



Isolation of selenate from selenite, carbonate, phosphate, and arsenate solutions for $\delta^{18}\text{O}$ -selenate determination

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ABSTRACT

Selenium and oxygen isotope systematics can be useful tools for tracing sources and fate of Se oxyanions in water. In order to measure $\delta^{18}\text{O}$ values of selenate, SeO_4^{2-} must first be sequestered from water by precipitation as $\text{BaSeO}_4(\text{s})$. However, other dissolved oxyanions insoluble with Ba^{2+} require removal. Dissolved selenate was separated from dissolved selenite, carbonate, phosphate, and arsenate by addition of Ce^{3+} cations that quantitatively removed these oxyanions by precipitation as insoluble $\text{Ce}_2(\text{SeO}_3)_3(\text{s})$, $\text{Ce}_2(\text{CO}_3)_3(\text{s})$, $\text{CePO}_4(\text{s})$, and $\text{CeAsO}_4(\text{s})$, respectively. $\delta^{18}\text{O}$ -selenate ($-8.19 \pm 0.17 \text{ ‰}$) did not change after four replicates of selenite removal by $\text{Ce}_2(\text{SeO}_3)_3(\text{s})$ precipitation and Ce^{3+} removal by cation exchange (-8.20 ± 0.14 , -8.32 ± 0.09 , -8.17 ± 0.13 , and $-8.29 \pm 0.13 \text{ ‰}$). $\delta^{18}\text{O}$ -selenate values ($-10.86 \pm 0.45 \text{ ‰}$) were preserved also when selenate was pre-concentrated on anion exchange resin, quantitatively retrieved by elution, and processed with Ce^{3+} to remove interfering oxyanions ($-10.77 \pm 0.07 \text{ ‰}$). The extraction and purification steps developed here successfully isolated dissolved selenate from interfering oxyanions while preserving $\delta^{18}\text{O}$ -selenate values. This method should be useful for characterizing $\delta^{18}\text{O}$ -selenate when present with the co-occurring oxyanions above in laboratory experiments and field sites with high Se concentrations, although further research is required for methods to eliminate any co-occurring sulphate.

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1. Introduction

The occurrence, exposure, and toxicity of selenium (Se) in natural systems are strongly linked to Se speciation. Se exists in nature as several oxidation states including the dissolved oxyanions selenate (Se(VI) , SeO_4^{2-}), selenite (Se(IV) , SeO_3^{2-}), and biselenite (Se(IV) , HSeO_3^- , $\text{pK}_a = 8.2$); in the solid form as elemental Se(0) or trapped within mineral sulphides as selenide (Se(-II) , Se^{2-}); or as gaseous hydrogen selenide (Se(-II) , H_2Se) or organoselenides. Selenate and selenite are of greater concern because of their mobility and bioavailability [1,2]. The Se oxyanions are usually found at low concentrations ($<10 \text{ ppb}$ or <0.13

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μM Se) within surface waters [3] but can have higher concentrations (>1 ppm or >13 μM Se) in groundwaters [4,5]. Dissolved Se occurrence is usually due to oxidative dissolution of selenide minerals or release from industrial activities, and can be spread around by agriculture irrigation channels [3,6,7]. Consequently, water resource management efforts focus on immobilizing Se oxyanions or otherwise removing them from the water column, such as taking advantage of natural attenuation processes like Se oxyanion reductive precipitation to Se(0) or uptake and transformation by microorganisms or plants [8–10].

Se and O isotope systematics could assist management practices by providing confirmatory evidence of *in situ* oxyanion removal processes through observed Se or O isotope fractionation. Kinetic isotope fractionation of Se isotopes in selenate or selenite can occur during Se–O bond breaking when heavier Se isotopes remain in unreacted oxyanions after oxyanions with lighter Se isotopes preferably react [11]. This heavy isotope enrichment has been detailed in the laboratory for a variety of biogeochemical reaction pathways including Se oxyanion reduction to Se(0) by microorganisms [12,13] or iron-bearing minerals [14,15], or biomethylation to organoselenides [16,17]. Phase transfer changes such as sorption to iron minerals can also fractionate selenate and selenite [15,18]. Moreover, Se isotope fractionation has been observed in field settings where oxyanion transformation was suspected [19–22]. In addition to Se, O isotope fractionation in selenate occurs during reduction to Se(0) by Fe(II)-bearing hydroxides [23] or by selenate-respiring bacteria [24]. Oxyanion $\delta^{18}\text{O}$ values and O isotope fractionation extents have not yet been observed in natural samples, however, due to lack of analytical methods.

Quantification of $\delta^{18}\text{O}$ values in Se oxyanions is constrained by the chemical condition of solvent water and still faces challenges for measurement of natural waters. Selenate exchanges its O with solvent H_2O molecules in acidic pH even at room temperature [25,26], requiring samples to remain in circumneutral pH to preserve O isotope signatures. In contrast, selenite rapidly exchanges its O with H_2O at any pH, and its measured $\delta^{18}\text{O}$ value should reflect the $\delta^{18}\text{O}$ value of H_2O [27]. To measure $\delta^{18}\text{O}$ values of Se oxyanions by isotope ratio mass spectrometry, the oxyanions must first be sequestered by precipitation with excess barium cations (Ba^{2+}) to form insoluble $\text{BaSeO}_4(\text{s})$ or $\text{BaSeO}_3(\text{s})$ [26,28] in a manner similar to $\text{BaSO}_4(\text{s})$ for S and O isotope analysis of sulphate. However, because both selenate and selenite precipitate with Ba^{2+} , separation of the oxyanions is required to avoid co-precipitation. Furthermore, other naturally occurring oxyanions including phosphate, carbonate, and arsenate are poorly soluble with Ba^{2+} and could co-precipitate and interfere with $\delta^{18}\text{O}$ analysis. Finally, the Se oxyanion precipitation method requires a relatively large sample volume and high oxyanion concentration (~ 10 mL of >1 mM Se at minimum) in order to precipitate enough mass for replicate measurements of ~ 300 μg BaSeO_4 required for IRMS [28]. Se stable isotope ratios, in contrast, can be quantified by mass spectrometry in lower samples volumes and far lower Se amounts of 10–100 ng Se, suitable for direct isotope analysis of Se in natural samples [17,19–21,29]. There is a clear need for an analytical chemical methodology that concentrates and isolates selenate for $\delta^{18}\text{O}$ analysis.

This work presents an approach to isolate selenate from solutions containing selenite and other oxyanions (carbonate, phosphate, and arsenate), while maintaining conditions that preserve selenate $\delta^{18}\text{O}$. The method is based on the removal of other oxyanions by precipitation with cerium cations (Ce^{3+}) owing to cerium's low solubility product constant

with selenite ($\text{Ce}_2(\text{SeO}_3)_3(\text{s})$, $K_{sp} = 10^{-24.4}$), phosphate ($\text{CePO}_4(\text{s})$ $K_{sp} = 10^{-23}$), and carbonate ($\text{Ce}_2(\text{CO}_3)_3(\text{s})$ $K_{sp} = 10^{-23.8}$), but high solubility with selenate ($\text{Ce}_2(\text{SeO}_4)_3(\text{s})$ $K_{sp} = 10^{0.5}$). Sulphate was not addressed because no metal cation selectively precipitates sulphate and not selenate at circumneutral pH and millimolar concentrations, due to their chemical similarities (K_{sp} of $\text{Ce}_2(\text{SO}_4)_3(\text{s})$ is $10^{-1.8}$). Chloride and nitrate do not interfere with BaSeO_4 precipitation and were not considered. Ce^{3+} also does not form a solid hydroxide at millimolar concentrations at pH ~ 7 [30], unlike other metal cations that selectively precipitate selenite over selenate, such as Cu^{2+} [31,32].

An earlier version of this method was used to separate selenate and selenite prior to $\delta^{18}\text{O}$ selenate measurements for samples of a biological reactor featuring microbial reduction of selenate to selenite and elemental selenium [24]. In the present work, the cerium precipitation step is compared to a selective reduction of selenite by glutathione (GSH), which does not reduce selenate at circumneutral pH. GSH has been studied as a model dissolved biological reductant for selenite [33]. However, its reaction with selenite could introduce different by-products such as the $\text{GSSG}^\cdot$ radical and GS-Se-SG during reduction [34–36], which might affect ^{18}O isotope analysis. Ion exchange resin is also evaluated for concentrating selenate from water with low dissolved Se. This report provides confirmation of complete removal of the interfering anions selenite (SeO_3^{2-}), phosphate (PO_4^{3-}), carbonate (CO_3^{2-}), and arsenate (AsO_4^{3-}) without selenate removal and with preservation of $\delta^{18}\text{O}$ selenate in solutions of mixed oxyanions.

2. Materials and methods

2.1. Cerium selenite precipitation

All stock solutions used for the experiments and chemical analysis were prepared using double-deionized (DI) water ($>18\text{ M}\Omega$) and the high purity chemicals listed in the Appendix. The initial concentrations of all solutions used in experiments are detailed in Table S1. All solutions were pH 7.0 in 45 mM MES buffer unless otherwise noted.

Batch reactors to determine optimal cerium:selenite ratios for cerium selenite precipitation were conducted with 8 mM sodium selenite or sodium selenate solution with calculated volumes of a Ce^{3+} stock solution were added to obtain the cerium:selenite or cerium:selenate ratios of approximately 0:1, 0.5:1, 0.66:1, 1:1, 2:1, 4:1, and 8:1. The 100 mM Ce^{3+} stock solution was prepared in 45 mM MES buffer, and the pH was raised to 7.0 by slowly adding 1 M NaOH to avoid the formation of Ce(III) hydroxide which is insoluble above pH 7.5. After 24 h of agitation by end-over-end rotating, aqueous samples were taken by centrifugation at 7,000 rpm for 10 min followed by filtration of the supernatant with 0.2 μm nylon syringe tip filters, and samples were stored at 4 °C until analysis. Identical control reactors of selenite and selenate were also prepared but equivalent volumes of deionized water were added instead of Ce^{3+} to estimate initial selenite and selenate concentrations prior to precipitation. A sample of white solids from the 4:1 cerium:selenite condition was analyzed by X-ray diffraction (XRD, Rigaku, $\text{CuK}\alpha$ radiation). Thermodynamic modelling of selected solutions was performed with visual Minteq software (v3.1) [37] in order to determine precipitation conditions for $\text{Ce}_2(\text{SeO}_3)_3(\text{s})$ and $\text{Ce(OH)}_3(\text{s})$ (Table S3). The optimal doses of Ce^{3+} to precipitate phosphate and arsenate

anions were similarly determined, and the optimal dose of Ce(III) to precipitate bicarbonate was determined with manual titration and without MES buffer to avoid its interference in dissolved carbon measurement.

The kinetics of cerium selenite precipitation were determined within a magnetically stirred solution of 5.6 mM selenite with volumes of the Ce^{3+} stock solution added for cerium:selenite concentration ratios of 2:1 and 8:1. At selected time points (0, 0.25, 0.5, 1, 2, 6, and 24 h), 5 mL suspension samples were removed, centrifuged, filtered, and stored as described above. Hundred microliter of 1 M nitric acid was added to each sample to prevent further Ce(III) selenite formation by lowering pH to a range of high $\text{Ce}_2(\text{SeO}_3)_3(\text{s})$ solubility (pH 3–4).

The second mode of selenite removal was examined using glutathione which rapidly and selectively reduces selenite and not selenate (Figure S1), but this method was found to cause a change in $\delta^{18}\text{O}$ -selenate values (Table S2) and no longer considered.

2.2. Ce^{3+} cation removal by ion exchange resin

Amberlite cation exchange resin (Acros Organics, Amberlite® IR120) was prepared by loading with Na^+ in a 500 mM NaCl solution and washing with deionized water while resting within an open-top low pressure chromatography column in a low pressure liquid chromatography unit (LPLC, BioLogic) equipped with a conductivity detector, until conductivity was below the chosen practical limit of 0.2 mS cm^{-1} . Batch reactors testing the removal of Ce^{3+} cations in the presence of 10 mM selenate used 2 g dried Na-IR120 resin in 30 ml solution. The batch reactors were mixed end-over-end for 2 h with samples collected at predetermined time points (0, 5, 15, 30, 60, and 120 min) for kinetic profiles. Aqueous samples for selenate and Ce^{3+} concentrations were separated from solids as described above. An identical control was prepared at 10 mM sodium selenate and 10 mM Ce^{3+} with the same sampling processes but no addition of ion exchange resin.

The removal of Ce^{3+} using Na-IR120 resin in the low pressure chromatography column by flow-through was also tested. In this experiment, 2 g of Na-IR120 resin was placed into the column, and solutions of selenate and Ce^{3+} identical to above were pumped through at 1.0 mL min^{-1} while monitoring conductivity. Effluent solution was collected once conductivity was greater than 0.2 mS cm^{-1} (indicating elution of dissolved ions such as Na^+ , Cl^- , and SeO_4^{2-}), filtered, and measured for dissolved Ce^{3+} and selenate concentrations.

2.3. Quantifying $\delta^{18}\text{O}$ -selenate values

Experiments that determined $\delta^{18}\text{O}$ values of selenate during exposure to Ce^{3+} cations and removal of Ce^{3+} by Na-IR120 resin were conducted as described above using varying concentrations of selenate and Ce^{3+} . Aqueous samples were taken and filtered for selenate $\delta^{18}\text{O}$ determination before and after exposure to 2 g Na-IR120 resin.

Experiments that monitored $\delta^{18}\text{O}$ values of selenate during exposure to the cerium selenite precipitation process followed by Na-IR120 resin processing were also conducted as described above. Solutions containing selenate and selenite were first exposed to Ce^{3+} for 24 h, after which cerium selenite solids were removed by centrifugation. Approximately, 2 g of Na-IR120 resin was then added to the solutions and mixed by rotating

end-over-end for 2 h. The resin was separated from the solution which was stored at 4 °C until analysis.

Experiments that tested the concentration and elution of selenate onto anion exchange resin, and any effect on selenate $\delta^{18}\text{O}$ values, were conducted using the LPLC system. BioRad AG4-X4 resin (100–200 mesh) was first prepared by loading with Cl^- using a 500 mM NaCl solution, placed in an open-top resin column, and washed with deionized water until conductivity in elution water was $<2 \text{ mS cm}^{-1}$. Solutions of varying selenate concentration and with or without other oxyanions (selenite, phosphate, arsenate, and carbonate) were slowly passed through the resin column at a 1.0 mL min^{-1} flow rate to allow for oxyanion exchange with surface sorbed Cl^- . De-ionized water was then passed through until conductivity was $<2 \text{ mS cm}^{-1}$ to remove test solution entrained in pores. Oxyanions were desorbed by introducing 500 mM NaCl solution. The effluent collection started after the effluent conductivity rose to $>2 \text{ mS cm}^{-1}$ and ended after 20 mL was collected. This 20 mL collection volume was predetermined to recover nearly all selenate.

Upon completion of all experiments, selenate was first collected by precipitation as solid $\text{BaSeO}_{4(s)}$ according to established methods [28]. Briefly, selenate solutions were transferred into glass tubes and received a volume of 1 M BaCl_2 solution for a final barium:selenate ratio of 5:1. The white solids precipitated immediately and were allowed to settle for 24 h. Solids were collected, centrifuged, washed successively with deionized water and methanol, and dried. Selenite recovery values were calculated by comparing the theoretical mass of $\text{BaSeO}_{4(s)}$ possible to the measured recovered mass after drying. Each sample was then homogenized, and triplicate or quadruplicate samples of approximately 300 μg were placed into Costech pressed silver capsules. $\delta^{18}\text{O}$ measurements for selenate and selenite were performed at the Earth Systems Center for Stable Isotopic Studies, a Yale Institute for Biospheric Studies research center, using a ThermoFinnigan thermal chemolysis elemental analyzer (reactor temperature 1455 °C, gas chromatograph column temperature 90 °C) and a ThermoFinnigan DeltaPlus XP mass spectrometer coupled via a Finnigan Conflo III continuous flow interface and a IVA straight path adapter. $\delta^{18}\text{O}$ -selenate measurements were also performed at the Environmental Biogeochemistry Lab, University of Delaware, using a Thermo thermal chemolysis elemental analyzer (reactor temperature 1450 °C, column temperature 95 °C) and a Thermo Delta V mass spectrometer coupled via a Conflo IV interface. The elemental analyzer at each laboratory utilized a graphite holder cup supported by glassy carbon beads within a glassy carbon tube, and He was the carrier gas. Each mass spectrometer was configured for detection of carbon monoxide (CO). Samples were calibrated to the Vienna Standard Mean Ocean Water (VSMOW) scale using in-house synthesized barium selenate (-14.0 ‰) and silver arsenate (1.9 and 14.1 ‰) standards [28], an in-house calibrated purchased BaSeO_4 standard (-10.3 ‰ , MP Biomedicals), and IAEA-601 benzoic acid with value of $+23.3 \text{ ‰}$. The $\delta^{18}\text{O}$ -selenate value for sodium selenate used in experiments was -8.0 ‰ . Standard deviations on replicate measurements of solids were $<0.2 \text{ ‰}$ for all standard materials and typically $<0.3 \text{ ‰}$ for samples (with exception of two $<0.6 \text{ ‰}$).

At times, the mean $\delta^{18}\text{O}$ -selenate values calculated from the replicate measurements were compared using the two-sample Student's *t*-test for comparing means with approximately equal variances. The null hypothesis was that no difference existed between the means before chemical processing and after chemical processing, and this null hypothesis failed to be rejected if the test statistic was less than the critical value at a significance

threshold level of 0.05 (95 % confidence level in an upper tail test). Calculated p -values are also provided for each comparison, and again the null hypothesis failed to be rejected if p is greater than the threshold 0.05. When the null hypothesis failed to be rejected, no significant statistical difference at the 95 % confidence level was concluded.

2.4. Analytical measurements

Concentrations of dissolved selenate, selenite, cerium, arsenate, and bicarbonate were analyzed by inductively coupled plasma mass spectrometer (ICP-MS) (Bruker Aurora M90). Aqueous samples were introduced either by Teflon tubing with a peristaltic pump for the measurement of total dissolved elements, or by streaming in eluent from a liquid chromatography (LC) system (Varian ProStar) used for speciation of selenate and selenite by LC-ICP-MS. Separation of selenate and selenite was achieved using a Hamilton PRP-X100 column at a 1 mL min^{-1} flowrate. The mobile phase contained 20 mM citric acid at pH 6 to provide buffer ability and oxyanion elution under 7 min and 5 mM ethylenediamine tetraacetic acid (EDTA) to provide complexing ligands for any dissolved metal cations (such as excess Ce^{3+}) to avoid metal complexing to column media or metal hydroxide precipitation. Se detection at 77, 78, and 79 m/z signals was made possible by introduction of H_2 gas into the collision-reaction interface to eliminate interferences caused by plasma-generated Ar-Ar dimers. Instrument response was calibrated to element concentrations using purchased standard solutions. The method detection limit for Se was $0.2 \text{ }\mu\text{M}$ for ICP-MS and $5 \text{ }\mu\text{M}$ for LC-ICP-MS.

Dissolved phosphate concentrations, and at times selenate and selenite concentrations, were measured by ion chromatography (IC) with a Dionex ICS-5000 system, an AS-20 column, ion suppression, and conductivity detection. An isocratic eluent method of 35 mM KOH for 20 min was used for phosphate determination, and a multi-step gradient eluent method was used for Se oxyanions consisting of 10 mM KOH for 20 min followed by a ramp to 30 mM for 15 min, all at a 1 mL min^{-1} flowrate. For all chromatographic analyses, only single sample measurements were performed for each experimental time point. Dissolved Na^+ and Cl^- concentrations were not determined. The method detection limit was $40 \text{ }\mu\text{M}$ for IC.

3. Results and discussion

3.1. Selenite and other oxyanions removal by precipitation with Ce^{3+}

The separation of selenate from other oxyanions in solution was first evaluated by confirming cerium-oxyanion precipitation and leaving selenate dissolved in solution. The ability of Ce^{3+} to precipitate HSeO_3^- and SeO_3^{2-} was tested at varying cerium:selenite ratios at circumneutral pH values (Figure 1(A)). Selenite removal from solution was first observed by the formation of white cerium selenite solids that was identified as $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ by XRD (Figure S2). Selenite removal was incomplete when cerium:selenite ratios were less than the stoichiometric value of 0.66:1. Ratios of 2:1 or higher showed 99.9–100 % selenite removal. Greater than stoichiometric Ce^{3+} amounts are, therefore, required to ensure complete $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ precipitation due to the common ion effect in which excess Ce^{3+} cations suppress the solubility of formed $\text{Ce}_2(\text{SeO}_3)_{3(s)}$. Similarly,

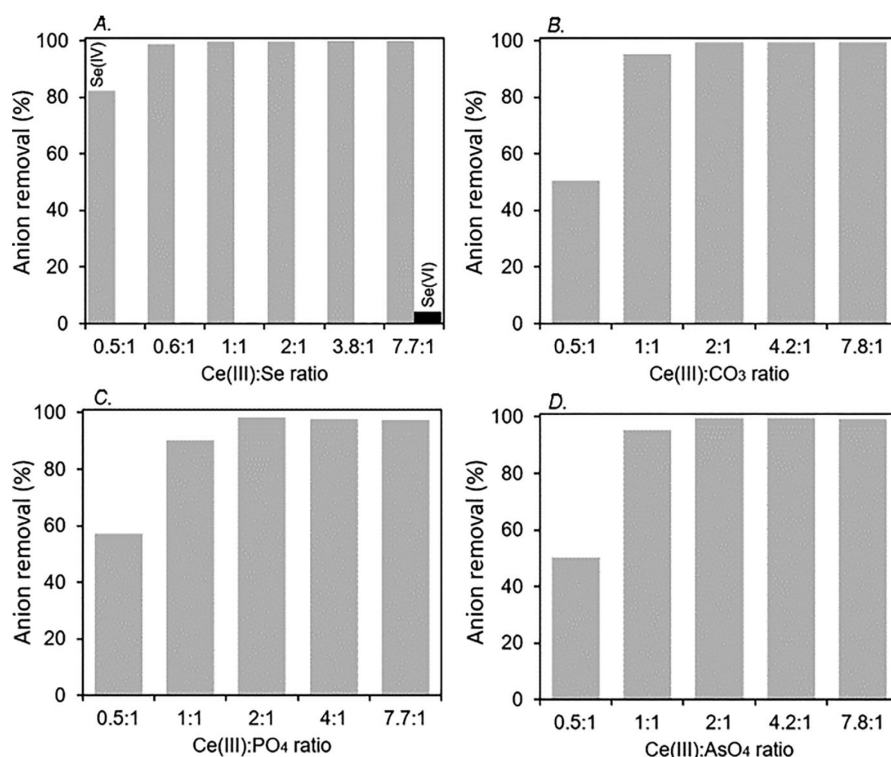


Figure 1. Percent removal of oxyanions during precipitation with Ce^{3+} at varying Ce(III):oxyanion ratios at pH 7 in 45 mM MES buffer solution after 24 h. (A) Selenite and selenate in separate solutions (any selenate removal was observed only at the 7.7:1 Ce(III):Se ratio, and at other ratios the selenate bars are not present), (B) carbonate, (C) phosphate, and (D) arsenate.

excess Ce^{3+} at a minimum cerium:oxygen ratio of 2:1 was also required to precipitate cerium carbonate ($\text{Ce}_2(\text{CO}_3)_3(\text{s})$), cerium phosphate ($\text{CePO}_4(\text{s})$), and cerium arsenate ($\text{CeAsO}_4(\text{s})$) from their individual solutions at pH 7 (Figure 1(B–D)). White precipitates were visually observed to form immediately in all solutions of phosphate and arsenate, but precipitates in the bicarbonate solutions evolved more slowly over several hours. A similar series of selenate solutions were prepared and exposed to varying concentrations of Ce^{3+} to examine the potential for Ce^{3+} to remove any selenate via accidental precipitation in our conditions (Figure 1(A)). No loss of selenate from solution was observed after addition of Ce^{3+} at cerium:selenate ratios between 0.6:1 and 4:1. However, at the highest Ce^{3+} amount added (8:1), selenate concentration decreased by 4.2 %, and a small white, unidentified precipitate formed. Thermodynamic calculations of this solution condition did not predict any $\text{Ce}_2(\text{SeO}_4)_3(\text{s})$ precipitation (Table S3). Nevertheless, larger excess Ce^{3+} could possibly induce some precipitation of cerium selenate despite its higher solubility ($\text{Ce}_2(\text{SeO}_4)_3(\text{s})$ $K_{sp} = 10^{0.51}$) compared to cerium selenite ($\text{Ce}_2(\text{SeO}_3)_3(\text{s})$ $K_{sp} = 10^{-24.4}$), and sample processing should avoid the cerium:selenate upper limit of approximately 8:1. In contrast, Ce(III):oxyanion ratios of at least 2:1 are required for removal of selenite, bicarbonate, phosphate, and arsenate, and increasing the ratios showed improved removal extent. As an initial approximation for separating

all selenate in natural samples, the optimal Ce^{3+} dose is recommended to be greater than twice the sum of precipitating oxyanion concentrations (e.g. selenite, carbonate, phosphate, arsenate) in order to precipitate them all, but less than the upper limit of eight times the selenate concentration.

Oxyanion precipitation with Ce^{3+} also requires strict pH control. In all cases, solution pH was carefully maintained under high concentrations of MES buffer that kept pH below the solubility limit for $\text{Ce}(\text{OH})_{3(s)}$ at these Ce^{3+} concentrations (pH 7.5). The buffer also prevented pH from dropping too low for $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ precipitation, caused by H^+ release from HSeO_3^- , HCO_3^- , and H_2PO_4^- which are the predominant oxyanion species at pH 7.0.

Similar pH ranges of $\text{Ce}(\text{OH})_{3(s)}$ precipitation [30] and $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ precipitation [38] have been reported. No precipitates were observed when Ce^{3+} was added to 45 mM MES buffer solution at pH 7.0 without any Se oxyanions, indicating no formation of $\text{Ce}(\text{OH})_{3(s)}$. Thermodynamic modelling of the solution conditions containing either 30 mM Ce^{3+} or 100 mM Ce^{3+} show no $\text{Ce}(\text{OH})_{3(s)}$ is expected to form at pH 6.0–7.0, although some $\text{Ce}(\text{OH})_{3(s)}$ will precipitate at pH 7.7 (Table S3). Thermodynamic calculations also demonstrate that $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ and not $\text{Ce}(\text{OH})_{3(s)}$ will form within pH 6.0–7.0 when 9.0 mM selenite is present. Under this solution condition, after $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ precipitates, only $\sim 4 \times 10^{-14}$ M is predicted to remain dissolved, so dissolution of $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ is not considered to be a concern. Furthermore, $\text{Ce}(\text{OH})_{3(s)}$ was not observed in the $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ XRD pattern in Figure S2. Other cerium (bi)selenite solids were not considered in thermodynamic modelling because these solids ($\text{CeH}(\text{SeO}_3)_2$, $\text{CeH}(\text{SeO}_3)_2 \cdot n\text{H}_2\text{O}$, $\text{Ce}(\text{HSeO}_3)_3$, and $\text{Ce}_2(\text{SeO}_3)_3 \times \text{H}_2\text{O}$ with $x = 3, 5$, or 7) have been observed to form by precipitation in water under different circumstances: only at acidic pH, typically with excess H_2SeO_3 , and with 4–30 days crystallization time [39].

The time required for complete $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ precipitation was determined with a kinetic experiment at two cerium:selenite ratios. $\text{Ce}_2(\text{SeO}_3)_{3(s)}$ precipitation is rapid and nearly complete within a few hours in the presence of excess Ce^{3+} at pH 7 (Figure 2). The selenite removal rate and extent was improved using a higher cerium:selenite ratio of 8:1 compared to 2:1. Only the ratio of 8:1 produced 100 % removal of selenite after 24 h with residual dissolved selenite below detection limit, as opposed to 99.9 % selenite

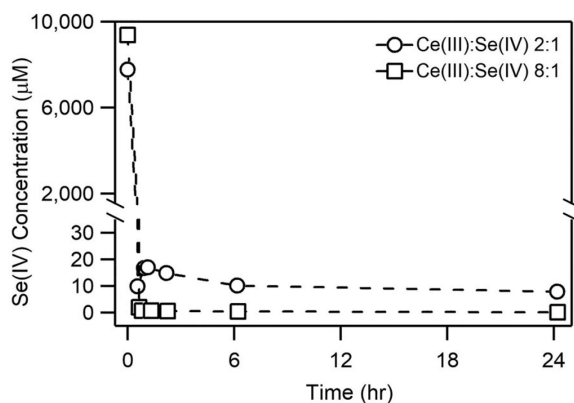


Figure 2. Kinetics of selenite removal by precipitation as $\text{Ce}_2(\text{SeO}_3)_3$ after addition of dissolved Ce^{3+} at the ratio of 2:1 and 8:1 at pH 7 in 45 mM MES buffer solution.

removal with 9 μM selenite remaining for the 2:1 ratio. Nevertheless, the 2:1 cerium:selenite ratio with 24-hour processing time was chosen for subsequent experiments with equimolar concentrations of selenate and selenite to avoid any possible cerium selenate precipitation.

3.2. Excess Ce^{3+} removal with cation exchange resin

Because excess Ce^{3+} cations are required to ensure complete selenite precipitation, residual Ce^{3+} concentrations could be far greater than selenate concentrations and could cause some selenate removal at high enough concentrations (Figure 1). Unintended selenate removal might induce an O isotope fractionation. The influence of excess dissolved Ce^{3+} on the $\delta^{18}\text{O}$ value of selenate was first examined using four solutions of freshly prepared, approximately 9 mM selenate containing varying concentrations of Ce^{3+} up to a cerium:selenate ratio of about 3.5 : 1. $\text{BaSeO}_4(\text{s})$ was precipitated within these solutions and measured for $\delta^{18}\text{O}$ -selenate (Table 1). The mean $\delta^{18}\text{O}$ -selenate values were compared in the *t*-test at the 95 % confidence level ($p = 0.05$). There was no significant statistical difference between $\delta^{18}\text{O}$ -selenate measured in the absence of Ce^{3+} ($-7.99 \pm 0.25 \text{‰}$) and $\delta^{18}\text{O}$ -selenate measured when Ce^{3+} was present (-7.68 ± 0.50 , -7.64 ± 0.27 , and $-7.79 \pm 0.33 \text{‰}$, with calculated *p* values of 0.20, 0.06, and 0.30, respectively, all > 0.05). Excess Ce^{3+} in the concentration range studied appears to have little influence on $\delta^{18}\text{O}$ -selenate values, although higher Ce^{3+} concentrations might produce problems described above, thus warranting Ce^{3+} removal.

Cation exchange resin Amberlite IR120 loaded with Na^+ were then evaluated for its ability to remove Ce^{3+} without removing any dissolved selenate. This same resin has been reported to be useful for heavy metal cation removal from water [40]. A second, identical set of 30 mL of ~ 9 mM selenate solutions with varying Ce^{3+} concentrations was prepared and exposed to 2 g of Na-IR120 resin for 2 h in batch reactors and monitored for selenate and Ce^{3+} concentrations and $\delta^{18}\text{O}$ -selenate values (Table 1). After treatment, 100 % of Ce^{3+} was removed and approximately 100 % of selenate was recovered, indicating the Na-IR120 resin binding sites removed only Ce^{3+} in exchange for Na^+ and had no affinity for selenate. Most importantly, the starting $\delta^{18}\text{O}$ -selenate value ($-7.82 \pm 0.58 \text{‰}$) did not change after Ce^{3+} removal by Na-IR120 resin because no significant statistical difference was observed when comparing means of $\delta^{18}\text{O}$ -selenate after processing with resin whether Ce^{3+} was present or not (-7.77 ± 0.10 , -7.60 ± 0.32 , $-7.76 \pm 0.32 \text{‰}$, with

Table 1. Effect of the dissolved Ce^{3+} removal process by Na^+ -loaded Amberlite IR120 resin on dissolved selenate concentrations and $\delta^{18}\text{O}$ -selenate values.

Solution composition	Ce(III):Se(VI) ratio	$\delta^{18}\text{O}$ -selenate (%Se(VI))	After treatment with Na-IR120 resin		
			recovery ^a (%)	Ce(III) removal ^b (%)	$\delta^{18}\text{O}$ -selenate (%)
9.0 mM Se(VI)	0	-7.99 ± 0.25	96	–	-7.82 ± 0.58
8.9 mM Se(VI) + 8.3 mM Ce(III)	1:1	-7.68 ± 0.50	101	100	-7.77 ± 0.10
8.6 mM Se(VI) + 18.3 mM Ce(III)	2.1:1	-7.64 ± 0.27	106	100	-7.60 ± 0.32
8.5 mM Se(VI) + 29.4 mM Ce(III)	3.5:1	-7.79 ± 0.33	108	100	-7.76 ± 0.32

^aRecovery values were determined as (final concentration/initial concentration) $\times$ 100 %, where the final concentrations were measured 2 h after addition of IR120 resin.

^bRemoval values were determined as (initial concentration – final concentration)/(initial concentration) $\times$ 100 %, where the final concentrations were measured 2 h after addition of IR120 resin.

calculated p values of 0.45, 0.15, and 0.39, respectively, all > 0.05). This reveals that no selenate reaction was occurring to cause any ^{18}O fractionation, and the Na-IR120 resin exposure does not interfere with $\text{BaSeO}_{4(s)}$ precipitation. And, within solutions of selenate alone, there was no statistical difference between $\delta^{18}\text{O}$ -selenate before ($-7.99 \pm 0.25 \text{ ‰}$) and after ($-7.82 \pm 0.58 \text{ ‰}$, with calculated p value of 0.33) resin treatment. Therefore, the ion exchange process in batch reactors can be applied to remove Ce^{3+} cations for $\delta^{18}\text{O}$ -selenate analysis without varying $\delta^{18}\text{O}$ -selenate values.

Furthermore, the Na-IR120 resin is suitable to remove Ce^{3+} when assembled into a column for flow-through sample processing. The same 30 mL, $\sim 9 \text{ mM}$ selenate solutions with varying Ce^{3+} concentrations were again exposed to 2 g Na-IR120 resin as a batch reactor for 2 h and as a column with a 1.0 mL min^{-1} flowrate (Table 2). Again, almost complete removal of Ce^{3+} (range of 99.7–99.9 %) and recovery of selenate (range of 96–108 %) was achieved for both modes of processing. Use of resin within a column may be advantageous when processing large sample volumes (to ensure resin contact) or for avoiding centrifugation and filtration steps after mixing in the batch configuration.

The kinetics of Ce^{3+} removal by Na-IR120 resin in batch configuration was more closely followed over 2 h to better describe the time needed to achieve complete Ce^{3+} removal. Two solutions containing 8 or 30 mM Ce^{3+} in the presence of selenate showed all Ce^{3+} was removed within 15 min without any disappearance of selenate after 2 g Na-IR120 resin was added (Figure 3(A,B)). Slightly faster removal was observed with the lower 8 mM Ce^{3+} solution (99.4 % in 5 min) compared to the 30 mM Ce^{3+} solution (88 % in 5 min), likely due to Ce^{3+} cations rapidly accessing the more readily available surface exchange sites, while higher Ce^{3+} concentrations require further diffusion to less accessible sites. For these millimolar range of Ce^{3+} concentrations, the resin concentration used here (67 g L^{-1}) is sufficient for complete removal of Ce^{3+} within 1 h. The presence of selenate did not prevent any Ce^{3+} surface exchange. No selenate removal was observed in the presence of resin whether Ce^{3+} surface exchange occurred or not (Figure 3(C)), and no selenate or Ce^{3+} removal occurred when no resin was provided (Figure 3(D)). These results help exclude the possibility of any interference of selenate on Ce^{3+} removal and of Ce^{3+} removal on remaining dissolved selenate.

Finally, the preservation of $\delta^{18}\text{O}$ -selenate values was demonstrated during the combined process of $\text{Ce}_2(\text{SeO}_3)_3(s)$ precipitation and Ce^{3+} removal (Table 3). Two sets of duplicate solutions containing $\sim 9 \text{ mM}$ selenate with selenite at either equimolar concentration to or half the concentration of selenate were processed to $\text{BaSeO}_{4(s)}$ harvesting. The Ce^{3+} precipitation step removed all dissolved selenite to below the LC-ICP-MS method detection limit of $5 \text{ } \mu\text{M}$. After removing excess Ce^{3+} with Na-IR120 resin, complete recovery of selenate as $\text{BaSeO}_{4(s)}$ was achieved for the 2:1 selenate:selenite condition, and up to 96 % recovery was observed

Table 2. Ce^{3+} removal percentages and selenate recovery percentages after 2 h treatment with Na^+ -loaded Amberlite IR120 resin in batch mode or column mode.

Solution composition	Ce(III):Se(VI) ratio	Ce(III) removal (%)		Se(VI) recovery (%)	
		Column	Batch	Column	Batch
9.0 mM Se(VI)	0	0.0	0.0	100	96
8.9 mM Se(VI) + 8.3 mM Ce(III)	1:1	99.8	99.8	102	101
8.6 mM Se(VI) + 18.3 mM Ce(III)	2.1:1	99.9	99.9	103	108
8.5 mM Se(VI) + 29.4 mM Ce(III)	3.5:1	99.7	99.9	101	106

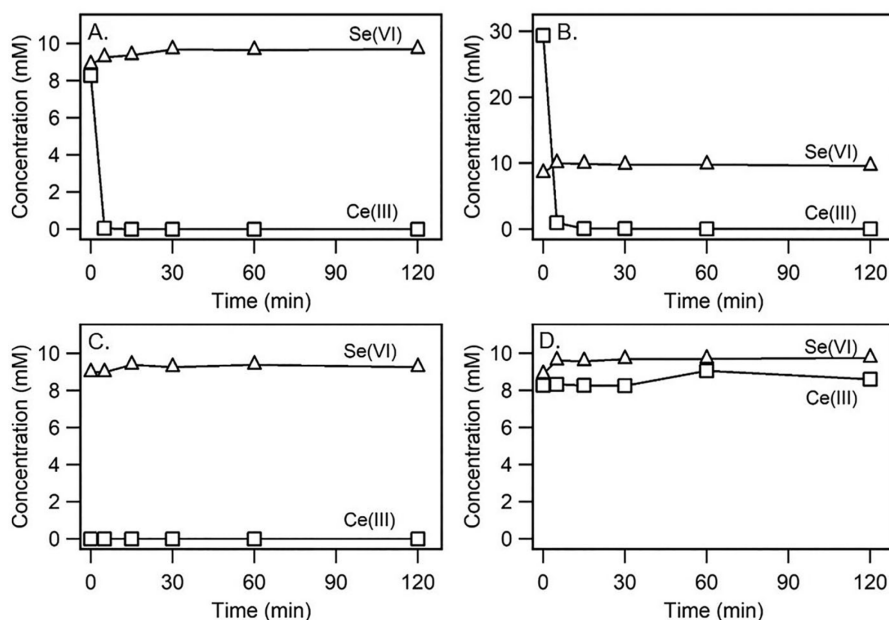


Figure 3. Kinetics of Ce^{3+} removal by 2 g Na-loaded IR120 Amberlite cation exchange resin in the presence of $\sim 9 \text{ mM Se(VI)}$ at pH 7 in 45 mM MES buffer and (A) 9 mM initial Ce^{3+} , (B) 30 mM initial Ce^{3+} , or (C) 0 mM initial Ce^{3+} as a control. (D) 9 mM Se(VI) and 9 mM initial Ce^{3+} without resin.

Table 3. $\delta^{18}\text{O}$ -selenate values measured before and after treatment to remove dissolved selenite by precipitation with Ce^{3+} and subsequent removal of excess Ce^{3+} with Na^+ -loaded Amberlite IR120 resin.

Initial solution composition	Se(VI):Se(IV) ratio	Dissolved Se(IV) remaining (mM)	Se(VI) recovery as BaSeO_4^a (%)	$\delta^{18}\text{O}$ -selenate (‰)
9.0 mM Se(VI)	–	–	99	-8.19 ± 0.17
8.9 mM Se(VI) + 9.2 mM Se(IV)	1:1	0	93	-8.20 ± 0.14
8.9 mM Se(VI) + 9.2 mM Se(IV)	1:1	0	94	-8.32 ± 0.09
9.0 mM Se(VI) + 4.0 mM Se(IV)	2:1	0	102	-8.17 ± 0.13
9.0 mM Se(VI) + 4.0 mM Se(IV)	2:1	0	102	-8.29 ± 0.13

^aSe(VI) recovery values were determined as (measured mass of BaSeO_4 formed/theoretical mass of BaSeO_4 expected based in initial Se(VI) concentration and volume) $\times 100\%$.

for the 1:1 condition. Some mass loss of selenate may have occurred during sample processing or due to $\text{BaSeO}_{4(s)}$ particles lost during the washing or drying procedure. No change in $\delta^{18}\text{O}$ -selenate values was observed for any of the four solutions tested (-8.20 ± 0.14 , -8.32 ± 0.09 , -8.17 ± 0.13 , -8.29 ± 0.13 ‰, with calculated p values of 0.47, 0.06, 0.43, and 0.14, respectively) compared to the solution containing selenate alone (-8.19 ± 0.17 ‰), that is all means were statistically indistinguishable. These results confirm that selenate can be isolated from a solution of mixed selenate and selenite oxyanions without significant loss of selenate or alteration of $\delta^{18}\text{O}$ -selenate values.

3.3. Selenate pre-concentration with anion exchange resin and purification by Ce^{3+} precipitation

Due to the requirement of millimolar amounts of selenate for $\text{BaSeO}_{4(s)}$ precipitation, samples of natural water containing typically micromolar selenate amounts would

require pre-concentration prior to precipitation. BioRad AG4-X4 resin pre-loaded with Cl^- (Cl-AG4-X4) was evaluated for quantitative sorption and subsequent release and recovery of selenate from dilute solutions. To first confirm complete selenate recovery, four test solutions containing varying concentrations of selenate (1–10 mM) were separately passed through Cl-AG4-X4 resin onto which the selenate was exchanged for previously sorbed chloride. The entirety of dissolved selenate was sorbed because only chloride and no selenate was detected after collecting the eluted test solution. Thirty mL of 500 mM NaCl solution was then passed through the resin column in order to determine the necessary volume to elute and recover all selenate ions. Eluent was collected in 2–8 mL increments, analyzed for selenate concentration, and cumulative μmoles of selenate recovered was tracked. In all four cases, approximately 20–25 mL of NaCl solution was needed to recover 100 % selenate (Figure S3). Quantitative recovery eliminates any possible isotope fractionation due to irreversible binding to resin.

The Cl-AG4-X4 resin under the predetermined operating conditions was then used to concentrate selenate from 2 L of a $63 \mu\text{M}$ (5 mg L^{-1}) selenate solution with other oxyanions and to demonstrate no isotope fractionation in $\delta^{18}\text{O}$ -selenate. This concentration was chosen because it is 100 fold higher than the US Environmental Protection Agency National Primary Drinking Water Standard ($50 \mu\text{g L}^{-1}$) and represents a high Se-concentrated wastewater such as from fossil fuel processing [41] which might be of interest for tracing source and fate of selenate. Equimolar amounts of selenite ($63 \mu\text{M}$) and phosphate ($63 \mu\text{M}$) and a typical environmental concentration of bicarbonate ($320 \mu\text{M}$) were also included to more closely represent natural waters. After concentration of the 2 L solution onto the resin and elution with 20 mL of 500 mM NaCl eluent, a total selenate recovery of 98 % was obtained along with trace amounts of selenite and phosphate. The eluent was processed by Ce^{3+} precipitation of remaining selenite and phosphate followed by $\text{BaSeO}_4(\text{s})$ precipitation. The $\delta^{18}\text{O}$ -selenate value was found to be $-10.99 \pm 0.07 \text{ ‰}$ (calculated p value of 0.32) which is nearly identical to and statistically insignificant from the original $\delta^{18}\text{O}$ -selenate value determined with the same Na_2SeO_4 salt dissolved in deionized water ($-10.86 \pm 0.45 \text{ ‰}$).

A second test solution of 20 mL containing a higher concentration of 20 mM selenate alone was then processed with Cl-AG4-X4 resin and $\text{BaSeO}_4(\text{s})$ precipitation. Here, 96 % of original selenate was recovered after sorption to resin, and again no statistically significant change in $\delta^{18}\text{O}$ -selenate was observed ($-10.55 \pm 0.11 \text{ ‰}$, calculated p value of 0.16) compared to the same initial solution above. The combined procedures of Cl-AG4-X4 resin concentration and Ce^{3+} precipitation purification, therefore, successfully demonstrated recovery of dissolved selenate while preserving $\delta^{18}\text{O}$ -selenate values.

3.4. Environmental implications

The selenate recovery method presented in this work can be applied to the determination of $\delta^{18}\text{O}$ -selenate in natural water samples subjected to certain restrictions. For one, the needed minimum amount of $\sim 10 \text{ mL}$ of $\sim 1 \text{ mM}$ selenate requires a pre-concentration of selenate from waters with much lower selenate levels. Pre-concentration with anion exchange resin, therefore, requires large volumes of natural samples for harvesting enough selenate. For example, a $1 \mu\text{M}$ selenate concentration requires a sample volume of 10 L minimum, and water resources can have an order of magnitude lower concentrations [3,22]. Such large volumes would provide a representative $\delta^{18}\text{O}$ -selenate value

only if $\delta^{18}\text{O}$ -selenate, and selenate concentration, are homogeneous within the sample. Large sample volumes may be problematic at groundwater sites where selenate concentrations (and presumably $\delta^{18}\text{O}$ -selenate) vary spatially across cm distances if reducing zones also exist only over short distances in sediments, e.g. [42,43]. Selenate concentrations of 1 μM or higher have been observed in groundwater wells monitoring plumes containing Se caused by irrigation activities [4,44], uranium waste leaks [45], or coal ash pond leachate [46]. Selenate concentrations much higher than 1 μM , though, would allow lower sample volumes.

The large sample volume requirement would not be a restriction when studying $\delta^{18}\text{O}$ -selenate values in surface waters over very large spatial scales, such as within individual lakes and rivers in the western United States [3], or in Se-rich wastewaters [47,48]. In such surface waters, selenate from industrial waste sources might have different $\delta^{18}\text{O}$ -selenate values than naturally occurring selenate because selenate might form under different water chemistries that imprint different $\delta^{18}\text{O}$ values. Selenate oxidation from selenite incorporates one additional O atom, either from ambient H_2O or an O-bearing oxidant. Therefore, $\delta^{18}\text{O}$ -selenate values would depend on $\delta^{18}\text{O}$ of solvent water and possibly $\delta^{18}\text{O}$ of the oxidant [49]. Consequently, following $\delta^{18}\text{O}$ -selenate values in nature may also help identify selenate formation processes in surface waters. Laboratory studies are needed to relate $\delta^{18}\text{O}$ values in selenate, H_2O , oxidants, and natural waters to elucidate these oxidation processes and to determine relationships between $\delta^{18}\text{O}$ of natural water and $\delta^{18}\text{O}$ -selenate.

The other interfering oxyanions, if at significantly higher concentrations, could also be minimized from solution by selective elution from anion exchange resins during the oxyanion pre-concentration step. More weakly bound oxyanions, such as bicarbonate and biselenite which sorb presumably in monodentate fashion due to monovalent charge, could possibly be eluted preferentially using less strong NaCl eluent. This ability is demonstrated in Figure S4 which shows preferential desorption from AG4-X4 resin of selenite oxyanions using only 50 mM NaCl eluent, followed by selenate desorption using 500 mM NaCl. Hundred percent of each Se oxyanion was recovered. Bicarbonate could also be avoided in the pre-concentrated selenate solution by preventing its sorption to AG4-X4 resin by lowering the pH of the water sample to well less than its pK_{a1} (6.3), promoting H_2CO_3 formation which should not exchange for Cl^- on the resin. Using anion exchange resin might also solve the problem of interfering sulphate. Anion exchange resins are expected to sorb the totality of dissolved S and Se oxyanions indiscriminately. Selective elution of selenate or sulphate might be possible using resin configured in a bench-scale chromatography column with large sample volume processing capabilities.

An alternative S and Se separation method could be selective reduction of sulphate to $\text{S}(0)$ or selenate to $\text{Se}(0)$ if a proper chemical reductant could be identified. While selenate can be reduced to $\text{Se}(0)$ under strongly acidic conditions [50], there is no known dissolved chemical reductant viable at the required circumneutral pH for either selenate or sulphate. Sulphate-respiring microorganisms tend to co-metabolize or fortuitously reduce selenate concomitantly [51], so finding a strictly sulphate-reducing bacterium may not be possible for selective sulphate removal within a biological reactor. Selective selenate removal might be possible in mineral-mediated reduction reactions such as with the Fe(II) -rich layered double hydroxide green rust [23] which does not reduce sulphate. Photocatalyzed reduction of selenate with TiO_2 [52] could also be explored for selective selenate

removal. Further work is required to confirm complete selenate removal, sulphate recovery, and $\delta^{18}\text{O}$ -sulphate preservation in such systems.

A complete methodology for isolating selenate from natural solutions containing diverse oxyanions would, therefore, contain the following steps: (i) pre-concentration of selenate and other oxyanions on Cl-loaded anion exchange resin followed by complete selenate recovery by elution with high concentrated NaCl eluent, (ii) precipitation of interfering oxyanions by addition of Ce^{3+} within the recommended cerium:oxygenion range and pH range of 4.0–7.0 over 24 h, (iii) removal of excess Ce^{3+} using Na-loaded cation exchange resin, (iv) removal of sulphate if necessary, possibly by large-scale anion chromatography, and (v) precipitation of selenate as $\text{BaSeO}_4(\text{s})$ followed by $\delta^{18}\text{O}$ measurement. Steps I–III have been demonstrated in the present work to not cause any change in $\delta^{18}\text{O}$ -selenate values.

This method is suitable for the isolation of selenate from selenite and characterization of $\delta^{18}\text{O}$ -selenate within laboratory waters with millimolar concentrations of both Se oxyanions, such as in studies examining Se oxyanion biogeochemical cycling processes. To help illuminate *in situ* transformations, isotope changes of selenate in field settings can be compared to a catalogue of stable isotope fractionations observed for selenate undergoing transformations in laboratory waters. Selenate reduction to $\text{Se}(0)$ was observed to induce ^{18}O -selenate fractionation values of 22 ‰ when reduced by $\text{Fe}(\text{II})$ hydroxides [23] and a range of 1–6 ‰ by microbial respiration [24], as determined by Rayleigh fractionation behaviour. This analytical method, and improved versions for sulphate removal, for $\delta^{18}\text{O}$ -selenate determination could help expand this catalogue by allowing processing of selenate in complex oxyanion solutions occurring in fungal biomethylation studies [17] and reduction by $\text{Fe}(\text{II})$ sulphides [15]. Moreover, this method is particularly suitable for examining selenite oxidation pathways to selenate using ^{18}O tracing techniques. The modes of selenite oxidation in nature are still unclear, and this method can remove unreacted selenite and trace the sources of O incorporation into selenate [49]. Knowledge on how environmental conditions induce $\delta^{18}\text{O}$ -selenate values could lead to a way to track different sources of selenate in nature.

4. Conclusions

A methodology was demonstrated for isolating selenate from solutions of diverse water chemistries for the purpose of quantifying selenate- $\delta^{18}\text{O}$ values. Oxyanions of selenite, carbonate, phosphate, and arsenate were removed from solution by precipitation with Ce^{3+} cations prior to precipitation of selenate as $\text{BaSeO}_4(\text{s})$ which was analyzed by TCEA-IRMS. The preferred mass of Ce^{3+} to add is an amount greater than the stoichiometric amount required to precipitate all interfering oxyanions, and here cerium:oxygenion ratios of 2:1–4:1 showed near complete removal of oxyanions without removal of selenate. Ce^{3+} precipitation with oxyanions was complete within a few hours for high cerium:oxygenion ratios. Excess Ce^{3+} was removed with Na^+ -loaded cation exchange resin to prevent Ce^{3+} interference with $\text{BaSeO}_4(\text{s})$ precipitation. Solution pH is required to be higher than 4.0 to avoid O mixing between selenate and H_2O , and lower than 7.5 to prevent $\text{Ce}(\text{OH})_3(\text{s})$ precipitation. Because >1 mM selenate is required for $\text{BaSeO}_4(\text{s})$ precipitation, pre-concentrating selenate onto Cl⁻-loaded anion exchange resin from larger volumes of low dissolved selenate was necessary, and complete recovery of selenate was possible with elution using 500 mM NaCl solution. No statistically significant change in selenate- $\delta^{18}\text{O}$ values was observed

during sample processing involving pre-concentration, elution, and precipitation steps. This methodology should be useful for separating selenate from selenite when co-existing in solution, such as within field samples with elevated Se concentrations or within laboratory experiments containing both oxyanions. Quantifying selenate- $\delta^{18}\text{O}$ values in addition to Se isotope values, as well as pathway-specific O and Se isotopic fractionation values, may be helpful in identifying selenate sources or transformation processes in environmental settings.

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