Environmental Science & Technology

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Sequential Extraction Method for Determination of Fe(II/III) and U(IV/VI) in Suspensions of Iron-Bearing Phyllosilicates and Uranium

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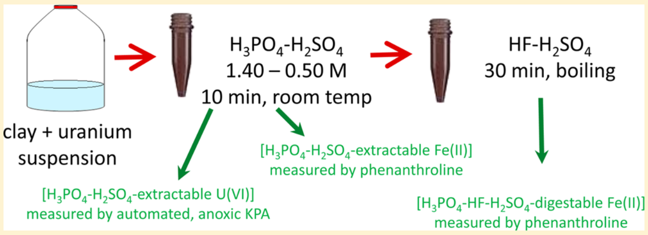
Supporting Information

ABSTRACT: Iron-bearing phyllosilicates strongly influence the redox state and mobility of uranium because of their limited hydraulic conductivity, high specific surface area, and redox reactivity. Standard extraction procedures cannot be accurately applied for the determination of clay-Fe(II/III) and U(IV/VI) in clay mineral-U suspensions such that advanced spectroscopic techniques are required. Instead, we developed and validated a sequential extraction method for determination of clay-Fe(II/III) and U(IV/VI) in clay-U suspensions. In our so-called "H_3PO_4−HF−H_2SO_4 sequential extraction" method, H_3PO_4−H_2SO_4 is used first to solubilize and remove U, and the remaining clay pellet is subject to HF−H_2SO_4 digestion. Physical separation of U and clay eliminates valence cycling between U(IV/VI) and clay-Fe(II/III) that otherwise occurred in the extraction solutions and caused analytical discrepancies. We further developed an "automated anoxic KPA" method to measure soluble U(VI) and total U (calculate U(IV) by difference) and modified the conventional HF−H_2SO_4 digestion method to eliminate a series of time-consuming weighing steps. We measured the kinetics of uraninite oxidation by nontronite using this sequential extraction method and anoxic KPA method and measured a stoichiometric ratio of 2.19 ± 0.05 mol clay-Fe(II) produced per mol U(VI) produced (theoretical value of 2.0). We found that we were able to recover 98.0−98.5% of the clay Fe and 98.1−98.5% of the U through the sequential extractions. Compared to the theoretical stoichiometric ratio of 2.0, the parallel extractions of 0.5 M HCl for clay-Fe(II) and 1 M $NaHCO_3$ for U(VI) leached two-times more Fe(II) than U(VI). The parallel extractions of HF−H_2SO_4 for clay Fe(II) and 1 M $NaHCO_3$ for U(VI) leached six-times more Fe(II) than U(VI).



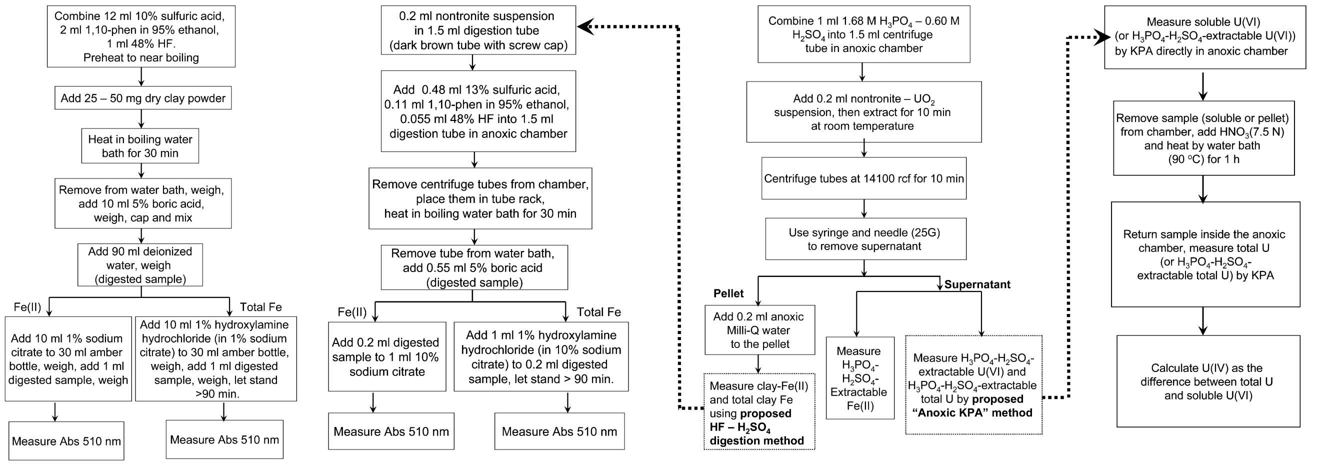
■ INTRODUCTION

Uranium contamination is a problem at many U.S. Department of Energy (DOE) sites and uranium ore-processing sites. One strategy for in situ remediation is to add electron donors to stimulate anaerobic conditions and reduce mobile uranyl (VI) to sparingly soluble uraninite ($U(IV)O_2(s)$).[1−3] However, further studies have shown that uraninite can be reoxidized by nitrate,[4] iron oxides,[5] and iron(III)-bearing phyllosilicates,[6] in addition to oxygen.[7] The mass of iron associated with phyllosilicate minerals is higher than the mass of iron associated with oxide minerals at several DOE sites.[8,9] Because phyllosilicates provide a large solid-phase reservoir of Fe(II/III) that can oxidize U(IV) or reduce U(VI) they may strongly influence the redox state and mobility of uranium.

Various operational extractions have been used for measuring Fe content and Fe(II/III) speciation in minerals and soils (Table S1). The combination of HF and H_2SO_4 has been used to dissolve clays and refractory minerals for over 50 years.[10] The method has evolved and become more standardized through a series of reports by Stucki and co-workers[11−13] and a report by Amonette and Templeton[14] (Figure 1a). In our current study we refer to this as the "conventional" HF−H_2SO_4 digestion method for clay-Fe(II). The conventional HF−H_2SO_4 digestion method has been used with a wide variety of iron-bearing phyllosilicates and has been shown to be superior to the 0.5 N HCl-ferrozine method because HCl cannot completely dissolve silicates which leads to erratic results and an underestimation of Fe(II) content.[15]

Various operational extractions have also been used for measuring solid-associated U concentrations in soils and sediments (Table S2). HNO_3 has been used to dissolve uranium dioxide in nuclear fuel processing for over 50 years.[16−18] HNO_3 is particularly effective because it will promote both proton-driven and oxidative dissolution of UO_2. Total U in soils and sediments has been measured using a 1 N HNO_3 extraction[19] but cannot be used for U(IV/VI) valence state determinations because NO_3^- can oxidize U(IV)[17,18,20] even under anoxic acidic conditions. A series of less harsh reagents has been used to extract U from soils including sodium bicarbonate (0−1.0 M, pH 8.2−8.6), ammonium carbonate (0.5 M, pH 9.0), ammonium acetate (1.0 M, pH 7.0), Na-EDTA (1.0 mM), and Na-citrate (2.0 mM).[19,21−24] In most cases the reagents are used because of

Received: August 15, 2012
Revised: October 12, 2012
Accepted: October 17, 2012
Published: October 17, 2012



a. Conventional HF-H_2SO_4 digestion method b. Modified HF-H_2SO_4 digestion method c. Proposed H_3PO_4-H_2SO_4 extraction method d. Proposed automated anoxic KPA method

Figure 1. Detailed flowcharts of extraction procedures. a. Conventional HF−H_2SO_4 digestion method; b. Modified anoxic HF−H_2SO_4 digestion method; c. Proposed H_3PO_4−H_2SO_4 extraction step to remove U; and d. Proposed automated anoxic KPA method. For sample suspensions containing both U and clay minerals, the H_3PO_4−HF−H_2SO_4 sequential extraction procedure begins at the top of c, where the sample is first extracted with H_3PO_4−H_2SO_4. The supernatant of that extraction is used to measure U(VI) using the automated anoxic KPA method (d), while the mineral pellet is extracted with HF−H_2SO_4 to measure clay-Fe(II) (b).

their ability to complex and solubilize U(VI). However, Zhou and Gu[24] showed that the redox condition of the soil-extract suspension had a significant effect on the forms of U extracted from contaminated soils. In particular, insoluble U(IV) phases were not extracted into $NaHCO_3$ solutions under anoxic conditions. However, under oxic conditions, where U(IV) oxidation is thermodynamically favorable in solutions with high $NaHCO_3$ concentrations,[5] U(IV) was oxidized and then complexed/extracted with $NaHCO_3$.

Several recent studies have used U L_{III}-edge extended X-ray absorption fine structure (EXAFS) to identify nonuraninite forms of U(IV) in bioreduced suspensions and sediments.[25−29] This sparingly soluble, monomeric U(IV) has been shown to be more susceptible to reoxidation as compared to biogenic UO_2.[29] Related to this, a very recent study has shown that monomeric U(IV) was effectively extracted into 1.0 M $NaHCO_3$ (pH 8.7) and formed aqueous U(IV)-carbonate species.[30] We have previously shown that natural organic matter can complex biogenic U(IV) such that soluble forms of U(IV) can exist in the aqueous phase.[31] Thus, the use of anoxic extraction solutions and the maintenance of anoxic conditions throughout U analysis is extremely important for accurate determination of operationally defined U(IV) and U(VI) concentrations.

The objectives of this research were to 1) develop and validate a new sequential acid extraction method to measure both Fe(II/III) and U(IV/VI) concentrations in systems containing iron-bearing phyllosilicates and uranium; 2) develop an automated "anoxic KPA" method such that both U(VI) and total U can be quantified in aqueous or extracted samples (U(IV) calculated by difference); and 3) modify the conventional HF−H_2SO_4 digestion method for more strictly anoxic operation (and simultaneously eliminate many gravimetric steps in the method). In this study we show that the conventional HF−H_2SO_4 digestion method for clay-Fe(II) produced erroneous results when uraninite ($U(IV)O_2(s)$) was included in sample suspensions. In addition, we show that the conventional 1 M $NaHCO_3$ extraction method for U(VI) produced erroneous results when iron-bearing nontronite was included in sample suspensions.

MATERIALS AND METHODS

A detailed flowchart of all extraction procedures is shown in Figure 1. A complete description of all materials, sample handling, and step-by-step analytical methods are provided in the Supporting Information while an abbreviated description is provided below.

Mineral Preparation. Nontronite NAu-2 was selected because it is a well characterized and studied specimen clay mineral. NAu-2 was suspended in 0.5 M NaCl for 24 h and then separated by centrifugation to obtain the 0.5−2.0 μm clay size fraction. Chemically reduced nontronite NAu-2 and H_3PO_4−H_2SO_4-washed nontronite NAu-2 were also prepared in this study.

Biogenic uraninite precipitates were produced with *Shewanella putrefaciens* strain CN32 (1×10^8 cell/mL; harvested at late log phase) using uranyl acetate (1.0 mM) as the sole electron acceptor and sodium lactate (5 mM) as electron donor in anoxic 30 mM $NaHCO_3$ buffer. Biogenic uraninite was 82.5% U(IV). Initial uraninite concentrations are reported as total U and total uraninite(IV).

Reactions between Nontronite and U. The first series of experiments was conducted with nontronite and U and used the conventional methods to measure clay-Fe(II) and U(VI). NAu-2 was dispensed into anoxic 30 mM $NaHCO_3$ (filled with 80:20% N_2:CO_2, pH 6.8) in 26 mL glass serum bottles with a final concentration of 0.5 g/L. Total solution volume in the serum bottles was 20 mL. Final U concentrations in the reactors were 90 to 720 μM uraninite(IV) or U(VI) (equivalent to 110 to 880 μM total U for UO_2-amended suspensions and 90 to 720 μM total U for U(VI)-amended suspensions). Clay-Fe(II) concentrations were measured using our modified anoxic HF−H_2SO_4 digestion and an anoxic 0.5 N HCl extraction. U(VI) concentrations were measured using the conventional 1 M $NaHCO_3$ extraction.

The second series of experiments was conducted with nontronite and uraninite and used the sequential extraction method to measure clay-Fe(II) and U(VI). NAu-2, chemically reduced NAu-2, or acid-washed NAu-2 was dispensed into anoxic 30 mM $NaHCO_3$ in 26 mL glass serum bottles (0.5 g/L). Experiments were initiated by the addition of 330 μM uraninite(IV) (400 μM total U). Samples were first extracted with H_3PO_4−H_2SO_4 to remove all U (U(VI) and total U were subsequently measured by automated anoxic KPA), and then clay-Fe(II) and total clay-Fe were measured using our modified anoxic HF−H_2SO_4 digestion method.

A third series of experiments was conducted to measure U mass balance recoveries with the sequential extraction method. NAu-2 was dispensed into anoxic 30 mM $NaHCO_3$ in 26 mL glass serum bottles (0.5 g/L). Final U concentrations in the reactors were 165 to 660 μM uraninite(IV) (equivalent to 200 to 800 μM total U).

A fourth and final series of experiments was conducted to measure Fe mass balance recoveries with the sequential extraction method. NAu-2 or acid-washed NAu-2 was dispensed into anoxic 30 mM $NaHCO_3$ in 26 mL glass serum bottles at final concentrations of 0.1, 0.5, or 1.0 g/L, and t = 0 samples were collected immediately. Samples were sequentially extracted with H_3PO_4−H_2SO_4 and HF−H_2SO_4 to measure Fe in both extraction solutions.

Proposed Sequential Extraction Method for U(IV/VI) and Clay-Fe(II/III) Measurement. The key feature of our proposed method is the physicochemical separation of U from the solid clay before the clay mineral is digested for the determination of clay-Fe(II) (Figure 1c). H_3PO_4 and H_2SO_4 were selected because they are widely used to dissolve and measure uranium oxides.[32−34] Both acids were purged with 99.995% N_2 for at least 1 h and then stored in the anoxic chamber for several days before use. Preliminary experiments during method development were conducted using variable concentrations and ratios of H_3PO_4 and H_2SO_4 and extraction times for comparison purposes. To determine the optimum acid concentrations, 0.2, 0.4, 0.6, 0.8, 1.0, or 1.2 mL of O_2-free H_3PO_4 (1.68 M)−H_2SO_4 (0.60 M) was added to a 0.2 mL sample suspension (0.5 g/L NAu-2, 400 μM UO_2). These mixtures yielded final H_3PO_4−H_2SO_4 concentrations (all M), respectively, of 0.84−0.30, 1.12−0.40, 1.26−0.45, 1.34−0.48, 1.40−0.50, and 1.44−0.51 in the extraction solutions. All samples were extracted for 10 min. To determine the optimum extraction time, extraction times were varied from 5 to 60 min, while the final concentrations of H_3PO_4−H_2SO_4 were fixed at 1.40 M−0.50 M, respectively.

Automated Anoxic KPA Method for Measurement of U(VI) and Total U. All U measurements were performed with a KPA-11 (ChemChek, Richland, WA) equipped with an autosampler where the whole unit was operated within an anoxic chamber. The KPA technique is valence state-specific, where it only measures luminescent hexavalent uranium.[35−37] The complete step-by-step automated anoxic KPA method is provided in the Supporting Information.

Multiple forms of U were operationally defined and measured in this study. For various experiments we measured $NaHCO_3$-extractable and H_3PO_4−H_2SO_4-extractable U(VI) under strictly anoxic conditions. Oxidized split samples of each of these operational fractions were used to measure total $NaHCO_3$-extractable and total H_3PO_4−H_2SO_4-extractable U, respectively. Corresponding U(IV) concentrations for each of these operational fractions were calculated as the difference between total U and U(VI).

H_3PO_4−H_2SO_4-extractable U(VI) and total H_3PO_4−H_2SO_4-extractable U were measured as follows. A 0.2 mL of sample suspension (0.5 g/L NAu-2, 0 to 720 μM total U) was added into a 1.5 mL centrifuge tube which contained 1.0 mL of H_3PO_4 (1.68 M)−H_2SO_4 (0.60 M) (1.40−0.50 M final concentrations). The tube was capped, shaken by hand, allowed to stand for 10 min, and then centrifuged at 14,100 g for 10 min (pelletized particles <0.02 μm). The supernatant was used to measure U and H_3PO_4−H_2SO_4-extractable Fe(II), while the mineral pellet was used to measure H_3PO_4−HF−H_2SO_4-digestable Fe(II). The anoxic supernatant was analyzed by automated anoxic KPA to measure H_3PO_4−H_2SO_4 extractable U(VI). Supernatant samples were removed from the anoxic chamber, oxidized with oxic HNO_3 (1 mL of conc. HNO_3 + 1 mL of sample; 7.5 N final concentration) at 90 °C for 1 h,[16−18] returned inside the anoxic chamber, and analyzed by automated anoxic KPA to measure total U H_3PO_4−H_2SO_4 extractable U (Figure 1d). We refer to Δ H_3PO_4−H_2SO_4-extractable U as the difference between the sample and the corresponding UO_2-only control.

Measurements of $NaHCO_3$-extractable U(VI) and total $NaHCO_3$-extractable U are described in theSupporting Information. We refer to Δ $NaHCO_3$-extractable U as the difference between the sample and the corresponding UO_2-only control.

Modified Anoxic HF−H_2SO_4 Digestion Method for Measurement of Clay-Fe(II) and Total Clay-Fe. Note that HF is an extremely corrosive and dangerous acid. We suggest researchers consult with their lab safety experts before following our procedures. In order to work under more strictly anoxic conditions we modified the conventional HF−H_2SO_4 digestion method used to measure clay-Fe(II) as follows (Figure 1b). 1) All operations were performed in an anoxic chamber until the point when the sample had to be heated in boiling water. 2) All solutions and reagents were deoxygenated, except HF. H_2SO_4 was purged with 99.995% N_2 for at least 1 h and then stored in the anoxic chamber for several days before use. Ethanol used for the preparation of 1,10-phenanthroline was deoxygenated by purging with 99.995% H_2 in the presence of a Pd catalyst (0.5 wt % Pd on 3.2-mm alumina pellets) inside the anoxic chamber. 3) Digestion tubes (1.5 mL dark brown centrifuge tubes with screw caps) were capped tightly before removal from the anoxic chamber to avoid loss of solution in the boiling water bath (and to avoid having to weigh tubes so frequently). We confirmed that less than 0.2% of solution (m/m) was lost during heating. 4) The boiling water bath was sealed and flushed with 99.995% N_2 to minimize oxygen contamination during this step. We refer to the Fe(II) concentration measured by the modified anoxic HF−H_2SO_4 digestion method as HF−H_2SO_4-digestable Fe(II).

For samples that were first extracted with H_3PO_4−H_2SO_4, the mineral pellet was subjected to anoxic HF−H_2SO_4 digestion as follows. 0.2 mL of sample suspension (0.5 g/L NAu-2, 90−720 μM UO_2 or U(VI)) was added into a 1.5 mL digestion tube which contained 1.0 mL of H_3PO_4 (1.68 M)−H_2SO_4 (0.60 M) and centrifuged at 14,100 g for 10 min. Needle and syringe were used to carefully remove all of the supernatant from the tube. In addition to removing and measuring U, the supernatant was used to measure H_3PO_4−H_2SO_4-extractable Fe(II) by 1,10-phenanthroline. 0.2 mL of anoxic Milli-Q water was added to the mineral pellet, and a pipet was used to resuspend the tube contents. H_2SO_4, 1,10-phenanthroline, and HF were then added to the tube, and samples were analyzed according to our anoxic HF−H_2SO_4 digestion method. We refer to this quantity as H_3PO_4−HF−H_2SO_4-digestable Fe(II). We refer to total clay-Fe(II) as the sum of H_3PO_4−HF−H_2SO_4-digestable Fe(II) plus H_3PO_4−H_2SO_4-extractable Fe(II). We refer to Δ total clay-Fe(II) as the difference between the sample and the corresponding NAu-2-only control.

■ RESULTS

U−Fe Valence Cycling in "Parallel Extraction" Solutions. With suspensions of unaltered nontronite (99.4% Fe(III)) and uranyl(VI) acetate, clay-Fe(II) concentrations measured in all initial samples using our modified anoxic HF−H_2SO_4 digestion method or an anoxic 0.5 N HCl extraction were reasonably low (Figure 2a, open symbols). In contrast, with suspensions of nontronite and uraninite (82.5% U(IV)), "parallel" extractions for clay-Fe(II) (in HF−H_2SO_4 or HCl) and U(VI) (in $NaHCO_3$) measured elevated concentrations of clay-Fe(II) in all initial samples (Figure 2). The extent of nontronite-Fe(III) reduction was not expected to be so rapid under these conditions. In fact, we show that these results were produced due to reactions that occurred during the acid extraction/digestion step (30 min in HF−H_2SO_4 or 24 h in HCl) and not during the first 1−5 min in the reactant suspensions. Using either the anoxic HF−H_2SO_4 digestion or the 0.5 N HCl extraction (Figure 2a), higher concentrations of clay-Fe(II) were measured with increasing concentrations of uraninite. With the anoxic HF−H_2SO_4 digestion, clay-Fe(II) concentrations measured at $t = 0$ ranged from 171 to 887 μM for all of the uraninite concentrations tested. In all of these experiments the nontronite concentration was 0.5 g/L, equivalent to 2,050 μM Fe(III), such that 8.3 to 43% of the clay-Fe(III) had been reduced in these samples. With the anoxic HCl extraction, clay-Fe(II) concentrations measured at $t = 0$ ranged from 112 to 200 μM for all of the uraninite concentrations tested (5.5 to 9.8% reduction of clay-Fe(III)). The elevated temperature of the HF−H_2SO_4 digestion (boiling water bath vs room temp for HCl extraction) likely increased the kinetics of uraninite oxidation by clay-Fe(III), consistent with results from Pierce et al.[38] We also suspect that Fe(III) dissolved by HF−H_2SO_4 could form Fe(III)-phenanthroline complexes that could oxidize uraninite.

The "parallel" extraction for U(VI) in $NaHCO_3$ also measured elevated concentrations of $NaHCO_3$-extractable U(VI) in all initial samples (Figure 2b). These were "direct" measurements where H_3PO_4−H_2SO_4 was not used to first remove U from the suspensions. Δ $NaHCO_3$-extractable U(VI) concentrations at $t = 0$ ranged from 29 to 52 μM and increased with increasing uraninite concentrations. For the initial uraninite concentrations of 90 to 720 μM total U, uraninite oxidation extent ranged from 32 to 7.2%, respectively, thus decreasing with increasing uraninite concentrations. Based on these "parallel" measurements of clay-Fe(II) and U(VI), the stoichiometry of clay-Fe(III) reduction coupled to uraninite oxidation could be evaluated. Theoretically, 2 mol of clay-Fe(II) should be produced per 1 mol of U(VI) produced. Stoichiometric ratios for Δ mol clay-Fe(II) per Δ mol U(VI) calculated from the measurements obtained with the "parallel" extractions of HF−H_2SO_4 or HCl for clay-Fe(II) and $NaHCO_3$ for U(VI) did not agree with this 2:1 mol Fe(II) per mol U(VI) ratio (Figure 2c). The Δ mol clay-Fe(II):Δ mol U(VI) average ratio was 12.9 ± 3.97 ($n = 7$) for the anoxic HF−H_2SO_4 digestion method and 3.87 ± 0.15 ($n = 7$) for the anoxic HCl extraction method. Because clay-Fe(II) and U(VI) concentrations were measured in different extraction solutions (from

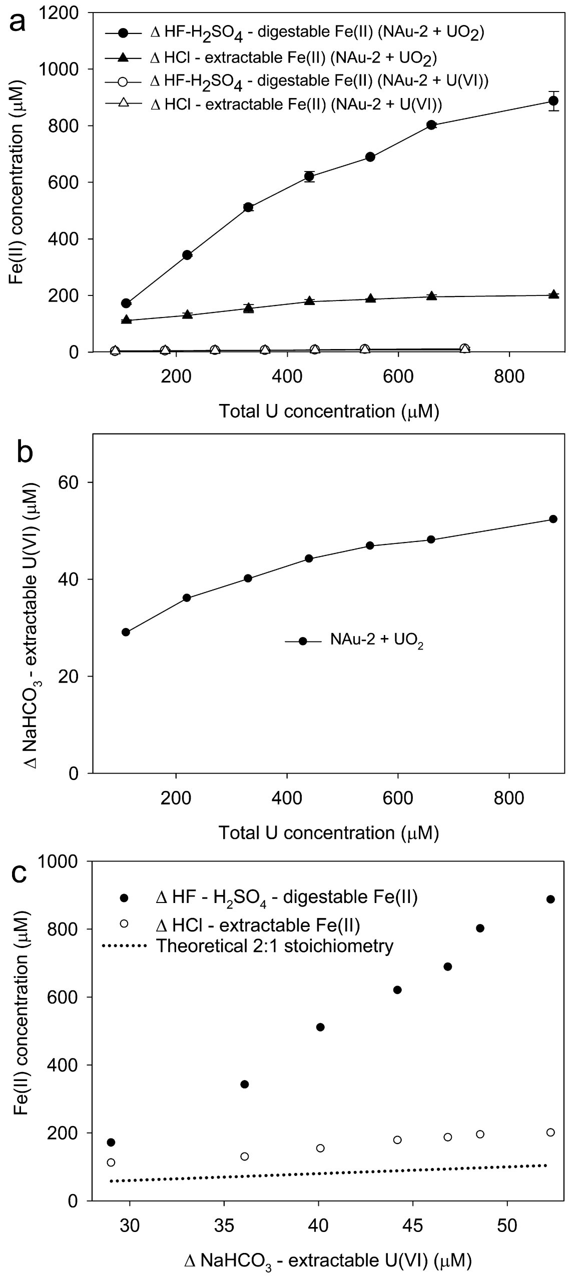


Figure 2. Measurements of clay-Fe(II) and U(VI) in suspensions of nontronite NAu-2 and U(IV) or U(VI) using conventional parallel methods of HF−H_2SO_4 or 0.5 N HCl for clay-Fe(II) and 1.0 M $NaHCO_3$ for U(VI). All samples collected at $t = 0$, equivalent to a reaction time of 1−5 min. Experiments conducted with 0.5 g/L NAu-2 and 90−720 μM total U in 30 mM $NaHCO_3$ (pH 6.8). a. Δ Fe(II) concentrations measured by modified anoxic HF−H_2SO_4 digestion method or 0.5 N HCl extraction method. b. Δ $NaHCO_3$-extractable U(VI) concentrations. c. Stoichiometric relationships between Δ mol Fe(II) and Δ mol U(VI) for the different clay-Fe(II) measurements. Dashed line represents theorectical stoichiometry of 2 Δ mol Fe(II) to 1 Δ mol U(VI). Concentration ratio of (Δ HCl-extractable Fe(II)/Δ $NaHCO_3$-extractable U(VI)) was 3.87 ± 0.15 ($n = 7$), while the concentration ratio of (Δ HF−H_2SO_4-digestable Fe(II)/Δ $NaHCO_3$-extractable U(VI)) was 12.9 ± 3.97 ($n = 7$).

the same $t = 0$ sample) stoichiometric agreement between these quantities does not have to occur. Our interpretations of these results are 2-fold. First, under acidic conditions in the HF–H_2SO_4 digestion solution or HCl extraction solution, uraninite oxidation by clay-Fe(III) is favorable and rapid. Second, under alkaline conditions (pH 8.4) in the $NaHCO_3$ extraction solution, uraninite oxidation by clay-Fe(III) is also favorable and rapid.[5]

U(VI) and Clay-Fe(II) Concentrations in Sequential Extractions. When using our proposed method of H_3PO_4–H_2SO_4 extraction for U(VI) followed by HF–H_2SO_4 digestion for clay-Fe(II), we measured significantly lower concentrations of both analytes in our $t = 0$ samples (values along y-axes in Figure 3a,b) as compared to the conventional parallel extractions (Figure 2a,b). In these experiments 0.5 g/L nontronite was reacted with 330 μM uraninite(IV). H_3PO_4–H_2SO_4-extractable U(VI), H_3PO_4–H_2SO_4-extractable Fe(II), and H_3PO_4–HF–H_2SO_4-digestable Fe(II) were measured over an 8 d incubation period. In the parallel extraction experiments, the most similar reaction conditions included 0.5 g/L nontronite and 360 μM uraninite(IV). At $t = 0$, we measured 25 μM Δ H_3PO_4–H_2SO_4-extractable U(VI) as compared to 44 μM Δ $NaHCO_3$-extractable U(VI) (Figure 2b) and 57 μM Δ total clay-Fe(II) as compared to 620 μM Δ HF–H_2SO_4-digestable Fe(II) (Figure 2a). The dramatic differences between the clay-Fe(II) concentrations measured in the presence (i.e., via "direct" method with HF–H_2SO_4) and absence of uraninite (i.e., removed with H_3PO_4–H_2SO_4) in these $t = 0$ samples demonstrate that the majority of clay-Fe(II) measured by the HF–H_2SO_4 digestion method was produced during the acid digestion step (30 min in boiling water bath) and not during the first few minutes in the reactant suspensions. With unaltered nontronite, Δ H_3PO_4–H_2SO_4-extractable U(VI) increased to 114 μM and Δ total clay-Fe(II) increased to 243 μM after 8 d. After 82 d reaction between uraninite and nontronite, we measured 162 μM Δ H_3PO_4–H_2SO_4-extractable U(VI) and 337 μM Δ total clay-Fe(II) (data not shown).

Because the H_3PO_4–H_2SO_4 extraction step does remove a small fraction of the clay-Fe (discussed below), we chose to repeat these kinetic experiments with H_3PO_4–H_2SO_4-washed nontronite to gauge this effect on clay reactivity (Figure 3a,b). Note that the H_3PO_4–H_2SO_4 extraction time was purposefully short (10 min plus 10 min centrifugation) to minimize redox reactions between U(IV) and clay-Fe(III) under the imposed acidic conditions and to minimize clay dissolution (Figure S1). In the absence of uraninite, approximately 5.3% (m/m) of clay Fe was solubilized into the H_3PO_4–H_2SO_4 extraction solution (Figure S2). With acid-washed nontronite and in the presence of uraninite, we measured even lower concentrations of U(VI) and clay-Fe(II) at $t = 0$. Specifically, we measured 12 μM Δ H_3PO_4–H_2SO_4-extractable U(VI) and 28 μM Δ total clay-Fe(II). We also found that the rate and extent of uraninite oxidation decreased with acid-washed nontronite as compared to unaltered nontronite (Figure 3a,b). We speculate that this acid-wash step removed a labile fraction of clay-Fe(III) that was particularly reactive with uraninite leading to these differences.

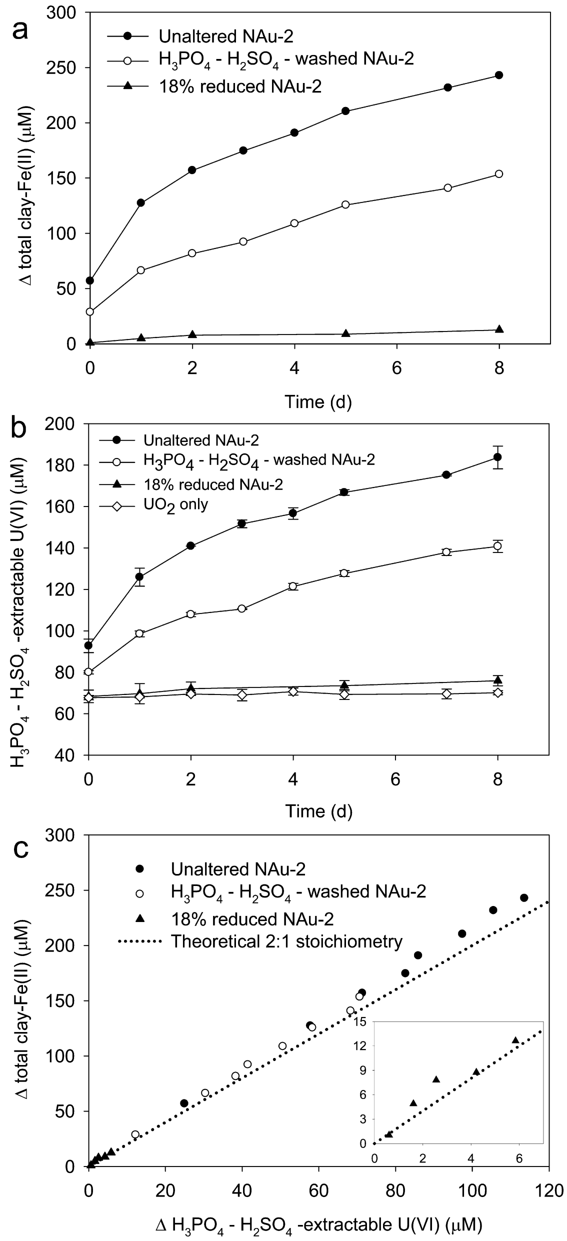


Figure 3. Kinetics of uraninite oxidation by various forms of nontronite NAu-2. Unaltered nontronite (99.4% Fe(III)), H_3PO_4–H_2SO_4-washed nontronite (99.4% Fe(III)) and chemically reduced nontronite (82.0% Fe(III)) were all used. Experiments conducted with 0.5 g/L NAu-2 and 330 μM uraninite(IV) in 30 mM $NaHCO_3$ (pH 6.8). Clay-Fe(II) and U(VI) concentrations measured by proposed sequential extraction procedure. a. Δ Total clay-Fe(II) concentrations versus time. b. Δ H_3PO_4–H_2SO_4-extractable U(VI) concentrations versus time. c. Stoichiometric relationships between Δ mol Fe(II) and Δ mol U(VI) for the proposed sequential extraction procedure. Dashed line represents theoretical stoichiometry of 2 Δ mol Fe(II) to 1 Δ mol U(VI). Concentration ratios of (Δ total clay-Fe(II) concentrations/Δ H_3PO_4–H_2SO_4-extractable U(VI)) were 2.19 ± 0.05 ($n = 8$) for the unaltered nontronite, 2.18 ± 0.08 ($n = 8$) for the acid-washed nontronite, and 2.40 ± 0.61 ($n = 5$) for the chemically reduced nontronite (inset with zoomed scale).

We chose to repeat these kinetic experiments with chemically reduced nontronite (18% Fe(II)) to evaluate how our proposed method would work under conditions of limited reactivity. Starting with chemically reduced nontronite, Δ H_3PO_4–H_2SO_4-extractable U(VI) increased only to 5.8 μM, while Δ total clay-Fe(II) increased to 12.6 μM after 8 d. These results suggest that only a small fraction of the structural Fe(III) in the chemically reduced nontronite could oxidize uraninite. A similar explanation is that, as a redox probe molecule, uraninite could only reduce a small fraction of structural Fe(III) in nontronite. The reaction constraint is most likely thermodynamic as results did not appreciably change after an 80 d incubation period (data not shown).

Most importantly with respect to method validation, we found excellent stoichiometric agreement between 2 mol of Δ clay-Fe(II) produced per 1 mol of Δ U(VI) produced using the sequential extraction method with all of the nontronites tested (Figure 3c). Nontronite-Fe(III) was reduced and uraninite was oxidized, and the increasing concentration of total clay-Fe(II) was consistent with the kinetics of U(VI) production. Specifically, the Δ mol Fe(II) per Δ mol U(VI) average ratio was 2.19 ± 0.05 ($n = 8$) for all of the kinetic samples measured with unaltered nontronite and uraninite, 2.18 ± 0.08 ($n = 8$) with acid washed-nontronite and uraninite, and 2.40 ± 0.6 ($n = 5$) with chemically reduced nontronite and uraninite.

U and Fe Mass Recoveries in Sequential Extractions. The proposed method has some intrinsic drawbacks (neglecting the labor efforts). The H_3PO_4−H_2SO_4 extraction step to remove U was also found to dissolve nontronite and remove clay Fe (Figures S2 and S3). We measured Fe(II) and total Fe in both the H_3PO_4−H_2SO_4 extraction solutions and the H_3PO_4−HF−H_2SO_4 digestion solutions to quantify mass balance recoveries for Fe in our proposed method. For these experiments we used both nontronite and H_3PO_4−H_2SO_4-washed nontronite with no U included in the suspensions. As expected, we found that H_3PO_4−H_2SO_4 removed more Fe from nontronite as compared to acid washed-nontronite and that proportionally more Fe was removed at lower nontronite suspension concentrations (Figure S2). The mass of Fe removed with H_3PO_4−H_2SO_4 was independent of the acid washed-nontronite suspension concentration. With 0.5 g/L nontronite, the clay suspension concentration used most often in all other experiments, $5.3 \pm 0.4\%$ ($n = 4$) of clay Fe was removed in the H_3PO_4−H_2SO_4 extraction solution and 91.4 to 94.4% of clay Fe was recovered in the H_3PO_4−HF−H_2SO_4 digestion solutions. Compared to our "direct" anoxic HF−H_2SO_4 digestion method to measure total Fe (in the absence of U), we were able to recover 98.0 to 98.5% of clay Fe in the H_3PO_4−H_2SO_4 and H_3PO_4−HF−H_2SO_4 solutions.

Mass balance recoveries for U were measured in a series of $t = 0$ samples with suspensions of 0.5 g/L nontronite and 165 to 660 μM uraninite analyzed according to the sequential extraction method. This experiment was conducted on two separate occasions for replication and validation purposes (Figure S3). Total U concentrations were measured in UO_2-only controls that were oxidized with HNO_3. We were able to recover 98.1 to 98.5% and 97.9 to 98.5% of total U in nontronite+UO_2 systems and nontronite+U(VI) systems, respectively, and consistently in replicate experiments. H_3PO_4−H_2SO_4 extraction efficiency of U was independent of the uraninite concentration.

DISCUSSION

Operational extractions for elements adsorbed to or incorporated into mineral matrices are often difficult to design and rarely perfect. For example, while high H^+ concentrations in acids can displace adsorbed metal cations (e.g., Fe^{2+}, Mn^{2+}), acidic conditions can also dissolve host mineral phases. Elevated concentrations of other host elements can interfere with the quantification of the target analyte (e.g., with high ratios of $[Fe^{3+}]$ to $[Fe^{2+}]$). Thermodynamic favorability of reaction(s) that alter analyte concentration(s) can occur in the extraction solution as compared to the sample suspension (e.g., with uraninite and ferrihydrite in 1 M $NaHCO_3$). It is difficult to identify redox-driven analytical artifacts unless strict controls on all oxidants and reductants are maintained, and a theoretical stoichiometric ratio between consumption/production of oxidants and reductants can be verified. An important conclusion from the current study is that one must carefully examine both the experimental conditions and the analytical conditions when examining systems with both Fe and U.

We have previously reported on the bioreduction of nontronite NAu-2 and U(VI)[6] and on the bioreduction of chlorite CCa-2 and U(VI)[39] before we developed our sequential extraction method. In those experiments we often relied on measurements of dissolved (<0.2 μm) U(VI) and Fe(II) and ferrozine-extractable Fe(II)[5] because we had encountered analytical difficulties but had not yet solved the problems. In those experiments, however, we also used parallel extractions for U(VI) (1 h in 1 M $NaHCO_3$) and clay-Fe(II) (24 h in 0.5 N HCl for NAu-2; 30 min in HF−H_2SO_4 for CCa-2) that now, in hindsight, were likely influenced by these artifacts. The general conclusion from those previous studies was that the oxidation of uraninite by clay-Fe(III) was the critical reaction that allowed U valence cycling to occur during bioreduction. Our current study demonstrates the thermodynamic feasibility of this reaction and supports those previous studies.

Oxidized forms of reactants (e.g., clay-Fe(III) with U(VI)) are often used to initiate bioreduction or chemical reduction experiments. Uninoculated or pasteurized or no-reductant controls are typically prepared with these same initial oxidized reactants. If a reduced product (e.g., uraninite) is the primary cause of a redox-driven analytical artifact, then that process will not be evident or important in these unreduced controls. The reduced product(s) will, however, affect measurements in the "active" reactant suspensions. Spectroscopic techniques can be employed on samples that have been manipulated to a minimum degree (and not subjected to any operational extractions) to avoid (or detect) these types of analytical problems. However, dedicated, sophisticated equipment is required and sample throughput is relatively slow compared to wet chemical techniques. Furthermore, the precision of valence state estimations by e.g., X-ray absorption near edge spectroscopy (XANES) are often only ±10% and the concentration of the target element needs to relatively high (e.g., >0.1% m/m). Even so, complementary spectroscopic and microscopic characterizations of reacted mineral suspensions would always be valuable.

Bioreduction experiments are further complicated because bacteria couple oxidation of an electron donor to reduction of, for example, Fe(III) and U(VI) if both are present. Analytical measurements can be attempted to evaluate the electron balance between the electron donor and these electron acceptors; however, even these measurements would not directly reveal if valence cycling was occurring in the extraction solutions. However, in many bioreduction experiments with both U(VI) and an alternative electron acceptor, abiotic experiments between U(VI) and the reduced alternative electron acceptor or U(IV) and the oxidized electron acceptor have been performed to better examine these complex reaction systems. Only in these abiotic subsystems, like ours with uraninite and nontronite, is it relatively straightforward to evaluate if the analytical measurements yield results consistent with proposed reaction stoichiometries.

As discussed above, uraninite oxidation by ferrihydrite is favorable in 1.0 M $NaHCO_3$ (pH 8.4) and in 0.5 N HCl.[5,40]

Knowing this, Ginder-Vogel et al.[40] decided to measure ferrozine-extractable Fe(II) rather than acid-extractable Fe(II) and dissolved U(VI) rather than $NaHCO_3$-extractable U(VI) to avoid analytical interferences when measuring the kinetics of uraninite oxidation by ferrihydrite. An empirical relationship was developed that found ferrozine extracted approximately 50% of the total Fe(II). Using this empirical relationship, Ginder-Vogel et al.[40] demonstrated good stoichiometric agreement between Δ mol Fe(II) produced and Δ mol U(VI) (approximately 2:1) produced in their experiments. However, if a sediment sample contains oxide-Fe(III) and/or clay-Fe(III) and is extracted in anoxic 1 M $NaHCO_3$, sediment-associated U(VI) will be tend to be overestimated (i.e., underestimate of U(IV)).

The sequential acid extraction procedure we propose for uraninite and phyllosilicates requires clay dissolution during the first extraction step to be limited to a mininum. Dissolution of Fe could be a complex function of phyllosilicate type, Fe concentration, and grain size distribution such that the extraction time and solid concentration may have to be adjusted for each specific material studied. Our method may not be readily adaptable to systems that contain U and poorly crystalline Fe(III) and Mn(III/IV) oxides because of their dissolution in $H_3PO_4-H_2SO_4$. However, the ability of solid-phase clay-Fe(III) to oxidize uraninite yet dissolve to only a limited extent may have important implications with respect to uranium ore processing. The majority of processes used to extract U from uranium-bearing minerals involve an oxidation/acid dissolution step.[41] The use of solid clay-Fe(III) instead of e.g. soluble ferric sulfate may facilitate separation of Fe and U during ore processing and allow for recovery and reuse of the clay-Fe. Future research will characterize and parametrize the kinetics of uraninite oxidation by iron-bearing phyllosilicates. Our results will hopefully improve this effort by identifying and overcoming analytical challenges and interferences encountered when working with these materials.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

A complete description of all materials, sample handling, and step-by-step analytical methods. Summary of operational extractions for measuring total Fe and Fe(II) in phyllosilicates. Summary of operational extractions for total U and U(VI). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the Subsurface Biogeochemical Research (SBR) Program, Office of Science (BER), U.S. Department of Energy (DOE) grant no. DE-SC0005333 to The Pennsylvania State University. We thank Chris Gorski, John Zachara, and four anonymous reviewers for their helpful comments on this manuscript.

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