**APPENDIX A: SUPPORTING INFORMATION**

**For**

**Isolation of selenate from selenite, carbonate, phosphate, and arsenate for 18O-selenate determination**

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*Isotopes in Environmental and Health Studies*

***Chemicals***

All stock solutions used for the experiments and chemical analysis were prepared using double-deionized (DI) water (>18 M) and the following high purity chemicals: sodium selenate (Acros Organics, 98%, Na2SeO4), sodium selenite (Sigma, 98%, Na2SeO3,), cerium(III) chloride heptahydrate (Acros Organics, 99.99%, CeCl3 • 7H2O), sodium chloride (Fisher Scientific, 99.80%, NaCl), barium chloride (J.T. Baker Chemical Co., BaCl2), disodium arsenate (Sigma Aldrich, Na2HAsO4), sodium bicarbonate (Fisher Scientific, Assay 99% - 100%, NaHCO3), sodium carbonate (Pharmacia, Na2CO3), trisodium phosphate (Mallinckrodt Chemical Works, Na3PO4 • 12H2O), and glutathione (Fisher BioReagents, stored at 4°C, GSH). The pH of the solution was adjusted by nitric acid (Fisher Scientific, trace metal grade, HNO3), hydrochloric acid (Sigma, 37% solution in water, HCl), and sodium hydroxide (Fisher, NaOH) and a calibrated Orion Star pH meter. Buffer solutions included 2-(N-Morpholino) ethanesulfonic acid (Acros Organics, 99%, p*Ka* = 6.10 at 25°C, MES) and 3-[Tris(hydroxymethyl)methylamino]-1-propanesulfonic acid (Acros Organics, 99%, p*Ka* = 8.4 at 25°C, TAPS). The initial concentrations of all solutions used in experiments are detailed in Table S1 below.

**Table S1.** A summary of initial solution conditions for all experiments. Experiments were conducted at room temperature (19-20°C).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Se(VI)** | **Se(IV)** | **Ce3+** | **MES** | **PO43-** | **CO32-** | **AsO43-** | **GSH** |
| **pH** | **mM** | **mM** | **mM** | **mM** | **mM** | **mM** | **mM** | **mM** |
| Cerium-oxyanion precipitation experiments (Figure 1) |  |
| 7.0 | 7.1 | -- | 3.6 | 45 | -- | -- | -- | -- |
| 7.0 | 7.1 | -- | 4.3 | 45 | -- | -- | -- | -- |
| 7.0 | 7.0 | -- | 7.0 | 45 | -- | -- | -- | -- |
| 7.0 | 6.8 | -- | 13.6 | 45 | -- | -- | -- | -- |
| 7.0 | 6.0 | -- | 22.8 | 45 | -- | -- | -- | -- |
| 7.0 | 5.5 | -- | 42.4 | 45 | -- | -- | -- | -- |
|  |  |  |  |  |  |  |  |  |
| 7.0 | -- | 8.0 | 4.0 | 45 | -- | -- | -- | -- |
| 7.0 | -- | 8.0 | 4.8 | 45 | -- | -- | -- | -- |
| 7.0 | -- | 7.9 | 7.9 | 45 | -- | -- | -- | -- |
| 7.0 | -- | 7.4 | 14.8 | 45 | -- | -- | -- | -- |
| 7.0 | -- | 6.5 | 24.7 | 45 | -- | -- | -- | -- |
| 7.0 | -- | 5.5 | 42.0 | 45 | -- | -- | -- | -- |
|  |  |  |  |  |  |  |  |  |
| 7.0 | -- | -- | 5.7 | 45 | 11.8 | -- | -- | -- |
| 7.0 | -- | -- | 10.7 | 45 | 10.6 | -- | -- | -- |
| 7.0 | -- | -- | 19.4 | 45 | 10.0 | -- | -- | -- |
| 7.0 | -- | -- | 32.4 | 45 | 8.2 | -- | -- | -- |
| 7.0 | -- | -- | 49.0 | 45 | 6.4 | -- | -- | -- |
|  |  |  |  |  |  |  |  |  |
| 7.0 | -- | -- | 3.5 | 45 | -- | 7.0 | -- | -- |
| 7.0 | -- | -- | 10.5 | 45 | -- | 10.5 | -- | -- |
| 7.0 | -- | -- | 21.3 | 45 | -- | 10.7 | -- | -- |
| 7.0 | -- | -- | 49.6 | 45 | -- | 11.8 | -- | -- |
| 7.0 | -- | -- | 65.5 | 45 | -- | 8.4 | -- | -- |
|  |  |  |  |  |  |  |  |  |
| 7.0 | -- | -- | 5.7 | 45 | -- | -- | 11.9 | -- |
| 7.0 | -- | -- | 10.7 | 45 | -- | -- | 10.5 | -- |
| 7.0 | -- | -- | 19.4 | 45 | -- | -- | 9.7 | -- |
| 7.0 | -- | -- | 32.4 | 45 | -- | -- | 7.8 | -- |
| 7.0 | -- | -- | 49.0 | 45 | -- | -- | 6.3 | -- |
|  |  |  |  |  |  |  |  |  |
| Kinetics of cerium selenite precipitation (Figure 2) |  |
| 6.9 | -- | 7.8 | 15.6 | 45 | -- | -- | -- | -- |
| 7.0 | -- | 9.4 | 75.2 | 45 | -- | -- | -- | -- |
|  |  |  |  |  |  |  |  |  |
| Ce3+ removal by ion exchange resin – batch kinetics (Figure 3) |  |
| 7.0 | 9.0 | -- | 8.3 | 45 | -- | -- | -- | -- |
| 6.9 | 10.0 | -- | 29.5 | 45 | -- | -- | -- | -- |
| 7.0 | 9.0 | -- | 0.0 | 45 | -- | -- | -- | -- |
| 7.0 | 9.0 | -- | 8.3 | 45 | -- | -- | -- | -- |
|  |  |  |  |  |  |  |  |  |
| Selenite removal with glutathione (GSH) – vary GSH concentration (Figure S1) |
| 7.0 | -- | 8.0 | -- | 32 | -- | -- | -- | 0.0 |
| 6.9 | -- | 7.6 | -- | 32 | -- | -- | -- | 7.6 |
| 6.7 | -- | 7.2 | -- | 32 | -- | -- | -- | 14.4 |
| 6.3 | -- | 6.6 | -- | 32 | -- | -- | -- | 26.4 |
| 6.1 | -- | 6.1 | -- | 32 | -- | -- | -- | 30.5 |
|  |  |  |  |  |  |  |  |  |
| Selenite removal with glutathione (GSH) – vary pH, kinetics (Figure S1) |
| 6.0 | -- | 13.8 | -- | 32 | -- | -- | -- | 53.3 |
| 7.0 | -- | 13.9 | -- | 32 | -- | -- | -- | 53.3 |
| 8.0 | -- | 13.9 | -- | 32*a*  | -- | -- | -- | 53.3 |
|  |  |  |  |  |  |  |  |  |
| Determining 18O-selenate after Ce3+ removal by ion exchange resin (Tables 1 & 2) |
| 7.0 | 9.0 | -- | 0.0 | 45 | -- | -- | -- | -- |
| 7.0 | 8.9 | -- | 8.3 | 45 | -- | -- | -- | -- |
| 6.9 | 8.6 | -- | 18.3 | 45 | -- | -- | -- | -- |
| 6.9 | 8.5 | -- | 29.4 | 45 | -- | -- | -- | -- |
|  |  |  |  |  |  |  |  |  |
| Determining 18O-selenate after Se(IV) precipitation and Ce3+ removal by ion exchange resin (Table 3) |
| 7.0 | 9.0 | 0.0 | -- | 45 | -- | -- | -- | -- |
| 7.0 | 8.9 | 9.2 | -- | 45 | -- | -- | -- | -- |
| 6.9 | 8.9 | 9.2 | -- | 45 | -- | -- | -- | -- |
| 7.0 | 9.0 | 4.0 | -- | 45 | -- | -- | -- | -- |
| 7.0 | 9.0 | 4.0 | -- | 45 | -- | -- | -- | -- |
|  |  |  |  |  |  |  |  |  |
| Concentrating selenate onto ion exchange resin (section 3.3) |  |
| 7.0 | 10 | -- | -- | 45 | -- | -- | -- | -- |
| 7.0 | 10 | -- | -- | 45 | -- | -- | -- | -- |
| 7.0 | 10 | -- | -- | 45 | -- | -- | -- | -- |
| 7.0 | 1 | -- | -- | 45 | -- | -- | -- | -- |
| 7.0 | 0.063 | 0.063 | -- | 45 | 0.063 | -- | 0.32 | -- |
| 7.0 | 20 | -- | -- | 45 | -- | -- | -- | -- |
|  |  |  |  |  |  |  |  |  |
| Selenite removal with glutathione (GSH) – 18O selenate (Table S2) |  |  |  | -- | -- | -- |
| 7.0 | 7.9 | 0.0 | -- | 32 | -- | -- | -- | 0.0 |
| 6.8 | 7.8 | 0.0 | -- | 32 | -- | -- | -- | 37.5 |
| 6.8 | 7.7 | 6.6 | -- | 32 | -- | -- | -- | 37.5 |
| 7.0 | 0.0 | 10.7 | -- | 32 | -- | -- | -- | 0.0 |
|  |  |  |  |  |  |  |  |  |

*a* The buffer used in this batch reactor was TAPS instead of MES.

***Selenite removal by reduction with glutathione***

Glutathione (C10H17N3O6S, GSH) was also evaluated as a reagent to chemically separate selenate and selenite because it readily reduces dissolved selenite to Se(0) but not selenate over a broad pH range [1]. Glutathione may be a preferred reagent for selenite separation when Ce3+ precipitation is not suitable, such as at higher pH where Ce(OH)3 may precipitate, or when other organic or inorganic compounds could interfere with cerium selenite precipitation. Other chemical reductants of selenite such as ascorbic acid [2], glucose [3] or borohydride were not considered due to their acidic pH or hydrothermal requirements which can overwrite selenate 18O values with H2O. The reaction generally takes four GSH molecules and results in the formation of the product diglutathione (GSSG):

 4GSH $+$ SeO32- $+$ 2H+ $ \rightarrow $4GSSG $+$ Se $+$ 3H2O (eqn 1)

Near complete selenite removal and Se(0) formation was observed at pH 7.0 when GSH was provided at or above stoichiometric amounts (GSH:selenite ratios of 4:1 and 5:1) (Figure S1a). The Se(0) was identified visually and typically appeared after a few hours as a dispersed red precipitate akin to amorphous red Se(0) but later evolved to a settled black solid after 24 hours. Both red elemental selenium [1] and monoclinic black elemental selenium [4] have been observed during abiotic GSH reduction of selenite. Less than stoichiometric GSH:selenite ratios resulted in incomplete selenite removal. Selenite removal was less than 100% for all solution conditions studied, however, and residual selenite could have been caused by reoxidation of Se(0) by reactive oxygen species such as H2O2 formed as intermediates [1].

A reaction kinetics experiment between GSH and selenite showed rapid removal of selenite at pH values 6.0 to 8.0 over 24 hours with a GSH:selenite ratio of 4:1 (Figure S1b). Selenite removal ceased within 0.5 h for pH 6.0 and 7.0 which is consistent with selenite reduction kinetics completed within 20 min under similar conditions [1].

Despite confirming selenite reduction kinetics and extent, GSH treatment was not able to preserve selenate 18O values as measured by IRMS from BaSeO4 solids. While GSH did remove the entirety of selenite and none of selenate within a solution of both oxyanions, the measured selenate-18O (-3.26 ± 0.28‰) increased by approximately 3‰ from unexposed selenate (-7.22 ± 0.11‰) (Table S3). A similar increase in selenate-18O was observed when GSH was present with selenate alone (-4.79 ± 0.19‰), and this indicates GSH molecules interfere with BaSeO4 precipitation. GSH could have influenced 18O values by co-precipitating with BaSeO4 due to GSH ability to form complexes with Ba2+ [5] and providing additional O of different isotopic values, or by creating H2O2 that oxidized small amounts of selenite to selenate which adjusted selenate 18O. Due to variable selenite removal extents and inability to preserve selenate 18O, glutathione should not be considered appropriate reagent for selective chemical removal of selenite.



**Figure S1.** Selenite removal from solution by reductive precipitation to Se(0) using glutathione. (A) Selenite removal percentage with respect to GSH:SeO3 ratio at pH 6-7 in 45 mM MES buffer. (B) Kinetics of selenite removal with a 4:1 ratio of GSH:SeO3 at varying pH.

**Table S2.** A summary of GSH precipitation methods applied to remove selenite at pH 7.0 with selenate δ18O isotope analysis. Three types of batch reactors were prepared including selenite, selenate, and both selenite and selenate solution. After combining with solutions, [GSH] = 37.5mM. Ratios were GSH:Se(IV) = 4.21:1, GSH:Se(VI) = 4.81:1, and GSH:Se(IV) = 5.68:1 & GSH:Se(VI) = 4.87:1.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Solution composition | Initial Se(IV)(mM) | Final Se(IV)(mM) | ΔSe(IV)(%) | Initial Se(VI)(mM) | Final Se(VI)(mM) | ΔSe(VI)(%) | δ18O-selenate(%) |
| Se(VI) alone | -- | -- | -- | 7.9 | 7.9 | 0.00 | -7.22 ± 0.11 |
| GSH+Se(VI) | -- | -- | -- | 7.8 | 7.9 | -0.51 | -4.79 ± 0.19 |
| GSH+Se(IV)+Se(VI) | 6.6 | 0.0 | 100.0 | 7.7 | 7.8 | -1.56 | -3.26 ± 0.28 |
| Se(IV) alone (control) | 10.7 | 0.0 | 99.9 | -- | -- | -- | -4.59 ± 0.28 |

**Table S3.** Thermodynamic modeling of species concentrations at equilibrium at three different pH values for four selected solution conditions. Units are molar concentrations (M).

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| **species** |  **pH 6.0** |  **pH 7.0** |  **pH 7.7** |
| 30 mM CeCl3, 45 mM MES solution |
|

|  |
| --- |
| Ce+3 |
| CeCl+2 |
| CeOH+2 |
| Cl-1 |
| H+1 |
| H-MES (aq) |
| MES-1 |
| Na+1 |
| NaCl (aq) |
| NaOH (aq) |
| OH- |

 |

|  |
| --- |
| 2.83E-02 |
| 1.57E-03 |
| 2.96E-05 |
| 8.74E-02 |
| 1.34E-06 |
| 2.56E-02 |
| 1.93E-02 |
| 4.39E-02 |
| 1.01E-03 |
| 3.94E-10 |
| 1.34E-08 |

 |

|  |
| --- |
| 2.81 x10-2 |
| 1.54 x10-3 |
| 2.90 x10-4 |
| 8.74 x10-2 |
| 1.34 x10-7 |
| 5.23 x10-3 |
| 3.97 x10-2 |
| 4.39 x10-2 |
| 1.01 x10-3 |
| 3.92 x10-9 |
| 1.35 x10-7 |

 |

|  |
| --- |
| 6.44 x10-3 |
| 4.46 x10-4 |
| 4.01 x10-4 |
| 8.84 x10-2 |
| 2.58 x10-8 |
| 1.22 x10-3 |
| 4.37 x10-2 |
| 4.38 x10-2 |
| 1.12 x10-3 |
| 2.08 x10-8 |
| 6.51 x10-7 |

 |
|  Ce(OH)3(s) |  0.0 |  0.0 |  2.27 x10-2 |
|  |  |  |  |
| 100 mM CeCl3, 45 mM MES solution |  |
|

|  |
| --- |
| Ce+3 |
| CeCl+2 |
| CeOH+2 |
| Cl-1 |
| H+1 |
| H-MES (aq) |
| MES-1 |
| Na+1 |
| NaCl (aq) |
| NaOH (aq) |
| OH- |

 |

|  |
| --- |
| 8.53 x10-2 |
| 1.45 x10-2 |
| 8.49 x10-5 |
| 2.82 x10-1 |
| 1.35 x10-6 |
| 2.45 x10-2 |
| 2.04 x10-2 |
| 4.21 x10-2 |
| 2.84 x10-3 |
| 3.41 x10-10 |
| 1.35 x10-8 |

 |

|  |
| --- |
| 8.46 x10-2 |
| 1.44 x10-2 |
| 8.46 x10-4 |
| 2.82 x10-1 |
| 1.35 x10-7 |
| 4.82 x10-3 |
| 4.01 x10-2 |
| 4.21 x10-2 |
| 2.84 x10-3 |
| 3.41 x10-9 |
| 1.35 x10-7 |

 |

|  |
| --- |
| 9.54 x10-3 |
| 1.71 x10-3 |
| 4.80 x10-4 |
| 2.95 x10-1 |
| 2.69 x10-8 |
| 1.14 x10-3 |
| 4.38 x10-2 |
| 4.18 x10-2 |
| 3.20 x10-3 |
| 1.84 x10-8 |
| 6.76 x10-7 |

 |
|  Ce(OH)3(s) |  0.0 |  0.0 |  8.82 x10-2 |
| 30 mM CeCl3, 9 mM Na2SeO3, 45 mM MES solution |
|

|  |
| --- |
| Ce+3 |
| CeCl+2 |
| CeOH+2 |
| Cl-1 |
| H+1 |
| H2SeO3 (aq) |
| H-MES (aq) |
| HSeO3-1 |
| MES-1 |
| Na+1 |
| NaCl (aq) |
| NaOH (aq) |
| OH- |
| SeO3-2 |

 Ce2(SeO3)3 (s) Ce(OH)3 (s) |

|  |
| --- |
| 2.26 x10-2 |
| 1.36 x10-3 |
| 2.51 x10-5 |
| 8.82 x10-2 |
| 1.32 x10-6 |
| 2.05 x10-16 |
| 2.58 x10-2 |
| 6.63 x10-13 |
| 1.91 x10-2 |
| 1.75 x10-2 |
| 4.25 x10-4 |
| 1.61 x10-10 |
| 1.33 x10-8 |
| 6.16 x10-15 |

 3.00 x10-3 0.0 |

|  |
| --- |
| 2.24 x10-2 |
| 1.32 x10-3 |
| 2.44 x10-4 |
| 8.82 x10-2 |
| 1.33 x10-7 |
| 2.10 x10-18 |
| 5.32 x10-3 |
| 6.83 x10-14 |
| 3.96 x10-2 |
| 1.75 x10-2 |
| 4.21 x10-4 |
| 1.60 x10-9 |
| 1.33 x10-7 |
| 6.41 x10-15 |

3.00 x10-3 0.0 |

|  |
| --- |
| 5.89 x10-3 |
| 4.35 x10-4 |
| 3.85 x10-4 |
| 8.91 x10-2 |
| 2.56 x10-8 |
| 1.60 x10-19 |
| 1.23 x10-3 |
| 2.58 x10-14 |
| 4.37 x10-2 |
| 1.75 x10-2 |
| 4.64 x10-4 |
| 8.44 x10-9 |
| 6.45 x10-7 |
| 1.09 x10-14 |

 3.00 x10-3 1.73 x10-2 |
|  |  |  |  |
|  |  |  |  |
| 42.4 mM CeCl3, 5.5 mM Na2SeO3 (7.7:1), 45 mM MES solution |
|

|  |
| --- |
| Ce+3 |
| CeCl+2 |
| CeOH+2 |
| Cl-1 |
| H+1 |
| H-MES (aq) |
| HSeO4-1 |
| MES-1 |
| Na+1 |
| NaCl (aq) |
| NaOH (aq) |
| OH- |
| SeO4-2 |
|  Ce2(SeO3)3 (s) |
|  Ce(OH)3 (s) |
|  |

 |  |

|  |
| --- |
| 3.93 x10-2 |
| 2.77 x10-3 |
| 3.78 x10-4 |
| 1.23 x10-1 |
| 1.36 x10-7 |
| 5.08 x10-3 |
| 1.09 x10-8 |
| 3.99 x10-2 |
| 5.43 x10-2 |
| 1.67 x10-3 |
| 4.69 x10-9 |
| 1.37 x10-7 |
| 5.50 x10-3 |

 0.0 0.0 |  |
|  |  |  |  |



**Figure S2**. X-ray diffractogram of cerium selenite (Ce2(SeO3)3) precipitated from a solution containing approximately 8 mM sodium selenite and 32 mM mM cerium(III) chloride at pH 7. Data in counts is the blue line, and the black bars are standard reflections for Ce2(SeO3)3 from particle diffraction card No. 00-051-1096. The two broad XRD reflections centered around 28 and 50 degrees are generally consistent with the multiple close spacing indicated by the reference data, although individual standard reflections were not discerned at this instrument resolution.



**Figure S3.**  Cumulative recovery of selenate with respect to volume of eluent (500 mM NaCl) passed through BioRad AG4-X4 ion exchange resin that was previously loaded with approximately 0.1 mmoles of selenate. The four data sets represent four trials of loading approximately 0.1 mmoles of selenate using varying volumes (10-100 mL) of solution with varying selenate concentrations (1-10 mM).

Selenite was first loaded onto the resin by filtering either 10 mL of 10 mM selenate solution (green open squares, blue closed diamonds, purple open circles) or 100 mL of 1 mM selenate solution (red open diamonds) for a total selenate loading of 0.1 mmoles. Sorbed selenate oxyanions were then replaced with chloride ions by passing through 500 mM NaCl solution. Samples were taken every 2-8 mL and measured for selenate concentration by ion chromatography. Cumulative selenate recovery was calculated as the sum of mmoles selenate measured in all prior aliquots of eluent divided by the total mmoles of selenate loaded on to the resin. The mmoles selenate in each aliquot was calculated as the measured concentration of selenate multiplied by the aliquot volume. The total mmoles of selenate loaded on to the resin was calculated by the concentration of selenate in the initial loading solution multiplied by the volume of loading solution. No selenate was observed in the loading solution after passing through the ion exchange column.

The flow rate of 500 mM NaCl eluent was 1.0 mL/min. The results of four replicate recovery tests show that 100% of loaded selenate can be recovered after elution of 20-25 mL of eluent.



**Figure S4.**  Cumulative recovery of selenite and selenate with respect to volume of eluent (50 mM NaCl for elution of selenite, and 500 mM NaCl for elution of selenate) passed through BioRad AG4-X4 ion exchange resin that was previously loaded with approximately 0.1 mmoles of selenite and 0.1 mmoles of selenate. The selenite and selenate oxyanions were loaded onto the resin by passing through 10 mL of solution with 10 mM of each oxyanion. Arrows indicate start of each eluent addition. Flow rate was 1.0 mL/min. 100% of each oxyanion was recovered. Cumulative Se recovery values were calculated as described in the caption of Figure S2.

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