeO_4^{2-} by SIC because of limitations in column selectivity and choice of eluents imposed by suppressor column compatibility[14,27]. To counter these interferences, SIC with UV detection has been reported since sulfate does not show appreciable absorption above 190 nm, but the LOD for SeO_4^{2-} was only 15 mg l^{-1} [28]. SIC has also been interfaced with AAS[14,29] but this system is only applicable to single element selectivity[18]. High costs of SIC–AAS and high-performance liquid chromatographic (HPLC)–ICP[18] instrumentation precludes their use for routine analyses.

The objective of this study was to develop a novel single-column ion chromatographic (SCIC) method for the determination of SeO_4^{2-} in aqueous soil extracts. The advantages in SCIC with conductimetric detection for analysis of SeO_4^{2-} over the other existing methods include: (i) single column simplicity and low costs in maintenance; (ii) direct determination of SeO_4^{2-} speciation; (iii) increased resolution over SIC due to the diverse column selectivity and choice of eluents; (iv) a rapid and routine analysis; (v) minimal sample pretreatment; (vi) high precision and high sensitivity; and (vii) simultaneous determination of other ions of interest. There appears to be very little work regarding HPLC analysis of soil samples[31]. To our knowledge, SCIC of selenium has not been reported. This study provides a routine analytical method for the detection of trace levels of SeO_4^{2-} in soils with high accuracy and precision.

EXPERIMENTAL

Chromatographic instrumentation

HPLC analysis was performed on a Beckman Model 332 liquid chromatograph, equipped with a Model 110A pump and a Model 210 sample injector. The SCIC system was composed of the following: a Vydac 3021C4.6 (The Separations Group, Hesperia, CA, U.S.A.) anion-exchange column (250 × 4.6 mm I.D.; batch No. 830616), a Wescan (Santa Clara, CA, U.S.A.) 269-003 ion-guard column (40 × 4.6 mm I.D.), an Eldex Model III thermostat column heater, a Wescan Model 213 conductivity detector, and a Hewlett-Packard Model 3390A printer–plotter integrator with variable input voltage. Sample injection loops of 100, 500, and 2000 μl were employed to test LODs.

Eluents and standards

The mobile phase consisted of phthalic acid (Mallinckrodt, St. Louis, MO, U.S.A.) prepared in 2.0–9.0 m*M* solutions adjusted to pH values between 4.2 and 5.1 with sodium borate. The flow-rate was 2 ml min^{-1}, the column inlet pressure was *ca.* 70 bar (1000 p.s.i.), the column temperature was 27°C, and the detector output was 10 mV. Column conditioning procedures are provided elsewhere[30].

All reagents were analytical grade. Analyte solutions were prepared by dissolving sodium dihydrogen phosphate monohydrate and potassium sulfate (Baker, Phillipsburg, NJ, U.S.A.), sodium nitrite and sodium chloride (Mallinckrodt, Paris, KY, U.S.A.), sodium selenate (Alfa, Denvers, MA, U.S.A.) and potassium nitrate (Aldrich, Milwaukee, WI, U.S.A.) in HPLC grade water. HPLC grade water was obtained by filtering deionized water through the sequence: HN Organic removal resin (Barnstead, Boston, MA, U.S.A.), HN Ultrapure DI exchange column (Barnstead), and a 0.22-μm Millipore GS filter. The selenium standards were made fresh from standard stock on a daily basis. The stock concentration was verified by hydride generation–AAS. All analytes were determined on an elemental basis.

Sulfate removal

Attempts were made to eliminate SO_4^{2-} interferences by precipitation with barium formate or reaction with a Ba^{2+}-loaded resin. Barium formate was synthesized by titrating 2875 mg l^{-1} of barium hydroxide with formic acid to pH 4.6. The barium saturated resin was composed of a strongly acidic cation exchanger (Dowex 50W-X8) loaded with barium chloride (0.73 g /g resin). Samples were shaken with the resin for 3 min and then injected through a 0.22-μm membrane filter for SeO_4^{2-} analysis.

Field samples

Soil extracts of Panoche clay loam (CL) (Typic Torriorthent) and Los Banos CL (Typic Haploxeralf) were prepared by adding 50 ml of deionized water to 10 g of soil, shaking for 1 h, and filtering the suspension through Whatman No. 42 filter paper. The filtrate was then passed through a 0.22-μm Millipore GS filter and introduced directly into the HPLC injector port. Simultaneous determination of Cl^-, NO_2^-, NO_3^- and SO_4^{2-} was also possible with the selected mobile phase in the described SCIC analysis. For comparative purposes, SeO_4^{2-} analysis by SCIC was verified by hydride generation–AAS and –ICP. Reduction of SeO_4^{2-} to SeO_3^{2-} with hydrochloric acid is a required preparatory step for the latter two methods.

Atomic absorption spectrometry

A Perkin-Elmer 5000 atomic absorption spectrophotometer equipped with a hollow cathode selenium lamp and a MHS-10 hydride generation system was employed. Samples were treated with 4 *M* hydrochloric acid to generate hydrogen selenide upon the addition of 3% sodium borohydride in 1% sodium hydroxide. The operational conditions consisted of the following: argon stripping gas, 70 cm^3 min^{-1}; burner gases, 10 l min^{-1} air and 15 l min^{-1} acetylene; lamp current, 8 mA; wavelength, 196.0 nm; slitwidth, 0.7 nm.

Inductively coupled argon plasma emission spectrometry

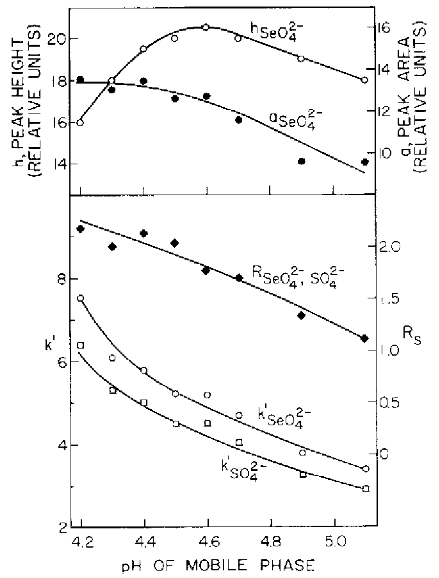
A Jarrell-Ash Atom Comp 800 ICP coupled with a custom-designed continuous flow hydride-generating system was used. The operational conditions were as follows: torch, fassel type; power forward, 1.75 kW; viewing height, 25 mm above blue tip of yttrium flame; coolant gas flow-rate, 16 l min^{-1} of argon; integration time, 17 s; pre-integration, 15 s. Generation of hydrogen selenide involved the following parameters: sample acidity, 6 *M* hydrochloric acid; sample flow-rate, 10.5 ml min^{-1}; reagent concentration, 1% sodium borohydride in 0.1 *M* sodium hydroxide; reagent flow-rate, 2.7 ml min^{-1}; carrier gas flow-rate, 0.8 ml min^{-1}; sample size, 25 ml.

RESULTS AND DISCUSSION

Optimization of mobile phase

Since the chemistry of SeO_4^{2-} and SO_4^{2-} is very similar, chromatographic parameters were assessed which would optimize resolution and detection of these two solutes. Fig. 1 illustrates that an increase in eluent pH (4 m*M* phthalic acid) decreased the k values (capacity factor) and R_s (column resolution) of SeO_4^{2-} and SO_4^{2-}. The solutes were not retained with the higher eluent pH. Improved separation was observed with higher k values upon decreasing the eluent pH, which also led to slightly longer analysis times. R_s between SeO_4^{2-} and SO_4^{2-} was relatively high ($R_s > 1.5$) when the eluent pH was less than 4.8. The optimum eluent pH range for detection of SeO_4^{2-} was considered to be from pH 4.4 to 4.8 since column efficiency of HPLC is generally greatest in the k' range 3–6. In terms of signal response, peak area of SeO_4^{2-} was somewhat constant from pH 4.2 to 4.4 and decreased thereafter, whereas peak height showed a dramatic maximum signal at pH 4.6 (Fig. 1). Because of this maximum signal in peak height, high R_s (1.8) and rapid elution ($k' = 5.0$), the eluent pH of 4.6 was selected to be optimum for determination of SeO_4^{2-} by SCIC.

The k', R_s and α (selectivity coefficient) decreased with increasing eluent concentration (Fig. 2); however, peak height increased, while peak area showed a linear decrease. Phthalic acid at 4.0 m*M* was selected as best suited for the SeO_4^{2-} analysis since it provided the optimum combination of parameters: $R_s = 2.0$, the signal re-



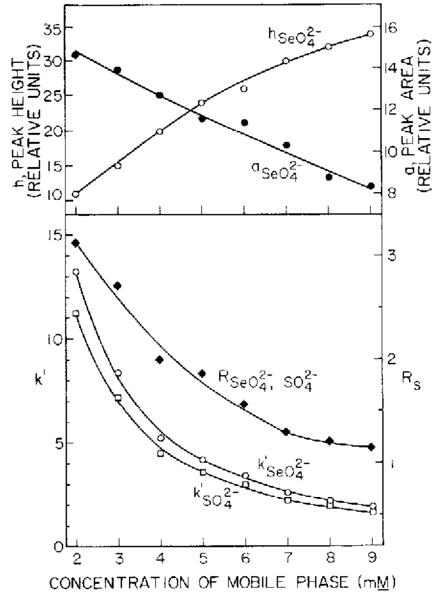


Fig. 1. Dependence of capacity factors, resolution and signal response of SeO_4^{2-} on eluent pH. Column, Vydac 302 IC (250 × 4.6 mm I.D.); eluent, 4 m*M* phthalic acid; flow-rate, 2 ml min^{-1}; sample size, 500 μl.

Fig. 2. Dependence of capacity factors, resolution and signal response of SeO_4^{2-} on the concentration of the mobile phase (phthalic acid, pH 4.6); other chromatographic conditions as Fig. 1.

TABLE I

PRECISION OF SCIC ANALYSIS AT VARYING ANION CONCENTRATIONS*

Column, Vydac 302 IC (250 × 4.6 mm); eluent, 4 m*M* phthalic acid, pH 4.6; detection, conductivity.

Sample injection	*Parameters*	*Anion*				
		Cl^-	NO_2^-	NO_3^-	SO_4^{2-}	SeO_4^{2-}
500 μl	Concentration (mg l^{-1})	5	2	2	20	5
	Relative standard deviation (%)	1.13	1.76	0.99	0.21	1.46
2000 μl	Concentration (μg l^{-1})	200	60	60	60	200
	Relative standard deviation (%)	18.0	8.9	9.7	17.2	13.5

* Based on ten injections of a standard.

sponse in both peak height and area were relatively high, and the migration rate (with $k' = 5.0$) was rapid enough for routine analyses.

Precision

The precision of the SCIC method for the analysis of SeO_4^{2-} in addition to Cl^-, NO_2^-, NO_3^- and SO_4^{2-} was tested by repeated (ten) injections made of standards ranging in concentration from 2 to 200 mg l^{-1} (Table I). Utilizing a 500-μl loop, the relative standard deviations ranged from 0.21 to 1.76% (SeO_4^{2-} = 1.46%).

Detection limits

For determination of SeO_4^{2-} by SIC using a 600-μl loop, LODs as low as 8 μg l^{-1} with standards, and 15–25 μg l^{-1} in groundwater had been reported[25]. The LOD by SCIC was calculated as the three-fold signal-to-noise ratio of the baseline (S/N = 3). Since the proposed SCIC method eliminates the need for a suppression system, reduced band spreading allows the use of a larger sample loop resulting in similar detection limits (Table II) as SIC. A comparable LOD range was reported with SIC–AAS[14]. Chromatographic conditions were not modified to increase the

TABLE II

DETECTION LIMITS OF SCIC ANALYSIS*

Chromatographic conditions as in Table I.

Sample injection	*Detection limit (μg l^{-1}) for:*				
	Cl^-	NO_2^-	NO_3^-	SO_4^{2-}	SeO_4^{2-}
100 μl	98	36	37	108	320
500 μl	31	7	5	16	65
2000 μl	9	3	1	4	18

* Assumed to be three-fold the signal-to-noise ratio of the baseline (S/N = 3).

sensitivity of the other inorganic anions since the emphasis of this report was to optimize the mobile phase for detection of SeO_4^{2-}.

Calibration plots

Calibration plots of peak area *vs.* solute ion concentration were linear for SeO_4^{2-}, Cl^{-1}, NO_2^-, NO_3^- and SO_4^{2-} ranging from 0.08 to 5 mg l^{-1}. A typical calibration plot for SeO_4^{2-} followed the equation of peak area $= 1.65\ C_{SeO_4^{2-}} - 0.03$; $r = 0.999$ ($p < 0.001$).

Interferences

The R_p between SeO_4^{2-} and SO_4^{2-} is highly concentration dependent. Chakraborti *et al.*[14] reported that only 0.3 mg l^{-1} of SO_4^{2-} reduced the SeO_4^{2-} signal by 5% with SIC. In another study with SIC (600-µl loop), poor resolution ($R_p = 0$) was reported for SeO_4^{2-} standards in the presence of a 50 mg l^{-1} SO_4^{2-} background[27]. In this work, we achieved an R_p of 100% (500-µl loop) when up to 80 mg l^{-1} of SO_4^{2-} was present (Table III). However, recovery of SeO_4^{2-} was reduced to 58% at this SO_4^{2-} level. Stoichiometric addition of barium formate increased the R_p from 33 to 100% in the presence of 100 mg l^{-1} of SO_4^{2-}, but did not improve the apparent recovery of SeO_4^{2-}. Similar results were obtained with the Ba^{2+}-loaded resin in quantities of $\geq$ 80 mg ml^{-1} of SO_4^{2-}. Thus SCIC provides a quantitative analysis of SeO_4^{2-} with background levels up to 40 mg l^{-1} of SO_4^{2-}. There were no observed interferences in the detection of SeO_4^{2-} by SCIC as a result of high levels of Cl^- and NO_3^- in the saline soil extracts.

Comparative methods of detection

A close relationship was observed between our SCIC method (X) and AAS (Y) and ICP (Y) using soil samples spiked with SeO_4^{2-} (200 and 500 µg l^{-1}). This relationship can be expressed as follows: $Y_{ICP} = 1.00X_{SCIC} + 3.9$, $r = 0.997$ ($p < 0.001$) and $Y_{AAS} = 0.70X_{SCIC} + 114$, $r = 0.992$ ($p < 0.001$). The unity in slope, particularly of

TABLE III

RESOLUTION AND APPARENT RECOVERY OF SeO_4^{2-}

Chromatographic conditions as in Table I.

SO_4^{2-} background (mg l^{-1})	*Practical resolution, R_p* (%)*	*Apparent SeO_4^{2-} recovery (peak area, %)*
0	100	100
40	100	100
60	100	81
80	100	58
90	55	46
100	33	21
150	0	0

* $R_p = (1 - A/B)\ 100$, where A is the distance of the valley between SO_4^{2-} and SeO_4^{2-} from the baseline, and B designates the peak height of SeO_4^{2-}. R_p was monitored with standards containing 3 mg l^{-1} SeO_4^{2-} in the presence of SO_4^{2-} ranging from 0 to 150 mg l^{-1}.

TABLE IV

COMPARISON OF SeO_4^{2-} VALUES IN AQUEOUS SOIL EXTRACTS DETERMINED BY SCIC, ICP AND AAS

Chromatographic conditions as in Table I. Figures in parentheses indicate percent recovery. ND = Not detected.

Soil	*SCIC**	*ICP* ($\mu g\ l^{-1}$)	*AAS*
Panoche CL, native	N.D.	2	N.D.
Spiked, 200 $\mu g\ l^{-1}$	201 (101)	202 (101)	235 (118)
Spiked, 500 $\mu g\ l^{-1}$	442 (88)	467 (93)	420(84)
Los Banos CL, native	N.D.	4	N.D.
Spiked, 200 $\mu g\ l^{-1}$	154 (77)	159 (80)	241 (121)
Spiked, 500 $\mu g\ l^{-1}$	538 (108)	534 (107)	498 (97)

* Based on four 2000-μl injections.

the former regression equation (ICP) indicates excellent agreement with SCIC. Recovery of SeO_4^{2-} in soil extracts by SCIC ranged from 77 to 108% (Table IV).

Soil analysis

A typical SCIC chromatogram for the aqueous extract of a soil sample is shown in Fig. 3. Solutes were separated into well defined peaks with a time of analysis of 24 min. Phosphate and SeO_3^{2-} were not retained under these chromatographic conditions. Calculations with external standards indicated that this soil extract contained 6.4 $\mu g\ l^{-1}$ Cl^-, 47 $\mu g\ l^{4-1}$ NO_2^-, 2.2 mg l^{-1} NO_3^-, 1.5 mg l^{-1} SO_4^{2-} and 538 $\mu g\ l^{-1}$ SeO_4^{2-}.

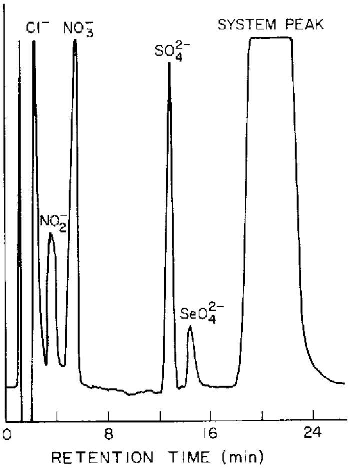


Fig. 3. SCIC chromatogram of a soil extract. Eluent, 4 m*M* phthalic acid, pH 4.6; other chromatographic conditions as Fig. 1, except 2000-μl sample size.

CONCLUSIONS

Some of the obvious advantages in the use of the SCIC method developed for the detection of SeO_4^{2-} over AAS, ICP and SIC include:

(1) Low detection limit without a large sample size requirement eliminating possible errors through dilutions;

(2) Direct determination of SeO_4^{2-} without the necessity of converting SeO_4^{2-} into SeO_3^{2-};

(3) The analysis allows greater amounts of SO_4^{2-} to be present in biological samples without interference in resolution and recovery of SeO_4^{2-} when compared to SIC;

(4) High background levels of Cl^- and NO_3^- in soil extracts showed no interferences in the SCIC analysis of SeO_4^{2-};

(5) SCIC proves to be a low cost, rapid and accurate routine method in the quantification of SeO_4^{2-} with the simultaneous determination of other inorganic anions.

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