ISOLATION OF SELENATE OXYANIONS AND CHARACTORIZATION OF SELENATE $\delta^{18}O$ FROM DIVERSE AQUEOUS SOLUTIONS

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ABSTRACT

The redox transformations, phase transformation, and mobility of selenium species play an important role in the environment in terms of the transport and bioavailability of selenium oxyanions, selenium contamination in waters, and toxicity to organisms. Several selenium removal techniques have been developed to decrease the selenium concentration in water such as ion exchange, reduction, precipitation, and bioremediation, among others. Stable isotope approach such as stable selenium isotopes (δ^{78}\text{Se}) has been used and reported in the literature to study kinetic isotope effects (KIE) of selenate reduction in abiotic and biotic environment.

Recently, a novel approach that observed KIE of stable oxygen isotopes in selenate (δ^{18}\text{OSeO}_4) was developed as a tool for characterizing selenate reduction. However, selenate can co-exist in solution with selenite and various oxyanions including arsenate, carbonate, phosphate, and sulfate, all of which can interfere with the isotopic analysis of selenate. These oxyanions can co-precipitate during barium selenate (BaSeO_4) precipitation used to measure δ^{18}\text{OSeO}_4. Therefore, selenite and other oxyanions must first be removed. This work tested glutathione reductive precipitation and cerium(III) precipitation methods that respectively reduced Se(IV) to Se(0) or precipitated cerium oxyanions to remove selenite and isolate selenate prior to BaSeO_4 precipitation. Both methods show that approximately 99% of selenite was removed under pH 7.0 within 10 mM selenite and 10 mM selenate. No removal of selenate with glutathione was observed but 4.2% of selenate was co-precipitated with cerium(III) chloride. This problem was improved by amending cerium(III) chloride addition techniques in a drop-by-drop addition manner for slower cerium-selenite precipitation. The isotope results show that the δ^{18}\text{OSeO}_4 values were affected by cerium(III) selenite precipitation and glutathione reductive precipitation methods compared to selenate alone samples, which could be due to co-precipitation of
cerium(III) into BaSeO$_4$ or unforeseen precipitation of cerium(III) hydroxide (Ce(OH)$_3$). Cerium(III) ions were removed with ion exchange resin, and little difference between $\delta^{18}$O$_{SeO_4}$ before and after was observed. Based on ease of use, less expense, ability to preserve $\delta^{18}$O$_{SeO_4}$, and statistically no difference of $\delta^{18}$O$_{SeO_4}$ values, cerium (III) chloride precipitation is recommended as the best selenite removal method.

Cerium(III) chloride also displayed an expected result of roughly 99% removal of arsenate, carbonate, and phosphate at pH 7.0. Sulfate is difficult to be isolated from selenate because of its similarity to selenate, which future work will be concentrated on the removal of sulfate and polish experimental methods to process natural water samples.
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CHAPTER I INTRODUCTION

1.1 Selenium and selenium oxyanions in the environment

Selenium and selenium oxyanions play an important role in the environment due to its wide range of redox states and phases, which encompass a selenium redox transformation cycle. The selenium phases cover dissolved, solid, and gaseous selenium, and redox states include +6, +4, 0, and -2. Selenate (+6, Se(VI), SeO$_4^{2-}$), selenite (+4, Se(IV), SeO$_3^{2-}$), and hydroselenite (+4, Se(IV), HSeO$_3^{-}$) are dissolved selenium oxyanions, and both selenite and selenate are highly soluble. However, comparing the sorption of selenite and selenate, selenite and hydroselenite tend to sorb to a greater extent than selenate, and both can be bonded to solid phase minerals such as soil, goethite, and hematite decreasing selenite or selenate mobility (Frankenberger 1999, Johnson et al. 2004, and Rovira et al. 2008). Solid selenium includes elemental selenium (0, Se(0)) and selenide (-2, Se(-II)), and selenide can also be a gaseous species as H$_2$Se (Dixon et al. 1972).

The mobility of selenium oxidized compounds in natural water and aquatic environments has led to selenium contamination in soil and water (Charlet 2007 and Rowland et al. 2003) as well as toxicity to animals and plants (Ohlendorf 1989 and Srivastava and Ma 2005). Therefore, there is a need to remove desorbed selenium from water or immobile if in the subsurface to minimum expansion. Most works have focused on the study of selenate and selenite reduction by different reductants to eliminate the harmful impacts of selenium oxyanions on aquatic environments. Se(VI) can be reduced by mineral reductants such as green rust (Johnson et al., 2003, Hayashi 2009, and Larese-Casanova and Schellenger 2013), TiO$_2$ and Ag-loaded TiO$_2$ with UV light (Tan et al. 2003), bacteria such as anaerobic bacteria (Oremland, 1989) and
Psedomonas stutzeri (Lortie 1992), and nitrate reductases of *Rhodobacter sphaeroides* (Sabaty *et al.* 2001). Moreover, Chung *et al.* (2006) has studied bioreduction to reduced selenate using a hydrogen-based membrane biofilm reactor, and a new developed technique, on-line pre-reduction of selenite by thiourea, was introduced by Qiu *et al.* (2006). For Se(IV) reduction, its reduction has been focused on the iron sulfide with clay minerals (Bruggeman *et al.* 2005, Charlet *et al.* 2007, and Scheinost *et al.* 2008), and selenite also can be reduced by glutathione (GSH) (Kessi *et al.* 2004 and Cui *et al.* 2008) that is a component in glutathione peroxide and superoxide in animals (Burau 1985). The reduction of Se(IV) to Se(0) can result in various selenium allotropes (Kessi *et al.* 1999 Johnson & Co. 1886), which have been known to be at least eight structurally distinct forms of selenium (Greenwood and Earnshaw 1997). The reduction of selenite also can be involved in bacteria such as *Rodospirillum rubrum* and *Rhodobacter capsulatus*, which observed the formation of orange-red selenium (Kessi *et al.* 1999 and Kessi 2006). Furthermore, some works have shown that few reductants can reduce not only selenate but also selenite under different conditions. Dowson (1968) found selenite can be reduced by hydrogen sulfide in weakly acid solution, but for selenate, only under a strongly acidified solution with hydrochloric acid, it is reduced to selenite and then precipitated as elemental selenium. In the environment, therefore, likely reductants for selenite are microbes and mineral sulfide, and for selenate are green rust, microbes, and physical and chemical combined techniques.

In addition, selenium and sulfur have similar chemical properties. Selenium and sulfur are in the same elements group below oxygen, therefore, they have the same oxidation states including -2, 0, +4, and +6 that form oxynions such as selenate (SeO$_4^{2-}$) and selenite (SeO$_3^{2-}$) as well as elemental selenium (Se(0)) and hydrogen selenium (H$_2$Se). Sulfur also includes the
oxidation states: -2, 0, +4, and +6, and its oxyanions are sulfate (SO$_4^{2-}$), sulfite (SO$_3^{2-}$), sulfur (S(0)), and hydrogen sulfide (H$_2$S). Selenium can form halides such as florine and chlorine, and also, it reacts with oxygen to form various oxidized selenium compounds (Hoffmann and King 1997). Additionally, selenate and selenite also can be precipitated by barium chloride as barium selenate and barium selenite. In order to study the transformation of selenium without the interference of sulfur, the decoupling of selenium and sulfur is necessary. However, due to the similarity of sulfur and selenium, they can react in similar ways, including precipitation, sorption, reduction, and ion exchange. They were found coexisting with sulfur in pyrite with similar properties (Yamamoto et al. 1983). Selenium is also commonly associated with sulfate in mineral like gypsum (Tanji 1986). In addition, techniques used to remove sulfur that are similar to those for removing selenium such as membrane filtration (reverse osmosis, and electrical dialysis), reductive precipitation in bioremediation, and ion exchange (Bowell 2004). Selenium releases from selenium rich sediments in the water, and is oxidized into selenium oxyanion, which result in the contamination in the water as well as in the ecosystem.

1.2 Selenium contaminations issues

Selenium was naturally found in a variety of soils, and it is also from coal, black shales and petroleum (Lakin 1973). It is commonly from the Cretaceous sedimentary rocks that contain rich selenium (Seiler et al. 1999 and Burau 1985). The oxidized selenium can be released from the sediment of Cretaceous-age shale into the ocean that results in the uptake by animals and microorganisms and can be accumulated in their body. Selenium environmental contamination has occurred in the Western United States such as Kesterson Reservoir in the San Joaquin Valley in California (Seiler 1992, Tanji et al. 1986, and Zhang et al. 2005), which led to
an elevation of selenium concentration in the drainage water. The wide spread selenium contamination with high concentration in the Western United States has been studied because of the residual selenium form the seepage of rocks and soil as well as the irrigation water from draining water (Seiler 1992). Irrigation practices applied to flush salts from the selenium contained soils can produce selenium rich effluent that caused the increasing of selenium concentration in draining water (Schoups et al. 2005). Furthermore, selenium is a constituent of petroleum, and it has been reported that crude oils from the San Joaquin Valley of California have a very high selenium concentration, and the selenium was discharged to the refinery wastewater treatment system to be removed by biological treatment (Gerhardt et al. 1992). Besides, the natural sources of selenium, Vorob’eva and Kushchevskaya (2007) also found the main anthropogenic source of selenium to drinking water, which is from enterprises discharging products and agricultural using fertilizers. Therefore, high selenium concentration can further contaminate the water that was taken by animals and livestock or plant, and even the indirect impacts on humankinds.

1.3 The health effects of selenium

Dissolved selenium has a very low concentration in natural water. The concentration in the seawater is about only 0.09 µg/l, and in the freshwater, the concentration is typically under 1 µg/L (Hem 1985). In addition, selenium was identified as an essential trace elemental nutrient to human and animals in the late 1950’s (Fordyce et al. 2000). However, the toxicity to organisms can depend on its concentration and speciation (Kessi 2004 and Johnson et al. 1999). There has been reported that the selenium heath effects include both selenium toxicity and selenium deficiency. For example, selenium toxicity can cause the deformities in embryos and larvae that
results in the failure reproduction in fish and wildlife (Seiler et al. 1999). For selenium deficiency, it is due to the insufficient taking selenium either for human or animals, which can induce growth depression (ill-thrift) and white-muscle disease (Burau 1985, Fordyce 2000, and Rayman 2000).

Overall, the selenium health impacts on either to human or animals and plant will relate to the selenium redox transformation that can provide a full understanding of how selenium oxyanions transported in the environment and led to selenium environmental impacts. Methods including selenate isolation and selenate precipitation were developed in this project based on some previous similar works.

1.4 Treatment of selenium-bearing water

Several selenium removal methods or technologies have been presented in different papers based on experiments including media filtration, chemical treatment, and biomediated removal (Moore 2011).

Physical treatments include filtration (such as sand filtration, clay filtration, and membrane filtration) (Kuan 1998 and Goh 2004), ion exchange (Moore 2011), and evaporation (Golder Associates 2009). Membrane filtration is a commonly used reliable treatment to purify water but can have a high operating cost. This techniques use pressure gradient to force water through the membrane but retaining the contaminants. Both reverse osmosis (RO) and nanofiltration (NF) are applied in the removal of selenium. For reverse osmosis, its membrane is non-porous, therefore, particles and some low molecular weight such as salt ions can be excluded by the membrane (Sagle and Freeman 2004). However, for the nanofiltration, a hydrophobic membrane layer is used to reject ions. Ion exchange is another common method used to remove
ions. It removes a contaminant ion from solution using acidic or alkaline resins by exchanging for an ineffective ion attached to an immobile solid surface. Evaporation has been considered for selenium treatment in California, which includes simple evaporation ponds and enhanced evaporation system (EES) (Golder Associates 2009). The EES accelerates evaporation by spraying water in the air, and the simple ponds that are solar evaporation ponds use large and shallow pond to provide a high evaporation potential. According to the National Strong-Motion Project (NSMP) at 2007, the studied site in California using evaporation technique only reduced 25% of selenium concentration.

Chemical treatments mainly include reductive precipitation to Se(0) and sorption onto engraved media. The reductive precipitation of selenite to Se(0) can occur with zero-valent iron (ZVI) (Zhang 2008), and with sodium sulfide (Geoffroy 2010). Sorption can occur on goethite and hematite (Rovira et al. 2008), on green rust (Hayashi 2009). Additionally, chemical treatments also include cementation, coagulation (Golder Associates 2009), and electrocoagulation (Mavrov 2006). The removal of selenium is associated with sorption and reduction but with different selected materials and chemicals. The Golder Associates Inc. (2009) and Lovett (2008) have studied the zero-valent iron method to remove selenium by reducing selenite or selenate to elemental selenium. The iron oxides hematite and goethite for sorption of selenate and selenite were studied by Rovira et al. (2008) under different parameters such as pH and initial selenium concentrations, which found pH could be an influential factor for sorption onto natural iron oxides. Another sorption and reduction treatment of selenium was by a Fe(II)-Fe(III) hydroxide-sulfate (green rust, GR), which found that the reduction of selenate can be either on GR surfaces by adsorption and reduction or in GR interlayers by coprecipitation and reduction (Johnson and Bullen 2003 and Myneni et al. 1997). Coprecipitation with ferrihydrite
was applied by USEPA in Best Demonstrated Available Technology to remove selenium from wastewater and is reviewed by (Twidwell et al. 2000). With this method, selenium is removed by adsorbing on the iron surface and followed by precipitation with ferrihydrite from solution.

Cementation with addition of catalyst to increase selenium removal was applied to remove heavy metals such as selenium and arsenic from water (MSE 2001). The catalyzed cementation is described the processes that selenium is removed from solution on the iron surface at a low pH.

Coagulation to remove selenium was found in using electrocoagulation that use electrolysis with graphite or stainless steel cathodes in conjunction with a metal anode forming insoluble precipitates, and a hybrid electrocoagulation-microfiltration process was tested to remove selenium and other have metals by Mavrov et al. (2006).

For biotreatment methods, it can be accomplished by a selection of microbial community to remove selenium by reducing selenium oxyanions. Some studies have shown that selenium was removed by microbial reduction of selenium oxyanions to elemental selenium (Maiers et al. 1988, Oremland 1990 and1989, and Lortie et al. 1992), and biofilms using sulfate-containing media with sulfate reducing bacteria (Hockin 2006). In addition, Ghung et al. (2006) has combined the bioreduction and biofilms methods to remove selenate reducing to Se(0) from wastewater.

1.5 Use of stable isotopes to characterize elemental selenium transformation

Contaminant stable isotope has been provides useful information of contaminant origin by analyzing their target elements such as selenium isotope (Philp 2007). Selenium geochemistry is complex which leads that it is difficult to fully understand and predict the transport, cycling, and bioavailability of selenium as well as its potential toxicity to organisms.
Furthermore, selenium released from sediments with high oxidation state selenium oxyanions has high mobility, bioavailability due to the reduction either by microorganism or by chemicals, which needs to be identified and quantified but it can not be satisfied by site management strategies. Selenium stable isotope study, therefore, was introduced to understand selenium transformation in both abiotic and biotic environment. Selenium stable isotopic ratios have been studied by Johnson’s group (1999, 2000, 2003, 2004, and 2008), which shows that it can serve as an indicator of selenium sources, reduction of selenium oxyanions, and biochemical processes in order to analyze and quantify selenium speciation. Moreover, the reduction of Se(VI) to Se(0) leads to the analysis of selenium isotopic fractionation that is expressed by kinetic isotopic effect by measuring the enrichment of heavier selenium in unreacted selenate (Johnson 2000). Therefore, it implies that the lighter selenium isotope is reduced during the reaction by breaking Se-O bond. Due to the reduction sensitivity of selenium stable isotope, the kinetic isotope effects showed that selenate reduction by green rust performed greater isotopic fractionation (Johnson and Bullen 2003).

In the selenium redox transformation study, most works focused on the measuring of $\delta^{78}$Se isotope values (Reamer and Veillon 1983 and Johnson 1999, 2004). Compared with the selenium isotope, a novel stable oxygen isotopic approach was developed for selenate reduction and transformation study by Larese-Casanova’s group. Oxygen is a common element, and $\delta^{18}$O values have been used as tracers of physical, chemical, and biological reactions in the oxyanions of nitrogen, sulfur, phosphorus, and chlorine (Xue et al., 2009, Farquhar et al., 2008, Jaisi et al., 2010, and Bohlke et al., 2005). There were no reports showed measuring $\delta^{18}$O values in selenium oxyanions to study its redox transformation cycle, and therefore, the quantifying of
$\delta^{18}\text{O}$ isotope values in selenium oxyanions is a prospective technique to trace chemical reaction pathways and to determine environmental sources of selenium.

In order to measure $\delta^{18}\text{O}$ values in selenate, Larese-Casanova and Blake (2013) have developed barium selenate precipitation method, which found that $\delta^{18}\text{O}$ isotope value is stable in the selenate oxyanions as barium selenate precipitates. Selenate was recovered after precipitation with barium chloride at the barium chloride:selenate ratio of 5:1 under pH 7. The barium selenate solids were transferred by centrifuging, washing with DI water and methanol, and drying covered by aluminum foil. Oxygen isotope measurement was performed on approximately 300 microgram of barium selenate using a thermal chemolysis elemental analyzer and a isotope ratio mass spectrometer.

Dissolved selenate in natural waters could be presented with selenite and other oxyanions of sulfur, phosphorus, arsenic, and carbon, which have impacts on the preparation of barium selenate because of the precipitation of barium chloride with other oxyanions, and also influence on the analysis of $\delta^{18}\text{O}_{\text{SeO}_4}$ values due to the impurity of obtained barium selenate. In order to remove the interfered oxyanions, methods are applied and developed based on other relevant studies. GSH reductive precipitation and cerium(III) selenite precipitation methods were used and developed to study the isolation of selenite from selenate. Meanwhile, cerium(III) chloride precipitates with other ions was presented as an extension of cerium(III) selenite precipitation method to remove other oxyanions. Barium selenate precipitation (Larese-Casanova and Blake 2013) used for selenate $\delta^{18}\text{O}$ isotopic values measurement was reviewed and applied in this project.
1.5.1 GSH reductive precipitation method

Glutathione is one of the most abundant thiols found in the eukaryotic cells, the cyanobacteria, and the proteobacteria, which has been known to be involved in the selenium metabolism (Kessi and Hanselmann 2004). Also, it is a component of glutathione peroxide in animals. Due to the inhibition of selenium in carbon dioxide production and oxygen uptake of yeast cells, the selenite oxidation with sulfhydryl was found (Painter 1941), and the selenite reduction by GSH precipitation was first demonstrated by Painter (1941) who has found a high reactivity of selenite with thiol group (Reaction 1). Furthermore, Ganther (1966, 1968, and 1971) has proposed glutathione could reduce selenite in mammalian tissues. According to Painter’s thiol group and selenite reaction, the reaction can be rewritten in terms of GSH (Reaction 2). Gather (1969), Kessi (2004) and Cui (2008) and others have studied the biotic (biological) and abiotic (chemical) selenite reduction and the reduced selenite species under acidic and neutral conditions using spectrophotometric methods and electrospray ionization tandem mass spectrometry method.

\[
4RSH + \text{SeO}_2 \rightarrow 2\text{RSSR} + \text{Se}(0) + 2\text{H}_2\text{O} \quad \text{(Reaction 1 (Painter 1941))}
\]

\[
4\text{GSH} + \text{H}_2\text{SeO}_3 \rightarrow 2\text{GSSG} + \text{Se}(0) + 2\text{H}_2\text{O} + 2\text{OH}^- \quad \text{(Reaction 2)}
\]

GSH reductive precipitation method was applied to isolate selenite from selenate based on the chemical selenite reduction. An optimum ratio and an effective selenite removal pH condition were determined in this study.

1.5.2 Cerium(III) precipitation method

A few works discussed the removal of selenite by selective precipitation methods such as cerium(III) selenite precipitation, however, some studies showed the properties of cerium(III)
chloride (Gospodinov et al. 2003, Sarju Prasad 1963, and Bouchaud et al. 2012), which presents a prospective method to remove selenite in order to compare with the GSH reductive precipitation method. The presented works focused on studying the environment of cerium(III) species and its formation that very help to understand the conditions of cerium(III) reactions such as pH and reaction temperature. However, it is still unknown whether cerium(III) chloride will precipitate with selenate mixed with either selenite or no selenite.

Cerium(III) selenite precipitation method was applied in this project to removed selenite selectively without precipitating seleante in a mixed selenite and selenate solution under a determined pH, and it was compared with the GSH reductive precipitation method. Moreover, other anions such as phosphate, arsenate, and carbonate also exist with selenite and selenate in an aqueous solution. Based on few studies of phosphate, arsenate, and carbonate precipitation with cerium(III) chloride from Ferri (1983) and Harron (2008), cerium(III) chloride may used to remove phosphate, arsenate, carbonate and other possible impact on ions as well.

1.5.3 Ion exchange method

Cerium(III) ions are likely to affect the results of δ^{18}O_{SeO_4} values by precipitating with cerium hydroxide, ion exchange was introduced to remove cerium(III) ions from selenate mixed solutions. Ion exchange method is an environmentally friendly and reusable method, and it has been applied in water softening and wastewater treatment (EPA 1981). Ion exchange resins are very small plastic beads, its structure is a polymer on which a fixed ion has been permanently attached and cannot be replaced during ion exchange (Rohn and Hass 2008). Ion exchange is a reversible chemical reaction that a cation or anion from solution is exchanged for a similarly
charged ion attached to immobile exchange particles (EPA 1981). Heavy metal removal
processes routinely use ion exchange to treat in industrial wastewater (Peters et al. 1985).

In order to completely isolate selenate and to avoid the interference from any other ions, ion exchange was applied to remove cerium(III) cations. Amberlite IR 120 sodium cation ion exchange resin was used to remove cerium(III) ions. It is a gel type strongly acidic cation exchange resin of the sulfonated polystyrene type that is used for water softening as well as water demineralization. Valverde et al. (2001) has studied the ion exchange equilibria of copper ion, cadmium ion, zinc ion and sodium ion using Amberlite IR-120 resin. The batch mode and column mode ion exchanges were applied to find a more beneficial technique to conduct cerium(III) cations removal.

Overall, after the application of the precipitation and ion exchange methods, the $\delta^{18}$O$_{SeO_4}$ values will be analyzed for all the applied methods to find a selenate isolation approach that does not change $\delta^{18}$O$_{SeO_4}$ value while using the methods not only GSH reductive precipitation or cerium(III) precipitation, but also ion exchange.

1.6 Objective and hypothesis

The overall objective of this project is to isolate selenate oxyanions from water containing other oxyanions such as selenite, carbonate, phosphate, arsenate, and other potential influential ions and processes $\delta^{18}$O value of selenate. Two precipitation methods including GSH reductive precipitation method and cerium(III) precipitation method were evaluated to remove selenite oxyanions from selenate contained water, and ion exchange method was used to separate cerium(III) cations from selenate contained water. Finally, selenate $\delta^{18}$O isotope values were
analyzed and determined to find the more effective selenite and cerium(III) removal methods that produced purified selenate solutions for further studies.

The hypothesis in this project are summarized as follows:

• Hypothesis 1: GSH reductively precipitates at least 99% of dissolved selenite, does not remove any selenate, and does not change $\delta^{18}O_{SeO_4}$ values of selenate at pH 7.0.

• Hypothesis 2: Cerium(III) precipitates at least 99% of dissolved, does not remove any selenate, and does not change $\delta^{18}O_{SeO_4}$ values of selenate at pH 7.0.

• Hypothesis 3: Cation exchange resin removes at least 99% of cerium(III) ions, does not remove any selenate ions, and does not change $\delta^{18}O_{SeO_4}$ values using batch methods.

• Hypothesis 4: Cerium(III) precipitation will removal 99.9% phosphate, carbonate, and arsenate, and does not change $\delta^{18}O_{SeO_4}$ values of selenate at pH 7.0.
CHAPTER II EXPERIMENT METHODS

2.1 Chemicals, Materials and Instruments

All stock solutions used for the experiments and chemical analysis were prepared using deionized (DI) water (Milli-Q® Direct 8 for production of pure (Type 3) water (8 L/hour) and ultrapure (Type 1) water 100 – 230 V ± 10 %, > 18 megaohm) and the following high purity chemicals: sodium selenite (Sigma, 98%, Na$_2$SeO$_3$), sodium selenate (Acros Organics, 98%, Na$_2$SeO$_4$), sodium arsenate disodium salt (Sigma Chemical Company, Na$_2$HAsO$_4$ • 7H$_2$O), sodium bicarbonate (Fisher Scientific, Assay 99% - 100%, NaHCO$_3$), sodium carbonate (Pharmacia, Na$_2$CO$_3$), and sodium phosphate tribasic (Mallinckrodt Chemical Works, Na$_3$PO$_4$ • 12H$_2$O), sodium chloride (Fisher Scientific, 99.80%, NaCl), barium chloride (J.T. Baker Chemical Co., BaCl$_2$), barium selenate (MP Biomedicals, LLC, BaSeO$_4$), and nitric acid (Fisher Scientific, trace metal grade, HNO$_3$). Glutathione (Fisher BioReagents, stored at 4°C, GSH) and cerium(III) chloride heptahydrate (Acros Organics, 99.99%, CeCl$_3$•7H$_2$O) were used as reactants for selenite removal. Methanol (Acros Organics, 99.9% HPLC grade, CH$_4$O), 2-(N-Morpholino)ethanesulfonic acid (Acros Organics, 99%, pKa = 6.10 at 25°C, MES) and 3-[Tris(hydroxymethyl)methylamino]-1-propanesulfonic acid (Acros Organics, 99%, pKa = 8.4 at 25°C, TAPS) were used as buffer. Sodium hydroxide solution (Fisher, 5M, NaOH) and hydrochloric acid (Sigma, 37% solution in water, HCl) were prepared to adjust the pH of stock solutions. Amberlite ion exchange resin (Acros Organics, Amberlite® IR120 Na form ion exchange resin) was prepared in a concentrated sodium chloride solution (Fisher Scientific, 99.80%, NaCl). Also, all the solution pH values were measured using a calibrated Thermo Scientific Orion Star pH meter.
2.2 Selenite removal by GSH reductive precipitation method experiment

2.2.1 GSH:Se(IV) ratio determination experiment

Batch reactors were conducted in 15-ml polypropylene tubes containing 8 ml of a solution of 8 mM sodium selenite and 45 mM MES buffer at pH 7.0. A 100 mM GSH stock solution was prepared in 45 ml MES buffer at pH 7.0 immediately before use to avoid GSH decomposition. Calculated volumes of GSH stock solution were added to achieve GSH:selenite ratios of 0:1, 1:1, 2:1, 4:1, and 5:1. The combined solutions were allowed to react 24 hours. Samples for dissolved selenite concentration measurement were prepared by centrifuging the solids in a Sorvall Legend XT centrifuge at 7000 rpm for 10 minutes, and filtering the supernatant with 0.2 µm nylon syringe tip filters, diluting to the linear range of calibration concentrations. Samples were stored at 4 degree Celsius until analysis by ICP-MS or ion chromatography. Identical control reactors were also set up but were spiked with equivalent volumes of DI water instead of GSH solution.

2.2.2 Se(IV) precipitation kinetics experiment

After the GSH precipitation ratio was determined, the GSH precipitation kinetics experiment was conducted at GSH:selenite ratio of 5:1 under the pH condition of 6, 7, and 8. Three batch reactors were prepared for three different pH conditions. Two batch reactors were prepared in 100-ml plastic bottles adding 24 ml of a solution of 13.3 mM sodium selenite and 40 mM MES buffer at pH 6 and pH 7. The other one was prepared in 100-ml plastic bottles containing 24 ml of solution of 13.3 mM sodium selenite and 40 mM TAPS buffer at pH 8. Three 100 mM GSH stock solutions were made in 50-ml polypropylene tubes at pH 6, 7, and 8 immediately before adding into three reactors to avoid GSH decomposition. 2.5-ml samples
were taken in 15-ml polypropylene tubes from each three reactors at 0 hour before adding GSH stock solution, and at 0.08, 0.25, 0.67, 1.0, 2.0, 6.0, and 24 hours after adding GSH stock solution. Each sample received 100 µl of 1 M sodium chloride solution to slow down the reaction by forming a complex with GSH before filtering with 0.2 µm nylon syringe tip filters, were diluted to the linear range of calibration concentrations, and were measured immediately on ICP-MS. The 6-hour and 24-hour samples were stored in a refrigerator at 4 degree Celsius until analysis by ICP-MS.

2.3 Selenite removal by Cerium(III) chloride precipitation method experiment

2.3.1 Cerium(III) chloride:Se(IV) or Se(VI) ratio determination experiment

This experiment was conducted in the reaction of cerium(III) chloride with sodium selenite and cerium(III) chloride with sodium selenate. Batch reactors were conducted in 15-ml polypropylene tubes adding 8 ml of a solution of 8 mM sodium selenite or sodium selenate and 45 mM MES buffer at pH 7.0. A 100 mM cerium(III) chloride stock solution was prepared in 45 mM MES buffer at pH 7.0 with slowly adding 1 M sodium hydroxide adjusting pH to avoid the formation of cerium(III) hydroxide which is insoluble above pH 7.5 under our condition. Calculated volumes of cerium(III) chloride stock solution were added to obtain the cerium(III) chloride:selenite or selenate ratio of 0:1, 0.5:1, 0.66:1, 1:1, 2:1, 4:1, and 8:1. The combined solutions were allowed to react 24 hours. Samples from selenite reactors and selenate reactors were prepared by centrifuging the solids in a Sorvall Legend XT centrifuge at 7000 rpm for 10 minutes, filtering the supernatant with 0.2 µm nylon syringe tip filters, diluting to the linear range of calibration concentrations, and storing at 4 degree Celsius until analysis by ICP-MS. Identical control reactors were also prepared but were added equivalent volumes of DI water.
without cerium(III) chloride. Samples from control reactors were prepared in ion chromatography vials, and measured on ion chromatography as initial concentrations.

2.3.2 Cerium(III) chloride addition methods experiment

Batch reactors were set up according to the cerium(III) chloride:selenite ratios of 1.15:1 and 2.10:1 containing 20 ml of a solution of 12 mM selenite and 12 mM selenate, and 45 mM MES buffer at pH 7.0 in 50-ml polypropylene tubes. A 100 mM of cerium(III) chloride stock solution was made in 45 mM MES buffer also at pH 7.0 with slowly addition of 1 M sodium hydroxide to avoid the formation of cerium(III) hydroxide solids that is insoluble above pH 7.5 considering our experimental condition. Calculated volumes of cerium(III) chloride stock solution based on the ratios of cerium(III) chloride to selenite was, first, slowly added drop by drop using a pasture pipette and continuous stirring with a cleaned pasture pipette. Second, cerium(III) chloride was added all at once with a pipette. The combinations of the solutions were allowed to react for 24 hours. Samples after 24 hours were centrifuged in a Sorvall Legend XT centrifuge at 7000 rpm for 10 minutes, and supernatant was filtered with 0.2 μm syringe tip filters and diluted to the linear range of the calibration concentrations. The diluted samples were stored at 4 degree Celsius until measured by ICP-MS.

2.3.3 Cerium(III) chloride kinetics experiment

Two cerium(III) chloride:Se(IV) ratios, low and high, at 2:1 and 8:1 were selected to conducted the cerium(III) chloride precipitation kinetics experiment. Two batch reactors were prepared based on the selected ratios in 250-ml plastic bottles containing 5.6 mM selenite stock solution and 45 mM MES buffer at pH 7.0, and solutions were well mixed by magnetic stirrer.
A 100 mM of cerium(III) chloride stock solution was prepared in 45 mM MES buffer at pH 7.0 with slowly adding 1 M sodium hydroxide to avoid cerium hydroxide precipitation which is insoluble above pH 7.5 under our experimental condition. 10 ml and 40 ml of 100 mM cerium(III) chloride stock solutions were added into the reactors at the ratio of 2:1 and 8:1, respectively. 5-ml samples were taken in 15-ml polypropylene tubes at 0 hour before adding any cerium(III) chloride stock solutions, and at 0.25, 0.5, 1.0, 2.0, 6.0, and 24 hours after adding cerium(III) chloride stock solutions. Samples prepared for dissolved selenite measurement were prepared by centrifuging the solids in a Sorvall Legend XT centrifuge at 7000 rpm for 10 minutes, filtering the supernatant with 0.2 μm syringe tip filters, diluting to the linear range of calibration concentrations, and storing at 4 degree Celsius in a refrigerator. After sampling, each sample was added 100 μl of 1 M nitric acid to prevent cerium(III) selenite formation by lowering pH to soluble range. Samples were measured by ion chromatography and ICP-MS.

2.4 Cerium(III) cation removal by ion exchange method experiment

2.4.1 Ion exchange in column mode

An ion exchange resin column was set up by loading 3 ml of Amberlite Na resin in a BIO-RAD column, and converted to a BioLogic low pressure chromatography unit and conductivity detector. Resin was prepared by regenerating with 500 mM sodium chloride and washing with DI water at the flow rate of 5.0 ml/min. 20 ml of each four solutions that were 8.98 mM sodium selenate, 8.87 mM sodium selenate + 8.28 mM cerium(III) chloride, 8.56 mM sodium selenate + 18.3 mM cerium(III) chloride, and 8.54 mM sodium selenate + 29.4 mM cerium(III) chloride were prepared in 50-ml polypropylene tubes. Solutions were passed through the resin column with a flow rate of 1.5 ml/min to exchange Ce$^{3+}$ for Na$^+$, and DI water was
added when sample’s water level fell to resin level. Eluent was collected in polypropylene tubes when conductivity was above 1000 µS. Waste was collected when the conductivity was below 1000 µS due to adding DI water with a 5.0 ml/min flow rate. After completing the first sample, the resin was set up by regenerating and washed with 500 mM sodium chloride and DI water for next sampling. Collected samples were filtered with 0.2 µm nylon syringe tip filters, diluted to the linear range of calibration concentrations, and stored at 4 degree Celsius for analysis by ICP-MS. 20 ml of identical control solutions without passing through the resin column were also prepared, and measured on ICP-MS as initial concentrations.

2.4.2 Ion exchange kinetics in batch mode

Ion exchange resin for batch reactors was prepared by regenerating with 500 mM sodium chloride, and washing with DI water in the ion exchange column, but was removed and dried. Three batch reactors were prepared by containing 30 ml of solutions of 8.98 mM sodium selenate, 8.87 mM sodium selenate + 8.28 mM cerium(III) chloride, and 8.54 mM sodium selenate + 29.4 mM cerium(III) chloride that were prepared by adding calculated volumes of 100 mM sodium selenate and 100 mM cerium(III) chloride into 50-ml polypropylene tubes, respectively. The solutions were well mixed with resin using a Cole-Parmer Roto-Torque rotator for 2 hours. 1 ml samples were taken into 2.0 ml microcentrifuge tubes at 0 hour before adding 2 grams of resin, and at 0.083, 0.25, 0.5, 1.0, and 2.0 hours after adding 2 grams of resin. The collected samples were prepared by centrifuging in an Eppendorf mini spin centrifuge at 10000 rpm for 10 minutes, filtering the supernatant with 0.2 µm nylon syringe tip filters, diluting to the linear calibration concentrations. Before the dissolved selenite concentration measurement on ICP-MS, the prepared samples were stored at 4 degree Celsius in a refrigerator. An identical
control was prepared at 10 mM sodium selenate + 10 mM cerium(III) chloride with the same sampling processes but no addition of ion exchange resin.

2.4.3 Ion exchange in batch mode

2 grams of dried ion exchange resin used for each reactor was prepared in the same way from section 2.4.2. In this experiment, four batch reactors were set up in 15-ml polypropylene tubes based on the solutions: 8.98 mM sodium selenate, 8.87 mM sodium selenate + 8.28 mM cerium(III) chloride, 8.56 mM sodium selenate + 18.3 mM cerium(III) and 8.54 mM sodium selenate + 29.4 mM cerium(III) chloride that were made by adding calculated volume of 100 mM sodium selenate stock solution and 100 mM cerium(III) chloride stock solution. Each 2 grams of prepared resin was added in the each reactor with 10 ml prepared solutions, and well mixed using a Cole-Parmer Roto-Torque rotator for 1 hour. After rotation, the samples were allowed to stand for 15 minutes after rotation. Ion exchange resins were settled immediately after stopping the rotation, centrifuging samples was no need of in the sampling processes but filtering supernatant with 0.2 µm nylon syringe tip filters, diluting to the linear range of calibration concentration, and storing at 4 degree Celsius in a refrigerator until analysis by ICP-MS. Four identical controls were prepared the same as the samples but no addition of resin.

2.5 Monitoring $\delta^{18}O_{\text{SeO}_4}$ values

This experiment was conducted on the basis of the GSH and cerium(III) chloride precipitation results. Three types of batch reactors were designed in 50-ml polypropylene tubes categorized by three types of solutions that were selenite solution, selenate solution, and mixed selenite and selenate solution. Type 1 batch reactor were prepared by adding 20 ml of a solution
of 12 mM sodium selenite and 45 mM MES buffer at pH 7. Type 2 batch reactor contained 20 ml of a solution of 12 mM sodium selenate and 45 mM MES buffer at pH 7. Type 3 batch reactor contained 20 ml of a solution of 12 mM sodium selenite, 20 ml of a solution of 12 mM sodium selenate and 45 mM MES buffer at pH 7. A 100 mM cerium(III) chloride stock solution was made in 45 mM MES buffer at pH 7 slowly adding 1 M sodium hydroxide adjusting pH to prevent cerium(III) hydroxide formation. A 100 mM GSH stock solution was prepared also in 45 mM MES buffer at pH 7 rapidly to avoid GSH decomposition. For the batch reactors of cerium(III) chloride addition, 4.8 ml of 100 mM cerium(III) chloride stock solution was added to achieve the Cerium(III) chloride:Se(IV) ratio of 2:1, and for the batch reactors of GSH addition, 12 ml of 100 mM GSH stock solution was added to obtain the GSH:Se(IV) ratio of 5:1. The combined solutions were allowed to react for 24 hours. Samples for dissolved selenium metal measurement were prepared by centrifuging the precipitates in a Sorvall Legend XT centrifuge at 7000 rpm for 10 minutes, filtering the supernatant with 0.2 µm nylon syringe tip filters, diluting to the linear range of calibration concentrations, and storing at 4 degree Celsius until analysis by ICP-MS and liquid chromatography. Equivalent controls were prepared as well but added same volumes of DI water without GSH and cerium(III) chloride stock solutions.

Barium selenate precipitation methods (Larese-Casanova and Blake 2013) were used to precipitate sodium selenite and sodium selenate from measured samples for selenate $\delta^{18}$O isotopic analysis after the measurement of dissolved elemental selenium concentration. Measured volumes and concentrations of samples for measuring $\delta^{18}$O isotope were transferred into glass tubes with the addition of excess 1 M barium chloride at 5:1 Ba(II):Se(VI) ratio. The white barium selenate precipitates produced immediately, but the white barium selenite precipitates appeared slowly with increasing pH at 8.0 (Larese-Casanova and Blake 2013), and
the reactions were allowed to be conducted for 24 hours. All the precipitates were carefully
removed into 2.0 ml microcentrifuge tubes for centrifuge at 10000 rpm for 10 minutes, and
washing with DI water and methanol to speed up drying process. After drying in a fume hood
covered with aluminum foil, each barium selenite and barium selenate solid was finely weighed
in quadruplicates of approximate 300 µg into Costech pressed silver capsules.

The batch reactors and control reactors were the same as those from the batch mode ion
exchange experiment (Section 2.4.3). Samples selected for measuring selenate δ¹⁸O isotope
from the four batch reactors and four control reactors were transferred into glass tubes,
precipitated with excess 1 M barium chloride solution at the ratio of 5:1, and allowed to react for
24 hours. After the precipitates were settled, the white barium selenate solids were carefully
removed into 2.0 ml microcentrifuge tubes centrifuging for at 10000 rpm for 10 minutes,
washing with DI water and methanol, and drying in an anoxic chamber covered with aluminum
foil for 24 hours. The dried samples were finely measured quadruplicates about 300 µg into
Costech pressed silver capsules for selenate δ¹⁸O isotopic analysis.

2.6 Arsenate, carbonate, and phosphate precipitation with cerium(III) chloride experiment

Batch reactors were conducted in 50-ml polypropylene tubes for arsenate, carbonate, and
phosphate precipitation. For arsenate and phosphate oxyanion’s reactor, it contains 20 ml of a
solution of 12 mM sodium arsenate disodium, and sodium phosphate as well as 45 mM MES
buffer at pH 7.0. For carbonate oxyanion’s reactor, it only contains 20ml of a solution of 12 mM
sodium carbonate and nitrogen gas purged DI water at pH 8.3 to avoid extra carbon sources. A
100 mM of cerium(III) chloride stock solution used for arsenate and phosphate was prepared in
45 mM MES buffer at pH 7.0 with slowly adding 1 M sodium hydroxide to avoid cerium
hydroxide formation which is insoluble above pH 7.5 under our experimental condition.

Another 100mM of cerium(III) chloride stock solution used for carbonate was prepared in nitrogen gas purged DI water at pH 7.0 with slowly addition of 1 M ammonia hydroxide. Calculated volumes of cerium(III) chloride stock solution were added to achieve cerium(III):carbonate ratios of 0.5:1, 0.66:1, 1:1, 2:1, 4:1, and 8:1, cerium(III):arsenate and cerium(III):phosphate ratios of 0.5:1, 1:1, 2:1, 4:1, and 8:1. The combined solutions were allowed to react 24 hours, and then samples for carbonate, arsenate, and phosphate concentration measurement were centrifuged in a Sorvall Legend XT centrifuge at 7000 rpm for 10 minutes, filtered with 0.2 µm nylon syringe tip filters, and diluted to the linear range of calibration concentrations. Phosphate cannot be measured on ICP-MS, its concentrations need to be measured by IC, which cannot include any cerium(III) ions passing through the KOH column and resulting in the precipitation of cerium(III) hydroxide.

After all the samples were diluted, phosphate samples must use Amberlite IR120 sodium ion exchange resin to remove cerium(III) ions. The resin preparation and cerium removal processes are the same in the section 2.4.3. ion exchange in batch mode. Samples were stored at 4 degree Celsius until analysis by ICP-MS or ion chromatography. Identical control reactors were also set up but were spiked with equivalent volumes of DI water instead of cerium(III) chloride solution.
CHAPER III RESULTS AND DISCUSSION

3.1 Selenite removal by GSH reductive precipitation results and discussion

3.1.1 Selenite removal by GSH reductive precipitation results

Glutathione is a highly reductive reagent to selenite, which can actively reduce selenite to element selenium at a high concentration (Cui et al. 2008). Selenite successfully removal was observed by the rapidly precipitation of red element selenium, and was accompanied by black element selenium after 7 hours. The characteristic of the color of elemental selenium varied with the ratio of GSH to selenite after the addition of GSH within 24 hours. It has been observed that the formation of orange-red selenium particle was performed at low GSH:Se(IV) ratio and the deep-red selenium particle was formed at high GSH:Se(IV) ratio. In addition, the samples at the GSH:Se(IV) ratio of 3.98:1 (approximately 4:1) and 4.98:1 (approximately 5:1) show 97.1 % and 96.3 % removal of selenite, which was not close to expected 99 % selenite removal, as seen in Figure 1. However, the percent selenite removal was improved when increasing the GSH:Se(IV) ratio, which suggest that higher initial concentration of GSH correlated with a more reduction of selenite (Table 1).

Selenite was transformed into elemental selenium rapidly in the presence of GSH at pH 7.0 in the GSH: Se(IV) ratio of 2:1 and 4:1 (Kessi and Hanselmann 2004). Similarly, the immediately selenite reduction into elemental selenium was observed under pH 6.0, 7.0, and 8.0 at 4.98:1 GSH:Se(IV) ratio. Based on the selenite removal kinetics (Table 1), pH 7.0 performed an ideal circumstance for selenite removal, which not only showed the evidence of a fastest selenite reduction rate between pH 6.0 and 8.0, but also displayed the most reduction of selenite into elemental selenium with equivalent GSH concentration (Figure 2). Furthermore, the slowest
selenite reduction rate was occurred at pH 8.0 but its reduction rate was increased and approached to the rate of pH 6.0 and 7.0 after 5 hours. After 24-hours selenite reductive precipitation, black selenium particles were aggregated in the bottom at the condition of pH 7.0 and 8.0. However, at pH 6.0, few black elemental selenium particles were observed accumulated in the bottom, and red selenium particles were stacked on the wall of the reactor.

3.1.2 Selenite removal by precipitation discussion

Selenite removal was confirmed by the evidence of the formation of allotrope elemental selenium such as amorphous red elemental selenium (Kessi and Hanselmann 2004) and monoclinic black elemental selenium (Hoffmann and King 1997). Red elemental selenium particles were observed before 7 hours, which is consistent with the observation of Kessi and Hanselmann’s (2004) results that the formation of red selenium particles within 2 hours. After 24 hours, black elemental selenium particles were aggregated in the reactor’s bottom, which became a dominant form of elemental selenium after 7 hours. The percent removal of selenite was much higher at the GSH:Se(IV) ratio above 3.98:1 than the selenite removal results from the GSH:Se(IV) ratio of 1.99:1 (Figure 1 and Table 1) at pH 7.0 condition, which is in accordance with Kessi and Hanselmann’s (2004) results of the increasing elemental selenium formation from GSH:Se(IV) ratio 2:1 to 4:1 at pH 7.0. Even though our selenite removal results indicate that selenite removal was a little lower at the GSH:Se(IV) ratio of 4.98:1 than it was at 3.98:1 ratio, an potential slightly improvement of selenite removal could occur with higher GSH and selenite concentrations according to Kessi and Hanselmann (2004).

Figure 2 shows that selenite was reduced by GSH 98.7%, 98.8%, and 97.5% in roughly 30 minutes at 4.98:1 GSH:Se(IV) ratio under pH 6.0, 7.0, and 8.0, respectively. Kessi and
Hanselmann also observed approximately 80 to 95% disappearance of the initial selenite concentration from the GSH:Se(IV) ratio of 4:1 at pH 7.0. These observations support that pH 7.0 is an ideal condition for selenite removal by GSH reductive precipitation at the GSH:Se(IV) ratio of 5:1. The various intermediates produced in the reaction of selenite and GSH reduction have been investigated by chemical approach at acidic and neutral conditions (Cui et al. 2008), which has a potential influence on the formation of different elemental selenium. The formed red and black element selenium will be determined by X-ray diffraction were likely due to the interferences of the complex reaction and various reduced selenium species (Cui et al. 2008 and Kessi and Hanselmann 2004).

3.2 Selenite removal by cerium(III) selenite precipitation results and discussion

3.2.1 Selenite removal by cerium(III) selenite precipitation results

Selenite removal was observed by the formation of slimy white cerium(III) selenite precipitates at pH 7.0. The cerium(III) chloride:Se(IV) ratio selected below and above stoichiometry ratio (0.66:1) performed a high percent of selenite removal. 84.4% and 98.9% of selenite were removed at 0.47:1 (approximately 0.5:1) and 0.63:1 (approximately 0.66:1) cerium(III) chloride:Se(IV) ratios (Figure 3). Above the stoichiometry ratio of cerium(III) chloride to selenite, the percent removal of selenite reached the proposed goal of at least 99% removal (Table 2). Few works have been devoted to the study of cerium(III) selenate precipitation. Based on the experimental results, no observation of cerium(III) selenate solids from the evidence of 0% selenate removal under the cerium(III) chloride:Se(VI) ratio from 0:1 to 4:1 (Figure 3). However, 4.23% selenate removal was observed with a small white cloud
precipitated preformed in the reactor, which imply that the likely of precipitation of selenate with cerium(III) chloride at a high cerium(III) chloride:Se(VI) ratio.

The cerium(III) chloride addition methods including drop-by-drop addition and all-at-once addition were considered due to the potential precipitation of cerium(III) selenate in a selenite and selenate contained solution. 100% selenite removal was congruous to the separately selenite removal results above. However, approximately 13 to 18% removal of selenate was observed in a selenite and selenate mixed solution, as seen from Figure 4 and Table 2. Furthermore, the addition of cerium(III) chloride drop by drop lessened the percent of selenate removal 25.9% and 3.4% at the cerium(III) chloride:Se(VI) ratio of 1.15:1 and 2.10:1, respectively (Table 2), which indicate that the drop-by-drop addition method improves the selenate preservation in a selenite and selenate contained solution. The minimization of cerium(III) selenate solids indicates that the drop-by-drop addition of cerium(III) chloride can lower the selenate removal percentage.

Selenite was precipitated rapidly in the presence of cerium(III) chloride at pH 7.0. The percent removal of selenite was improved by increasing the cerium(III) chloride:Se(IV) ratio from 1.91:1 to 7.65:1 (approximately 2:1 to 8:1). 99.87% and 99.97% selenite removals were observed within 30 minutes in samples containing cerium(III) chloride:Se(IV) ratios of 1.91:1 and 7.65:1, respectively (Figure 5). Moreover, selenite concentration remained approximately 3-4% higher at the cerium(III) chloride:Se(IV) ratios of 1.91:1 compared with the concentration at 7.65:1 cerium(III) chloride:Se(IV) ratio, which indicate that higher concentration of cerium(III) chloride correlated with a faster and more precipitation of selenite.
3.2.2 Selenite removal by cerium(III) selenite precipitation discussion

Selenite was successfully removed by 99% in support of the hypothesis of at least 99% removal of selenite using cerium(III) selenite precipitation method at pH 7.0. The precipitation was conducted under pH 7.0 considered the formation of cerium(III) hydroxide while the selenite contained solution was greater than pH 7.5. Few studies show the precipitation of cerium(III) selenite at various pHs in chemical approach, however, Bouchaud (2012) used electrochemical method found the precipitation of cerium(III) hydroxide starting at pH 7.6 that supports the pH value of 7.5 from Visual Minteq model precipitating cerium(III) as white cerium(III) hydroxide. S. Jolin (1874) observed the precipitate of amorphous cerium selente till the addition of ammonia (Prasad and Kumar 1963) that further proves selenite removal should below pH 7.5 to avoid the formation of cerium(III) hydroxide.

Even though much more selenite was precipitated with a cerium(III) chloride:Se(IV) ratio of 7.65:1 compared with a ratio of 1.91:1, the 1.91:1 cerium(III) chloride:Se(IV) ratio with 99.87% selenite removal (Figure 3) was used in further $\delta^{18}$O$_{SeO4}$ study to avoid the interference of cerium(III) selenate solids formation. On the other hand, precipitating selenite with cerium(III) chloride in a selenite and selenate contained solution probably accompanied by the precipitation of selenate based on our observation (Figure 4). According to the observed evidence from cerium(III) chloride addition methods, 4.62% of selenate was avoided to be precipitated with drop-by-drop addition method at the cerium(III) chloride:selenate ratio of 1.15:1 (VI) than it was precipitated at 2.01:1 cerium(III) chloride:Se(VI) ratio (Table 2). Therefore, the drop-by-drop addition has a potential of decreasing more cerium(III) selenate precipitation at low cerium(III) chloride:Se(IV) or Se(VI) ratio.
3.3 Cerium(III) cations removal by ion exchange method results and discussion

3.3.1 Cerium(III) cations removal by ion exchange method results

Ion exchange is an effective approach of removing heavy metals such as lead and silver (Semmens and Martin 1980 and Peters 1985). Cerium(III) cations was successfully removed without an influence on selenate anions by using both column and batch mode ion exchange methods (EPA 1981) by Amberlite IR 120 sodium acidic resin from a selenate and cerium(III) chloride mixed solution. Above 99% of cerium(III) cations was removed with varying cerium(III) chloride concentrations that were 8.28 mM, 18.3 mM, and 29.4 mM (Figure 10), which suggest the relative higher removal of cerium(III) cations in batch mode compared with the cerium (III) ions removal in column mode. Moreover, the consistency of selenate concentration shown in Figure 11 indicate that the removal of cerium(III) cations has no impact on selenate ions in column and batch mode ion exchange. Overall, considering the cerium(III) cation removal and the remaining selenate concentrations, batch mode ion exchange method is more preferable to be used to remove cerium(III) cations.

Cerium(III) cations rapidly disappeared in the combination of resin within 5 minutes (Figure 7 and 8). However, the removal of cerium(III) cations rate was much faster in the sample containing low cerium(III) chloride concentration than that it in the sample high cerium(III) chloride concentration. 99.4% and 88.4% of the initial cerium(III) chloride concentrations disappeared in 5 minutes from the mixtures of sodium resin with the sample of 8.87 mM selenate and 8.28 mM cerium(III) chloride and 8.54 mM selenate and 29.4 mM cerium(III) chloride, respectively (Figure 7 and 8). After 1 hour, the percent removal of cerium(III) cations was close to 99.9% that support the high removal of cerium(III) cation in batch mode (Table 3). No changes of cerium(III) chloride and selenate concentrations were
observed while no addition of resin in the sample of 8.87 mM selenate and 8.28 mM Ce(III) as a control, as seen in Figure 9, as well as no varies of selenate concentration when only selenate was mixed with resin (Figure 6), which excluded the interference of resin and selenate ions in the removal of cerium(III) cations.

3.3.2 Cerium(III) cations removal by ion exchange method discussion

The ion exchange results provide evidence of cerium(III) cations removal in batch and column modes. Higher cerium(III) cations removal percentage in batch mode was more effective in the removal of cerium(III) ions than in column mode. On the other hand, the concentrations of selenate after the mixture of resin remained the same concentration before the addition of resin in batch mode ion exchange except the loss of a small volume while sampling the supernatant. In terms of time saving, according to the conduction of batch mode and column mode ion exchange experiment, it is more time consuming to run a sample in column mode. In general, batch mode ion exchange method is more effective to remove cerium(III) ions and more convenient to operate for the removal of cerium(III) cations.

Although the immediate approximately 88 to 99% removal of cerium(III) cations was observed within 5 minutes, the exchanging of most 99% cerium(III) cations with sodium ions occurred at least in 30 minutes and 1 hour for the samples containing cerium(III) chloride with 8.28 mM and 29.4 mM, respectively (Table 3). This observation implies that an overall slow ion exchange process correlated with a fast removal of cerium(III) cations. High cerium(III) chloride concentration was possibly associated with cerium(III) ion exchange rate. The cerium(III) ion exchange rate was slower in the samples of 29.4 mM cerium(III) chloride than it was in the sample of containing 8.28 mM cerium(III) chloride (Figure 7 and 8), and it indicates
that the exchange of cerium(III) ions with sodium ions is likely relevant to cerium(III) chloride concentrations but all the cerium(III) ions can be exchanged with sodium ions in the end.

3.4 Monitoring $\delta^{18}\text{O}_{\text{SeO}_4}$ values results and discussion

3.4.1 Monitoring $\delta^{18}\text{O}_{\text{SeO}_4}$ values results

Oxygen stable isotope has been used in the reduction of selenate to elemental selenium as a characterization tool for monitoring $\delta^{18}\text{O}_{\text{SeO}_4}$ value (Schellenger and Larese-Casanova 2013). The methods of selenite removal including GSH reductive precipitation or cerium(III) selenite precipitation were evaluated for their effects on $\delta^{18}\text{O}_{\text{SeO}_4}$ values. According to the $\delta^{18}\text{O}_{\text{SeO}_4}$ values, the values of selenate alone were -7.30‰ and -7.22‰ observed in GSH reductive precipitation and cerium(III) selenite precipitation, respectively (Table 4). However, compared with selenate alone samples, for both precipitation methods, roughly -3.5 to -4‰ difference were observed in samples of Ce(III)/GSH + Se(VI) and Ce(III)/GSH + Se(IV) + Se(VI). This difference is likely because of the chemical reactions between selenite and GSH or cerium(III) chloride that possibly decreased the enrichment of $^{18}\text{O}$ in selenate. Moreover, the decreasing of $^{18}\text{O}$ in selenate enrichment presumably because, first, GSH was co-precipitated with barium selenate; second, the possibility of cerium(III) hydroxide was also co-precipitated with barium selenate; third, cerium(III) ions could be trapped in the barium selenate precipitates. Even though the $\delta^{18}\text{O}_{\text{SeO}_4}$ values was lessened, the presented values observed in cerium(III) selenite precipitation method was more consistent between the samples of Ce(III) + Se(VI) and Ce(III) + Se(IV) + Se(VI) than the values obtained from GSH reductive selenite precipitation method. This results indicates that the cerium(III) selenite precipitation method is more preferable to be applied to selenite removal in terms of the consistency of $\delta^{18}\text{O}_{\text{SeO}_4}$ values. Furthermore, a
hypothesis test at 95% confidence interval showed the evidence that the $\delta^{18}$O$_{\text{SeO}_4}$ value analyzed from cerium(III) selenite precipitation method had no difference for the two samples of Ce(III) + Se(VI) and Ce(III) + Se(IV) + Se(VI). Therefore, cerium(III) selenite precipitation method was preferentially used to remove selenite for the isolation of selenate in diverse aqueous water.

In order to solidify the evidence that the $\delta^{18}$O$_{\text{SeO}_4}$ values measured from cerium(III) chloride contained samples was consistent with the selenate alone sample, ion exchang-batch mode method was used to remove the likely interference of cerium(III) cations for improvement. As expected, after cerium(III) cations removal, the $\delta^{18}$O$_{\text{SeO}_4}$ value observed in cerium(III) chloride and selenate contained solutions was much more close to the value of the selenate alone sample (Table 5). Furthermore, an incremental concentration of cerium(III) chloride was presented as cerium(III) chloride:Se(VI) ratios of 0:1, 1:1, 2:1 and 3:1, which also displayed the consistency of $\delta^{18}$O$_{\text{SeO}_4}$ values between -7.60 and -7.80 ‰ (Table 5). The ion exchange – batch mode test has confirmed the presumed reason from the previous cerium(III) selenite precipitation, which observed a difference between the selenate alone sample and the samples contained cerium(III) chloride and Se(VI) or both Se(IV) and Se(VI).

3.4.2 Monitoring $\delta^{18}$O$_{\text{SeO}_4}$ values discussion

$\delta^{18}$O$_{\text{SeO}_4}$ values from both cerium(III) selenite precipitation method and ion exchange - batch mode method were in support of the primarily choice of selenate isolation and cerium(III) cations removal. Hypothesis tests were conducted to proof no difference that was observed using ion exchange – batch mode method between the selenate alone sample and cerium(III) chloride contained samples. At 95% confidence interval, the hypothesis tests shows the evidence that the $\delta^{18}$O$_{\text{SeO}_4}$ values before and after cerium(III) cations removal by ion exchange - batch
mode method had no difference, which more convincingly indicate that batch mode with ion exchange method can remove cerium(III) ions without changing $\delta^{18}O_{\text{SeO}_4}$ values as well as to keep the stability of $\delta^{18}O_{\text{SeO}_4}$ values between samples. pH is a major factor considered for all the experiment in this project, however, the solutions pH in the ion exchange method was not adjusted. The consistency of $\delta^{18}O_{\text{SeO}_4}$ values observed from the ion exchange – batch mode method suggests that pH has no influence on the cerium(III) removal with ion exchange method in a cerium(III) chloride and selenate or both selenite and selenate contained solutions. Therefore, these data from ion exchange – batch mode test suggest pH is not likely a factor to lower the $\delta^{18}O_{\text{SeO}_4}$ values from the cerium(III) selenite precipitation. Overall, monitoring $\delta^{18}O_{\text{SeO}_4}$ values under precipitation methods and ion exchange method implies that the precipitation treatment has the possibility increase $^{18}O$ enrichment in selenate compared with the ion exchange treatment.

3.5 Other oxyanions precipitation with cerium(III) chloride results and discussion

3.5.1 Other oxyanions precipitation with cerium(III) chloride results

The precipitation of arsenate and phosphate with cerium(III) chloride have been observed white cloud precipitates after addition of cerium(III) chloride rapidly, but for carbonate, its precipitates presented slowly after addition of cerium(III) chloride. In general, at the stoichiometry cerium(III) chloride:As(V), cerium(III) chloride:C(IV), and cerium(III) chloride:P(V) ratios of 1:1, 0.66:1, and 1:1, respectively, it observed that approximately 99% of arsenate and phosphate were removed (Figure 12 and 13). Furthermore, Figure 12 and 13 show that the oxyanions of arsenic and phosphorus optimum removal was found at 2:1 and 1.94:1 of the cerium(III) chloride:As(V) and cerium(III) chloride:P(V) ratios that were 99.59% and
98.20%, respectively. However, the percent removal of arsenate and phosphate with cerium(III) chloride has a slightly decreasing trend when increasing cerium(III) chloride concentrations, as seen in Figure 12 and 13. For carbonate, it was hard to be precipitated out in the acidic condition with cerium(III) chloride, the precipitates was observed slowly precipitated and stack on the wall of reactors at pH 7.0.

In addition, the samples of arsenate and phosphate were also observed white precipitates stacked on the bottom of reactors, but it was found about two days later and the pH of both samples were between 5.6 and 5.9. This implies that the white precipitates may not be cerium(III) hydroxide according to the measured pH range from each sample. After centrifuging the solids and filtering the supernatants, more white precipitates would not appear.

3.5.2 Other oxyanions precipitation with cerium(III) chloride discussion

Even though few works have studied on the precipitation of arsenate, carbonate, and phosphate using cerium(III) chloride at a certain pH, the percent removal of the oxyanions of arsenic and phosphorus was close to 99% removal as expected in our hypothesis. However, compared with these two oxyanions removal percentage, arsenate precipitated with cerium(III) chloride showed a prospective removal of 99% at pH 7.0 which suggests that arsenate can be effectively removed by cerium(III) chloride. For phosphate, even though the percent removal did not reach 99% by precipitating with cerium(III) chloride at pH 7.0, a presumable reason could be explained by the chemical reaction equilibrium. During overnight reaction, the chemical reaction equilibrium leads to precipitates reappeared and stacked on the wall of tubes based on the revisable chemical reaction. The reappearance of phosphate were associated with
the concentration of cerium(III) chloride. The higher concentration of cerium(III) chloride, the easier observation of the precipitate after filter.

Carbon is a common element and exists in water and chemicals especially in organic chemicals. For the carbonate samples, due to various sources of carbon, the measured carbon concentration was too high (data do not show here) to analysis its removal by cerium(III) chloride. During carbon oxyanions removal analysis, the ICP-MS cannot distinguish all the sources of carbons such as samples, water, and MES buffer, and therefore, it is likely to affect the accuracy of carbonate concentration measurements. The analyze carbon of samples and controls was mainly from MES buffer and DI water, respectively, which was likely to vary the concentration of carbon in the measurements, and has a potential to impact on the precipitation of cerium(III) carbonate. Therefore, methods need to be developed further for minimum the carbon concentration from other sources.
CHAPTER IV CONCLUSIONS AND ENGINEERING AND SCIENCE SIGNIFICANCE

4.1 Conclusions

This project has demonstrated successfully removal of selenite using glutathione (GSH) to reductively precipitate selenite to Se(0) and by using cerium(III) to precipitate selenite under the condition of pH 7. Optimal doses were determined at the GSH:Se(IV) ratio and cerium(III) chloride:Se(IV) ratio of 4.98:1 and 1.91:1, respectively. With the cerium(III) selenite precipitation method, the percent removal of selenite was at least 99% as hypothesized but approximately 13 to 17% of selenate was observed to co-precipitate in a either 9.32 mM selenite and 8.10 mM selenate or 9.20 mM selenite and 7.79 mM selenate solution. In order to avoid cerium(III) selenate formation, the methods of addition cerium(III) chloride was adjusted as drop-by-drop addition as opposed to all-at-once addition, which lessened to roughly 4% and 1% in the formation of cerium(III) selenate at the cerium(III) chloride:Se(IV) or Se(VI) ratio of 1.15:1 and 2.10:1, respectively. For the GSH reductive precipitation method, selenite reduction to Se(0) was rapid with 99% selenite removal without remaining selenate. The same red selenium precipitates as Kessi’s (2004) selenite abiotic GSH reductive precipitation experiment were observed.

Both GSH reductive precipitation and cerium(III) selenite precipitation methods can be useful to remove selenite, however, they have advantages and disadvantages. For cerium(III) selenite precipitation method, cerium(III) chloride is easy to prepare and cheap to purchase but it has to be carefully monitoring pH in stock solutions to avoid cerium(III) hydroxide formation. For GSH reductive precipitation method, GSH is an effective reductant to precipitate selenite.
without removing any selenate but it is expensive and has to be stored at 4 Celsius degree and freshly prepared as needed.

The ideal precipitation method for isolation of selenate from selenite should not change the $\delta^{18}$O$_{\text{SeO}_4}$ isotopic values in order to measure accurate values of selenate in nature. Although both precipitation methods showed a difference of $\delta^{18}$O$_{\text{SeO}_4}$ isotopic values compared with the selenate alone sample, cerium(III) selenite precipitation led to the most consistent and accurate $\delta^{18}$O$_{\text{SeO}_4}$ isotopic values. The $\delta^{18}$O$_{\text{SeO}_4}$ isotopic values from cerium(III) selenite precipitation including selenate alone, selenate and cerium(III) chloride, and selenate, selenite and cerium(III) chloride were observed to be $-7.30 \pm 0.05$ ‰, $-3.50 \pm 0.04$ ‰, and $-3.88 \pm 0.17$ ‰, respectively. The approximately -4.00 ‰ differences in $\delta^{18}$O$_{\text{SeO}_4}$ isotopic values after adding cerium(III) chloride could be caused by the excessive cerium(III) ions interference. However, the excess of cerium(III) ions was efficiently removed by sodium-loaded Amberlite cation exchange resin in a batch mode reactor, which minimized the change in $\delta^{18}$O$_{\text{SeO}_4}$ isotopic values. The values presented in the samples of selenate alone, 8.28 mM Ce(III) + 8.87 mM Se(VI), 18.3 mM Ce(III) + 8.56 mM Se(VI), and 29.4 mM Ce(III) + 8.54 mM Se(VI) were $-7.99 \pm 0.28$ ‰, $-7.77 \pm 0.10$ ‰, $-7.60 \pm 0.32$ ‰, and $-7.76 \pm 0.32$ ‰, respectively. Therefore, the batch-mode ion exchange method not only has no impacts on the analysis of $\delta^{18}$O$_{\text{SeO}_4}$ isotopic value, but also minimizes the cerium(III) interference of lowering the enrichment of $^{18}$O value. Overall, cerium(III) selenite precipitation method followed by ion exchange removal of cerium(III)ions is an ideal selenite removal method for further transformation of $^{18}$O in selenate study.

Arsenate, carbonate, and phosphate anions have been successfully precipitated by cerium(III) chloride. Even though few works study on the precipitation of arsenate, phosphate, and carbonate using cerium(III) chloride, this project has observed some expected results in
order to remove these oxyanions from selenate contained water. Arsenate was 99% precipitated by cerium(III) chloride at pH 7.0 when the cerium(III) chloride:As(V) ratio was greater than 1:1, and the 2:1 cerium(III) chloride ratio presented an optimum removal of arsenate 99.59%.

However, phosphate and carbonate were close and less than 99% removal above the cerium(III) chloride:P(V) and cerium(III) chloride:C(IV) ratios of 0.66:1 and 1:1, respectively, at pH 7.0. Although their removal was not close to expected 99% removal, the optimum ratios of precipitating carbonate and phosphate by cerium(III) chloride that were 1:1 and 2:1, respectively.

The chemical reactions equilibrium is likely to have an influence on the phosphate and carbonate precipitation with high concentration of cerium(III) chloride as the increasing of reaction time. In addition, the observation of more white precipitates of each oxyanions after removing the overnight precipitated solids has a possible effect of chemical equilibrium.

4.2 Further work and improvement

The project has successfully isolated selenate from selenite with GSH reductive precipitation and cerium(III) selenite precipitation methods. However, any selenate removal during cerium(III) selenite precipitation is expected to be lessened under 1%. Even though the addition cerium(III) chloride method has been adjusted, it did not reached to the goal of less than 1% selsnate co-precipitation, this adjustment provides an evidence of the possibility of avoiding co-precipitating of selenate during cerium(III) selenite precipitation for further study.

In addition to removing arsenate, phosphate, and carbonate, sulfate also needs to be removed. However, the similarity of chemical properties of sulfate and selenate causes the difficulty to separate sulfate from selenate. It has been found that sulfate and selenate have the similar solubility with the same metal cations such as barium, copper, etc. Both of them behave similarly during precipitation process. For example, the measuring of oxygen isotope in selenate
requires precipitating selenate with barium chloride but barium sulfate precipitates are commonly known, which is hard to distinguish these two precipitates if they are precipitated at the same time. Additionally, ion exchange method cannot be applied to two similar ions for concentrating selenate ions due to their similarity. Therefore, it is essential to find new methods to remove sulfate for the further analysis of oxygen isotope in selenate.

This novel oxygen stable isotope study will applied to natural water samples from Western United States. All the developed methods need to be polished and make them available to be applied to processes natural water samples, and quantify selenium redox transformation in the environment utilizing oxygen isotopic values.
FIGURES AND TABLES

3.1 Selenite removal by GSH reductive precipitation results and discussion

GSH reductive precipitations:

Figure 1. Percent removal of selenite with 8.04 mM selenite and varying amounts of GSH at pH 7.0. Precipitates rapidly presented deep-red color after the addition of GSH. Samples were measured after 24 hours on ICP-MS.

Table 1. A summary of selenite removal percent at various GSH:Se(IV) ratios at pH 7.0. The initial selenite concentration before adding GSH was 8.04 mM in reactors

<table>
<thead>
<tr>
<th>GSH:Se(IV) ratio</th>
<th>Se(IV) (mM)</th>
<th>GSH concentration (mM)</th>
<th>Removal percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:1</td>
<td>8.04</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.99:1</td>
<td>7.44</td>
<td>7.41</td>
<td>42.2</td>
</tr>
<tr>
<td>1.99:1</td>
<td>6.93</td>
<td>13.79</td>
<td>68.8</td>
</tr>
<tr>
<td>3.98:1</td>
<td>6.09</td>
<td>24.24</td>
<td>97.1</td>
</tr>
<tr>
<td>4.98:1</td>
<td>5.74</td>
<td>28.57</td>
<td>96.3</td>
</tr>
</tbody>
</table>
GSH reductive precipitation kinetics:

![Figure 2. Kinetics of selenite removal by addition of GSH prepared at pH 6.0, 7.0, and 8.0. pH 6.0: [Se(IV)]\textsubscript{initial} = 13.8 mM, [MES] = 32 mM, and [GSH] = 53.3 mM; reactor’s solution at pH 7.0: [Se(IV)]\textsubscript{initial} = 13.9 mM, [MES] = 32 mM, and [GSH] = 53.3 mM; reactor’s solution at pH 8.0: [Se(IV)]\textsubscript{initial} = 13.9 mM, [TAPS] = 32 mM, and [GSH] = 53.3 mM. The kinetic samples were taken up to 24 hours and measured by ICP-MS for Se(IV) concentrations.](image-url)
3.2 Selenite removal by cerium(III) selenite precipitation results and discussions

Ce(III) ratio determination:

Figure 3. Percent removal of selenite and selenate during precipitation as white $\text{Ce}_2(\text{SeO}_3)_3$ precipitates for solutions that containing 8.37 mM selenite, 7.45 mM selenate and varying amounts of $\text{CeCl}_3$ based on the ratios at pH 7. Samples were measured after 24 hours on ICP-MS.

Figure 4. Percent removal of selenite and selenate by precipitation as white $\text{Ce}_2(\text{SeO}_3)_3$ precipitates at the ratio of 1:1 and 2:1 adding cerium(III) chloride solution with drop-by-drop and addition all-at-once methods. A solution for 1:1 ratio containing 9.32 mM selenite and 8.10 mM selenate, and a solution for 2:1 ratio containing 9.20 mM selenite and 7.79 mM selenate. Samples were allowed to react 24 hours and measured on ICP-MS.
Table 2. A summary of selenite removal by cerium(III) chloride at varying ratios including drop-by-drop addition and all-at-once addition methods

<table>
<thead>
<tr>
<th>Actual Ce(III) : Se(IV) or Ce(III) : Se(VI) ratio</th>
<th>Cerium(III) chloride concentration (mM)</th>
<th>Initial Se(IV) (mM)</th>
<th>Initial Se(VI) (mM)</th>
<th>Se(IV) removal percentage (%)</th>
<th>Se(VI) removal percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:1</td>
<td>0</td>
<td>8.37</td>
<td>7.45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.47:1</td>
<td>4.99</td>
<td>8.05</td>
<td>7.16</td>
<td>84.42</td>
<td>-5.28</td>
</tr>
<tr>
<td>0.63:1</td>
<td>7.41</td>
<td>7.96</td>
<td>7.08</td>
<td>98.90</td>
<td>-7.70</td>
</tr>
<tr>
<td>0.96:1</td>
<td>13.79</td>
<td>7.22</td>
<td>6.42</td>
<td>99.24</td>
<td>-3.99</td>
</tr>
<tr>
<td>1.91:1</td>
<td>24.24</td>
<td>6.34</td>
<td>5.64</td>
<td>99.97</td>
<td>-0.52</td>
</tr>
<tr>
<td>7.65:1</td>
<td>39.02</td>
<td>5.10</td>
<td>4.54</td>
<td>99.99</td>
<td>4.23</td>
</tr>
<tr>
<td>1.15:1(drop by drop)</td>
<td>10.71</td>
<td>9.32</td>
<td>8.10</td>
<td>100</td>
<td>13.13</td>
</tr>
<tr>
<td>1.15:1(all at once)</td>
<td>10.71</td>
<td>9.32</td>
<td>8.10</td>
<td>100</td>
<td>17.75</td>
</tr>
<tr>
<td>2.10:1(drop by drop)</td>
<td>19.35</td>
<td>9.20</td>
<td>7.79</td>
<td>100</td>
<td>15.58</td>
</tr>
<tr>
<td>2.10:1(all at once)</td>
<td>19.35</td>
<td>9.20</td>
<td>7.79</td>
<td>100</td>
<td>16.14</td>
</tr>
</tbody>
</table>

Ce(III) kinetics:

![Ce(III) kinetics graph](image)

Figure 5. Kinetics of selenite removal by precipitation as Ce₂(SeO₃)₃ after addition of dissolved CeCl₃ at the ratio of 2:1 and 8:1 under pH 7. Ce(III):Se(IV) = 2:1, [Se(IV)]<sub>initial</sub> = 7764.0 µM, [Ce(III)] = 10 mM, [MES] = 45 mM; Ce (III):Se (IV) = 8:1; [Se(IV)]<sub>initial</sub> = 9381.5 µM, [Ce(III)] = 10 mM, [MES] = 45 mM. The kinetics samples were taken up to 24 hours and measured by ICP-MS for Se(VI) concentrations.

3.3 Cerium(III) cations removal by ion exchange method results and discussions
3.3 Cerium(III) cations removal by ion exchange method results and discussions

Kinetics:

Figure 6. Kinetics of a solution containing 8.98 mM selenate mixed with 2 grams of Amberlite Na cation exchange resin at pH 7.1 within 2 hours: (a) shows the concentration of cerium(III) ions our time, \([\text{Ce(III)}]_{\text{initial}} = 0 \text{ mM}\); (b) shows the concentration of selenate our time, \([\text{Se(VI)}]_{\text{initial}} = 8.98 \text{ mM}\).

Figure 7. Kinetics of a solution containing 8.87 mM selenate + 8.28 mM Ce(III) mixed with 2 grams of Amberlite Na cation exchange resin at pH 6.25 within 2 hours: (a) shows the concentration of cerium(III) ions our time, \([\text{Ce(III)}]_{\text{initial}} = 8.28 \text{ mM}\); (b) shows the concentration of selenate our time, \([\text{Se(VI)}]_{\text{initial}} = 8.87 \text{ mM}\).
Figure 8. Kinetics of a solution containing 8.54 mM selenate + 29.4 mM Ce(III) mixed with 2 grams of Amberlite Na cation exchange resin at pH 5.73 within 2 hours: (a) shows the concentration of cerium(III) ions our time, \([\text{Ce(III)}]_{\text{initial}} = 29.4 \text{ mM}\); (b) shows the concentration of selenate our time, \([\text{Se(VI)}]_{\text{initial}} = 8.54 \text{ mM}\).

Figure 9. Kinetics of a solution containing 8.87 mM selenate + 8.28 mM Ce(III) without mixing of 2 grams of Amberlite Na cation exchange resin at pH 5.45 within 2 hours: (a) shows the concentration of cerium(III) ions our time, \([\text{Ce(III)}]_{\text{initial}} = 8.28 \text{ mM}\); (b) shows the concentration of selenate our time, \([\text{Se(VI)}]_{\text{initial}} = 8.87 \text{ mM}\).
Table 3. A summary of cerium(III) cation removal kinetics of samples containing 8.54 mM selenate + 29.4 mM cerium(III) chloride and 8.54 mM selenate + 8.28 mM cerium(III) chloride in batch mode ion exchange.

<table>
<thead>
<tr>
<th>Time</th>
<th>Ce (III) removal percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.87 mM selenate + 8.28 mM Ce(III)</td>
</tr>
<tr>
<td>0.083</td>
<td>99.34</td>
</tr>
<tr>
<td>0.25</td>
<td>99.96</td>
</tr>
<tr>
<td>0.5</td>
<td>99.98</td>
</tr>
<tr>
<td>1.0</td>
<td>99.98</td>
</tr>
<tr>
<td>2.0</td>
<td>99.98</td>
</tr>
</tbody>
</table>
Column and batch ion exchange:

Figure 10. Percent removal of cerium(III) cation by Amberlite IR 120 Na cation exchange resin in column mode (blue) and batch mode (red). 3 ml of resin was set up in ion exchange for column mode and 2 grams resin was prepared in 50-ml polypropylene for batch mode. Samples were included 8.98 mM Se(VI), 8.87 mM Se(VI) + 8.28 mM Ce(III), 8.56 mM Se(VI) + 18.3 mM Ce(III), and 8.54 mM Se(VI) + 29.4 mM Ce(III).

Figure 11. Selenate concentrations before and after contacting with Amberlite IR 120 Na cation exchange resin in column and batch modes. Initial and Final concentrations of selenate: [Se(VI)]$_{initial}$ = 8.98 mM, 8.87 mM, 8.56 mM, and 8.54 mM; [Se(VI)]$_{column final}$ = 9.01 mM, 9.04 mM, 8.82 mM, and 8.64 mM; [Se(VI)]$_{batch final}$ = 8.60 mM, 8.98 mM, 9.10 mM, and 9.26 mM that were listed for 8.98 mM Se(VI), 8.87 mM Se(VI) + 8.28 mM Ce(III), 8.56 mM Se(VI) + 18.3 mM Ce(III), and 8.54 mM Se(VI) + 29.4 mM Ce(III), respectively.
3.4 Monitoring δ¹⁸O SO₄ values results and discussions

Table 4. A summary of GSH and cerium(III) precipitation methods applied to remove selenite at pH 7.0 with selenite δ¹⁸O isotope analysis. Three types of batch reactors were prepared including selenite, selenate, and both selenite and selenate solution. After combining with solutions, [Ce(III)] = 19.35 mM and [GSH] = 37.5 mM. Ratios were in Ce(III):Se(IV) = 1.81:1, Ce(III):Se(VI) = 1.90:1, and in Ce(III):Se(IV) = 2.08:1 & Ce(III):Se(VI) = 1.89:1; GSH:Se(IV) = 4.21:1, GSH:Se(VI) = 4.81:1, and GSH:Se(VI) = 5.68:1 & GSH:Se(VI) = 4.87:1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial Se(IV)</th>
<th>Final Se(IV)</th>
<th>ΔSe(IV)</th>
<th>Initial Se(VI)</th>
<th>Final Se(VI)</th>
<th>ΔSe(VI)</th>
<th>δ¹⁸O-selenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerium (III) chloride treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se(VI) alone</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>9.9</td>
<td>10.2</td>
<td>-0.94</td>
<td>-7.30 ± 0.05</td>
</tr>
<tr>
<td>Ce(III)+Se(VI)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>10.2</td>
<td>10.8</td>
<td>-5.9</td>
<td>-3.50 ± 0.04</td>
</tr>
<tr>
<td>Ce(III)+Se(IV)+Se(VI)</td>
<td>9.3</td>
<td>0.3</td>
<td>96.8</td>
<td>10.3</td>
<td>8.5</td>
<td>16.11</td>
<td>-3.88 ± 0.17</td>
</tr>
<tr>
<td>GSH treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se(VI) alone</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7.9</td>
<td>7.9</td>
<td>0.00</td>
<td>-7.22 ± 0.11</td>
</tr>
<tr>
<td>GSH+ Se(VI)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7.8</td>
<td>7.9</td>
<td>-0.51</td>
<td>-4.79 ± 0.19</td>
</tr>
<tr>
<td>GSH+ Se(IV)+Se(VI)</td>
<td>6.6</td>
<td>0.0</td>
<td>100.0</td>
<td>7.7</td>
<td>7.8</td>
<td>-1.56</td>
<td>-3.26 ± 0.28</td>
</tr>
</tbody>
</table>

Table 5. A summary of cerium(III) cations removal with batch mode ion exchange method for selenate δ¹⁸O isotope analysis. The pH in initial samples without the addition of resin were 7.3, 5.4, 5.1, and 5.0. The pH in final samples with addition of resin were 7.4, 5.1, 5.2, and 5.3. 2 grams Amerblite Na resin was prepared in each reactors. Ce(III):Se(VI) ratios for each reactor were 0:1, 0.93:1, 2.14:1, and 3.44:1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial Se(VI)</th>
<th>Final Se(VI)</th>
<th>ΔSe(VI)</th>
<th>δ¹⁸O-selenate no resin</th>
<th>δ¹⁸O-selenate after resin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce(III) removed by</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ion exchange – batch mode</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.98 mM Se(VI)</td>
<td>8.98</td>
<td>8.59</td>
<td>0.38</td>
<td>-8.08 ± 0.26</td>
<td>-7.99 ± 0.28</td>
</tr>
<tr>
<td>8.28 mM Ce(III)+8.87 mM Se(VI)</td>
<td>8.87</td>
<td>8.98</td>
<td>-0.10</td>
<td>-7.68 ± 0.50</td>
<td>-7.77 ± 0.10</td>
</tr>
<tr>
<td>18.3 mM Ce(III)+8.56 mM Se(VI)</td>
<td>8.56</td>
<td>9.11</td>
<td>-0.55</td>
<td>-7.64 ± 0.27</td>
<td>-7.60 ± 0.32</td>
</tr>
<tr>
<td>29.4 mM Ce(III)+8.54 mM Se(VI)</td>
<td>8.55</td>
<td>9.26</td>
<td>-0.72</td>
<td>-7.79 ± 0.33</td>
<td>-7.76 ± 0.32</td>
</tr>
</tbody>
</table>
3.5 Other oxyanions precipitation with cerium(III) chloride results and discussion

Figure 12. Percent removal of arsenate with 12 mM arsenate and varying amounts of cerium(III) chloride at pH 7.0. White precipitates were presented rapidly after the addition of cerium(III) chloride. Samples were measured after 24 hours on ICP-MS.

Figure 13. Percent removal of phosphate with 12 mM phosphate and varying amounts of cerium(III) chloride at pH 7.0. White precipitates were presented rapidly after the addition of cerium(III) chloride. Samples were measured after 24 hours on ICP-MS.
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APPENDIX I – GENERAL INSTRUMENTS INTRODUCTION

An general summary of used instruments in this experiment including a Sorvall Legend XT centrifuge from Thermo Scientific Company, an Eppendorf mini spin centrifuge, a BioLogic low pressure chromatography (LC) unit, a Cole-Parmer Roto-Torque rotator, and a Cole-Parmer Mini magnetic stirrer.

An inductively coupled plasma mass spectrometer (ICP-MS) (BRUKER aurora M90) was used through all the experiments to analyze the concentration of dissolved selenium, cerium, arsenate, and carbonate. 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. The chromatography system consisted of a Varian ProStar autosampler and solvent delivery pump and a Hamilton PRP-X100 column. The eluent was a solution of 20 mM citric acid at pH 6 to provide buffer ability and a selenium oxyanion elution time of under 7 minutes, and 5 mM ethylenediamine tetraacetic acid (EDTA) to provide complexing ligands for any dissolved metal cations to avoid metal hydroxide precipitation inside the column. Machine response was calibrated to element concentrations using purchased standard solutions.

In addition, dissolved metals such as selenate and selenite concentrations were also measured by Dionex ICS-5000 ion chromatography system. An EGC KOH cartridge was used in the system, and the eluent generator concentrations were applied a multi-step gradient method consisting of 10 mM for 20 minutes followed by a ramp up to 30 mM at 15 minutes. If the IC was used to measured phosphate, there were some adjustment in the instrumental methods. First, the eluent generator concentration was switched to isocratic method. Second, the eluent
concentration was set up at 35 mM. Third, the time needed to be extend to 20 minutes for sufficient time to analysis.
APPENDIX II–EXAMPLES OF STANDARD CURVE IN THE EXPERIMENT

Standard solutions were included selenate, selenite, phosphate, arsenate, and carbonate that were measured on ICP-MS to make standard curve. Figure # and # show examples of standard curves used in GSH reductive precipitation experiment.

Figure 15. Se (IV) standard curve in the GSH reductive precipitation ratio determination experiment, the concentrations of standard solutions were 0, 0.5, 5, 50, 100, and 150 µM.

Figure 16. Se (IV) standard curve in the GSH reductive precipitation kinetic experiment, and the concentrations of standard solutions were 0, 0.5, 5, 50, 100, and 150 µM.