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Determination of ferrous and ferric iron in aqueous biological solutions

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A solvent extraction method was employed to determine ferrous and ferric iron in aqueous samples. Fe³⁺ is selectively extracted into the organic phase (n-heptane) using HDEHP (bis(2-ethylhexyl) hydrogen phosphate) and is then stripped using a strong acid. After separation, both oxidation states and the total iron content were determined directly by ICP-MS analysis. This extraction method was refined to allow determination of both iron oxidation states in the presence of strong complexing ligands, such as citrate, NTA and EDTA. The accuracy of the method was verified by cross-checking using a refinement of the ferrozine assay. Presented results demonstrate the ability of the extraction method to work in a microbiological system in the presence of strong chelating agents following the bioreduction of Fe³⁺ by the Shewanella alga BrY. Based on the results, a robust approach was defined to separately analyze Fe³⁺ and Fe²⁺ under a wide range of potential scenarios in subsurface environments where radionuclide/metal contamination may coexist with strongly complexing organic contaminants.

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1. Introduction

The importance of iron oxides in controlling the fate and transport of many environmentally important radionuclide/metal species has long been recognized in the literature [1–2]. In the storage and disposal of radioactive wastes, which are often envisioned in steel containers, a variety of iron(II) and iron(III) oxides are generated as the container corrodes [3–6]. The presence of iron(II) generates a reducing environment, and in the case of transuranic waste, the actinides are maintained in reduced and therefore less mobile forms, for example Pu(III) or (IV) vs. Pu(VI) [7–13]. To establish a mechanistic understanding of these systems, it may also help differentiate between enzymatic and abiotic mechanisms. In this context, a reliable method to quantify the iron oxidation state that is suitable for the complex systems generally encountered under subsurface conditions is of primary importance.

The detection of oxidation states of iron in solutions is most often accomplished using spectrophotometric methods, such as the ferrozine assay [15] and the 1,10-phenanthroline method [16]. Both techniques rely on the formation of a colored Fe²⁺-complex at almost neutral pH and its absorbance is measured in relation to a set of standard solutions. However, these spectrophotometric methods can be limited by the presence of strong complexing agents and possibly by other interferences (e.g., competition with other divalent cations or strongly absorbing co-contaminants). A further obstacle in the Fe²⁺ determination is often neglected is the effect of residual oxygen in the reagents used, especially when the concentration of Fe²⁺ is lower than 0.5 mM.

A common method for the separation of metal ions having different oxidation states is solvent extraction [17–21]. The solvent extraction technique is based on the distribution of the analyte of interest between two immiscible liquids, usually an aqueous solution and an organic solvent containing an extracting species. Many organic molecules were developed and utilized in the extraction process. On the basis of our experience in separation science, bis(2-ethylhexyl) hydrogen phosphate (HDEHP) was chosen as the extractant for this study since it was used to separate various metals including americium, curium, neptunium, plutonium and uranium [22–28]. This acidic extractant allows separation of cations in acidic medium. This extraction approach is likely able to deal with interferences from strong complexants such as NTA (nitrilotriacetate) and citrate, and to a lesser extent EDTA, which can co-exist with radionuclide contaminants in environmental biologically active systems.

In this context, the aim of this paper is to define a solvent extraction procedure to detect ferrous and ferric iron with a view to coupling this method to the detection of different oxidation
states of multivalent radionuclides, such as uranium, neptunium or plutonium. The extraction method could permit the simulta-
neous determination of all metal/actinide species using one analytical
approach. Additionally, we modified the ferrozine assay [29] for the
detection of Fe2+ to extend its applicability to these complex
systems and use this analytical approach to confirm the results
obtained in the HDEHP extraction approach.

2. Experimental

The following reagents were used in the present work: fer-
rozine monosodium salt of 97% purity supplied by Aldrich,
(2-ethylhexyl)hydrogen phosphate (HDEHP) 97% purity, also
from Aldrich, solvents: cyclohexane, toluene and n-heptane, all
of HPLC grade from Fisher Scientific, HCl (certified ACS Plus)
from Fisher Scientific, nitrilotriacetic acid (NTA) 99+ % from
Aldrich, sodium dihydrogen citrate 99% from Aldrich, EDTA dis-
odium salt dihydrate 99+ % from Sigma-Aldrich, sodium L-lactate
approx. 98% from Sigma-Aldrich, lactate reagent kit from Trin-
ity Biotech., hydroxylamine hydrochloride 99% Reagent Plus from
Sigma-Aldrich.

All solution preparation and experiments involving Fe2+ were
performed in a nitrogen negative pressure anoxic glove box
(MBraun Labmaster 130 with re-circulating copper shaving oxygen
purification system) equipped with an oxygen sensor. The oxygen
levels in the glove box were maintained below 3 ppm O2 at all
times and were typically 0.1 ppm O2. It was found that at or below this
concentration of oxygen, significant (~1%) oxidation of Fe2+ did not
occur in the timeframe of a couple of months.

Ferric and ferrous iron solutions were prepared in 0.1 M
hydrochloric acid to prevent precipitation of the oxide phases.
With the exception of the ferrous iron solution, which was pre-
pared in the glove box, all solutions were prepared outside the
glove box and transferred into the box, where they were purged
to remove oxygen over several weeks by equilibrating with the
nitrogen atmosphere. The ferrous solution was prepared with a
fresh FeCl2 solid (Sigma, analytical grade) and the ferric solution
was prepared using the certified iron(III) oxide (Alfa Aesar stock#
44666, lot# H121R005). Since a standard reference for the iron oxida-
tion state mixture is commercially unavailable, these solutions
were used as secondary standards for the preparation of iron oxida-
tion state mixtures with different ferric to ferrous ratios. The
oxidation states and iron concentrations in these standards were
measured using the ferrozine method, a combination of extrac-
tion with ICP-MS assay and direct ICP-MS measurements. The Fe2+
concentration in the secondary standard solution was equal to
58.9 ± 0.3 mM and the Fe3+ concentration in the secondary stand-
ard solution was equal to 99.7 ± 0.4 mM. These two solutions were
kept in a nitrogen-controlled atmosphere and were used for further
dilutions.

Aqueous metal concentrations were determined by ICP-MS
(Agilent Model 7500ce), fitted with a hydrogen reaction cell, to
minimize the interference of the argon–oxygen peak with the iron
peak at mass of 56 and to extend the sensitivity of analysis. The ICP-
MS determination limit of iron was 5 ppb, which corresponds to an
iron concentration of 10⁻⁷ M. The ICP-MS apparatus was calibrated
prior to each analytical run. The certified iron standard solution
(High Purity Standards) contained 1000 ppm of Fe⁴⁺ and further
dilutions of this sample were used for calibration. Each calibration
used a minimum of six points over the iron concentration range
of 0–500 ppb. The R² of calibration linearity was on the level of
0.9999 ± 0.0001. Each result reported by ICP-MS was an average of
five measurements and the precision of single point measurement
was better than 0.5%. The highest relative difference between the
synthetic samples having initially the same composition was 9%
and was caused by an experimental uncertainty of the extraction
and stripping steps.

Considerable experimental work was carried out to obtain the
optimum conditions that would enable both ferric and ferrous iron
to be determined in aqueous solution and in the presence of com-
plexing ligands such as citrate, NTA and EDTA. The effects of acid
concentration for the extraction and stripping steps, contact time
for extraction and type of solvent were investigated. Once the pro-
cedure was established, a series of experiments were performed to
assess its performance in mixed oxidation state solutions and in
more complex “real” experimental systems.

2.1. Modified ferrozine assay

A modified version of the ferrozine assay [29] was used to
crosscheck the results of the extraction experiments. Briefly,
0.9 mL of 4.06 × 10⁻⁴ M (0.2 g L⁻¹) ferrozine in 0.25 M HEPES (N-2-
hydroxethylpiperazine-N’-2-ethanesulfonic acid) buffer (enzyme
grade) was added to 0.1 mL of acidified sample (0.5 M HCl) and
the purple color was allowed to develop. The absorbance of the com-
plex was measured within 15 minutes at 562 nm, using either a
CARY 5000 spectrophotometer or a Thermo Spectronic GeneSys 20
spectrophotometer, and compared to a calibration curve obtained
from a series of standards prepared in a similar fashion. No
difference in absorption readings was noted between the two
instruments. The molar extinction coefficient for the ferrous fer-
rozine complex was calculated as the average of several calibra-
tions and was equal to 27.400 ± 1000, in good agreement with the lit-
erature value [15] of 27.900. A good linear dependence of the
Beer–Lambert law was observed for Fe⁴⁺ concentrations ranging
from 10 to 80 μM of Fe⁴⁺ with the precision of the Fe⁴⁺ determina-
tion being equal to ±2 μM. The determination limit was assessed
to be on the level of 6 μM Fe⁴⁺.

For samples containing up to 8 mM of iron, the total iron con-
dentration was determined as follows: to 0.1 mL of sample 0.9 mL
of 0.28 M hydroxylamine hydrochloride in 0.28 M HCl was added.
After 30 minutes, all the Fe³⁺ was reduced and 0.1 mL of this
solution was added to 0.9 mL of the ferrozine solution and the
absorbance measured after 20 minutes. The Fe⁴⁺ concentration
was determined by the difference between the total iron concentration
and the Fe⁴⁺ concentration directly measured in the sample.

2.2. Procedure for the HDEHP solvent extraction method

The following procedure is applicable to sample aliquots con-
taining up to 5 mM of ferric iron. The analytical range for Fe²⁺ was
assessed and it was determined that up to 8 mM Fe²⁺ could be
detected, although it is believed that this amount could be much
higher given that Fe²⁺ does not partition into the organic phase. The
following procedure is also applicable to solutions containing cit-
rate and NTA at concentrations up to 5 times that of iron. All steps
were carried out in glass vials with PTFE lids.

Step 1: To 0.5 mL of sample, 1.5 mL of 0.67 M HCl was added to
obtain a final acid concentration of 0.5 M. An aliquot of the sample
was removed for analysis to determine the total iron concentra-
tion.

Step 2: To the remaining sample, an equal volume of 0.1 M
HDEHP in n-heptane was added and shaken for 1 hour. The phases
were allowed to completely separate and an aliquot of the aqueous
phase was removed for determination of Fe²⁺ content, taking care
to not to contaminate the extracted portion of the solution with the
organic phase.

Step 3: To a portion of the organic phase, an equal volume of
4 M HCl was added and shaken for 15 minutes. The phases were
allowed to completely separate and an aliquot of the aqueous phase
was removed for determination of Fe³⁺ content, again taking care
to minimize contamination by the organic phase.
Total iron, ferrous and ferric, concentrations were determined directly from the analysis of the acidified sample by ICP-MS measurements.

2.3. Coupled effects of both oxidation states and complexing ligands

A series of solutions containing a total iron concentration of 0.5 mM were prepared using the secondary standard solutions described above and were treated as a reference material since concentrations of Fe²⁺ and Fe³⁺ were precisely known. In the absence of complexing ligands, the Fe²⁺ content was varied from 0 to 0.5 mM. In the presence of citrate, NTA or EDTA, the ratio of Fe²⁺ to Fe³⁺ was kept constant at 1:1 and the concentration of the ligands was varied from 0.05 to 2.5 mM, giving a total iron to ligand ratio of 1:0.1 to 1:5.

2.4. Reduction of Fe³⁺ by Shewanella alga under anoxic conditions

Full details of the experimental procedure can be found in Reed et al. [14]. Under anaerobic conditions, aqueous Fe³⁺, as a stabilized Fe³⁺-NTA complex was added, at an approximate concentration of 6 mM, to a solution containing the metal-reducing bacteria S. alga (strain BrY), which had been grown anaerobically [30]. Lactate, as sodium L-lactate, was used as the electron donor. Total iron, ferric and ferrous iron concentrations were measured as the experiment progressed using both methods as described above. Lactate was analyzed using a lactate reagent and standards kit (Trinity Biotech) based on the colorimetric technique as recommended by the manufacturer.

3. Results and Discussion

The experimental results we report were obtained for solutions that were carefully degassed to remove all trace levels of oxygen. We note that the presence of even trace-levels of oxygen can lead to significantly higher errors when the total Fe concentrations are 0.1 mM or below due to the oxidation of Fe²⁺ by this residual oxygen content leading to spurious and inconsistent analytical results.

3.1. Experimental refinement of the HDEHP solvent extraction method

HDEHP is an acidic extractant, thus the distribution ratio of the metal, defined as the ratio of metal in the organic phase to metal in the aqueous phase, depends on the acidity in the aqueous phase. Therefore, the effect of the acid concentration on the extraction of 0.4 mM solutions of ferric and ferrous iron using 0.1 M HDEHP was measured. Between 0.05 M and 1.0 M hydrochloric acid concentration, the extraction of Fe³⁺ into the organic phase was essentially quantitative (>98.5%). Above 1.0 M HCl, the amount of Fe³⁺ extracted decreased rapidly and was almost zero at 4 M. In the case of Fe²⁺, less than 8 ± 1% was extracted into the organic phase at all acid concentrations investigated. Consequently, it was decided that acidifying the solutions to 0.5 M HCl would provide the optimal conditions for separation of Fe³⁺ from Fe²⁺. Under these conditions, the Fe³⁺/Fe²⁺ separation factor is 1000 ± 150. These results are in agreement with previous studies that have demonstrated that HCl concentrations below 1.0 M give the best separation between Fe³⁺ and either Fe²⁺ [31] or divalent cobalt and nickel, which behave similarly to Fe²⁺ [32].

The extraction of Fe³⁺ into the organic phase (Fig. 1) is relatively slow compared to other metals; for example, the trivalent lanthanides, trivalent actinides, and U(VI) were extracted in minutes [33–35]. Almost quantitative partitioning (greater than 98.5%) of Fe³⁺ occurs after 1 hour and is in agreement with previous studies [36–37]. Extraction rate can be increased by increasing the temperature [33]; however the effect of temperature was not investigated in the present study.

The effects of the three solvents, cyclohexane, toluene, and n-heptane, on the extraction of 0.4 mM Fe³⁺ from a 0.5 M HCl solution by 0.1 M HDEHP were also established (Table 1). The relative polarities of these solvents are: cyclohexane = 0.006, n-heptane = 0.012, and toluene = 0.099 [38]. Altering the solvents has a small but significant effect on the rate of partitioning of Fe³⁺ into the organic phase. When cyclohexane is the solvent, the partitioning occurs on the same timescale as n-heptane, i.e., one hour. Toluene appears to increase the time for Fe²⁺ partitioning; after one hour only 75% of the Fe³⁺ has transferred into the organic phase. This trend is correlated with the polarity of the solvents. In solvents of low polarity, HDEHP via the formation of dimers [39] extracts trivalent cations according to the following mechanism [40]:

\[
M^{3+}(aq) + 3(HDEHP)_{2}^{3-}(org) \rightarrow [M(HDEHP)_{2}]^{+}(org) + 3H^{+}(aq)
\]

Cyclohexane and n-heptane have low and comparable polarities that enable HDEHP to form dimers more rapidly, which leads to a more rapid extraction of Fe³⁺. Toluene, however, with its somewhat higher polarity, causes a decreased rate of Fe³⁺ extraction.

The Fe³⁺ extraction yield is a function of free HDEHP concentration and decreases with increasing Fe³⁺ concentration in the organic phase. Fig. 2 shows the isotherm for Fe³⁺ extraction. The extraction efficiency of Fe³⁺ is significantly influenced by its initial concentration in the aqueous phase. As mentioned before, the extraction is better than 98.5% for Fe³⁺ concentrations below 1 mM. A 5 mM initial concentration leads to the extraction of 92% Fe³⁺. The amount of Fe⁴⁺ extracted decreases as the initial concentration increases; 77% of Fe⁴⁺ is extracted at an initial concentration of 10 mM, and 48% of Fe⁴⁺ is extracted at 35 mM. From the data

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>% Fe³⁺ extracted into organic phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyclohexane</td>
</tr>
<tr>
<td>10</td>
<td>35.92 ± 0.45</td>
</tr>
<tr>
<td>20</td>
<td>56.78 ± 2.67</td>
</tr>
<tr>
<td>30</td>
<td>71.84 ± 1.97</td>
</tr>
<tr>
<td>45</td>
<td>84.53 ± 0.03</td>
</tr>
<tr>
<td>60</td>
<td>93.68 ± 0.03</td>
</tr>
</tbody>
</table>
presented in Fig. 2, the organic phase is saturated at an initial Fe$^{3+}$ concentration of 35 mM, with only $\sim$17 mM extracted. In these experiments, an HDEHP concentration of 100 mM was used. Since six HDEHP molecules are required to extract one Fe$^{3+}$ cation (see Eq. (1)), this concentration of HDEHP allows a maximum of 16.7 mM Fe$^{3+}$ to be extracted. The net effect of this Fe$^{3+}$ “capacity” is that there will be a limiting Fe$^{3+}$ concentration that depends on the concentration of HDEHP used in the organic phase, and this limit will, in part, define the error in the analytical approach. Therefore, under the conditions of the procedure presented here, a maximum concentration of 5 mM Fe$^{3+}$ is recommended, which results in an error of less than 10%. To measure higher concentrations of Fe$^{3+}$, the sample could be diluted or a higher concentration of HDEHP could be used up to a maximum of 0.3 M. For the range of concentrations investigated, the Fe$^{2+}$ remains in the aqueous phase and does not interfere with the extraction of Fe$^{3+}$, which is discussed in Section 3.2. For this reason, the Fe$^{2+}$ concentration limit in this extraction procedure is defined simply by its solubility in the aqueous phase.

### 3.2. Mixed oxidation state system and the effect of highly complexing ligands

In all the experiments reported herein, the ferrous and ferric secondary standard solutions were used as the reference. The separation of iron oxidation states in the synthetic mixtures and analytical sample preparations were conducted in accordance with the modified ferrozine method and the extraction method using our anoxic nitrogen glovebox. The results of the experiments performed on mixed Fe$^{2+}$/Fe$^{3+}$ oxidation state systems using these two methods are shown in Fig. 3. The total iron concentrations measured by ICP-MS were always greater than those determined by the ferrozine method. The explanation for this observation is that the reduction of Fe$^{3+}$ was not complete. However, agreement to within our target experimental error could be achieved. There is also a slight difference between the two methods in the determination of Fe$^{3+}$ concentration, since the solvent extraction method determines the concentration directly, whereas the ferrozine assay determines the concentration by difference. The results obtained by both methods, however, agree to within $\pm$ 4%.

In the presence of citrate and NTA (Table 2), the detection of both oxidation states is not significantly affected. However, the presence

**Table 2**

<table>
<thead>
<tr>
<th>System</th>
<th>Fe:ligand ratio</th>
<th>Total iron (mM)</th>
<th>Fe$^{2+}$ (mM)</th>
<th>Fe$^{3+}$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe/citrate</td>
<td>1:0.1</td>
<td>0.518</td>
<td>0.209</td>
<td>0.322</td>
</tr>
<tr>
<td></td>
<td>1:1</td>
<td>0.518</td>
<td>0.222</td>
<td>0.263</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>0.520</td>
<td>0.242</td>
<td>0.278</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>0.567</td>
<td>0.259</td>
<td>0.290</td>
</tr>
<tr>
<td>Fe/NTA</td>
<td>1:0.1</td>
<td>0.529</td>
<td>0.250</td>
<td>0.276</td>
</tr>
<tr>
<td></td>
<td>1:1</td>
<td>0.512</td>
<td>0.242</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>0.516</td>
<td>0.228</td>
<td>0.235</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>0.524</td>
<td>0.274</td>
<td>0.256</td>
</tr>
<tr>
<td>Fe/EDTA</td>
<td>1:0.1</td>
<td>0.517</td>
<td>0.244</td>
<td>0.252</td>
</tr>
<tr>
<td></td>
<td>1:1</td>
<td>0.519</td>
<td>0.320</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>0.515</td>
<td>0.368</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>0.500</td>
<td>0.411</td>
<td>0.109</td>
</tr>
</tbody>
</table>
of EDTA affects both of these methods with the influence increasing with increasing EDTA concentration. In the HDEHP solvent extraction procedure, the determination of total iron concentration in the presence of EDTA is unaffected but the measurement of the relative concentration of each oxidation state is affected. More specifically, the measured concentration of Fe^{2+} is somewhat elevated whereas the concentration of Fe^{3+} is lower than the value expected. In the extractions carried out on systems containing EDTA and only one iron oxidation state, the presence of EDTA did not affect Fe^{2+} determination. However, EDTA caused Fe^{3+} to remain in the aqueous phase during the initial extraction step. In the solvent extraction method, EDTA interferes with the formation of Fe^{3+}-HDEHP dimers, thus prevents the complete extraction of Fe^{3+} into the organic phase, resulting in a lower Fe^{3+} concentration than expected. Altering the experimental conditions, for example increasing the acid concentration and increasing the time for partitioning to occur in the initial extraction step, did not resolve this problem. Therefore, in the mixed oxidation state system, the apparent increase in Fe^{2+} concentration is due to Fe^{3+} that remained in the aqueous phase, which led to a correspondingly lower Fe^{3+} concentration in the organic phase.

In the ferrozine assay, the measurement of Fe^{2+} was unaffected by the presence of EDTA but the total iron measurement and therefore the determination of Fe^{3+} content were impacted. Experiments performed on systems containing EDTA and only one oxidation state of iron (results not shown) revealed that hydroxylamine hydrochloride was unable to quantitatively reduce Fe^{3+} to Fe^{2+} because of EDTA complexation and as a result the Fe^{3+} concentration determined was lower than expected. The effect of EDTA on both these methods can be explained in terms of differences in stability constants. The strength of the complexes formed between Fe^{2+} or Fe^{3+} and the three ligands investigated in this study are compared in Table 3. The Fe^{3+}-EDTA complex is much stronger than the other Fe^{3+} organic complexes as well as all the Fe^{2+} organic complexes.

In summary, for the range of experimental conditions investigated, both the solvent extraction procedure and the ferrozine method could not overcome the effects of strong EDTA complexation to permit reliable detection of Fe^{3+}. Determination of ferrous and ferric iron in the presence of EDTA could, however, be done by using ICP-MS to measure the total iron concentration and the ferrozine method to establish the Fe^{3+} content.

### 3.3. Reduction of Fe^{3+} by S. alga under anoxic conditions

The analytical procedures described herein to determine different oxidation states of iron are applied to biologically active environmental samples in the presence of a moderately strong complexant, NTA. Fig. 4 shows the reduction of Fe^{3+} (as an NTA complex) to Fe^{2+} by S. alga that was performed in separate experiments. It was impractical, due to the time-intensive nature of the analyses in the glovebox, to perform the ferrozine method and extraction simultaneously. There is an excellent correlation between the utilization of lactate, as an electron donor, and the reduction of Fe^{3+}. There is a strong relationship between the production of Fe^{3+} and the growth of the cells over the course of the experiment (data not shown). Both the solvent extraction procedure and the ferrozine method, Fig. 4a and 4b respectively, were able to track the reduction of Fe^{3+} to Fe^{2+} over the course of the experiments and gave very similar results. This agreement in a relatively complex media application confirms the applicability of both these analytical methods to the oxidation state specific analysis of iron in environmental media.

### 4. Conclusions

The simultaneous measurement of Fe^{2+} and Fe^{3+} is needed to establish and understand key redox processes in complex environmental systems and must be done very carefully to get the correct results. All reagents used in the analytical procedure must be oxygen free, since residual oxygen will easily oxidize trace-levels of Fe^{2+} and introduce significant error into the analysis. When the Fe^{2+} concentration is lower than 0.1 mM, this error can reach 100% in the ferrozine and extraction methods indicating that the ferrous ions have disappeared from the system. Alternatively, the presence of a strong complexing agent in the system can mask the Fe^{3+} concentration leading to an overestimation of the Fe^{3+} content. For these reasons, an incorrect result in the Fe^{2+} and Fe^{3+} determination is obtained if analytical conditions are not carefully controlled.
The results presented herein show that a solvent extraction procedure that utilizes HDEHP for the separation of ferrous and ferric iron in aqueous solutions, when combined with ICP-MS analysis, can provide at least the same degree of accuracy and sensitivity as the ferrozine method. This approach has the added benefit of also separating oxidation states of multivalent actinides so that it can be combined with radioanalytical methods (e.g., liquid scintillation counting) to analyze these oxidation states at the same time. Both the ferrozine and HDEHP extraction methods were capable of detecting both oxidation states of iron in systems containing complexing agents with log K values lower than 17, in this case citrate and NTA, and in a more complex system containing microorganisms.

However, the presence of EDTA affected the oxidation–specific analysis of iron in both methods. The extent of this effect increased with increasing EDTA concentration. In the solvent extraction procedure, total iron concentration was unaffected, whereas the Fe(II) concentration was higher than expected and the Fe(III) lower than expected. In both cases, this was attributed to the strength of the Fe(III)-EDTA complex (log K = 25.1). On the basis of these observations, we proposed to use the following method in the presence of EDTA: determine the total iron concentration directly from the sample using ICP-MS, equipped with a hydrogen reaction cell, determine the Fe(II) concentration, using the modified ferrozine method we described, and determine the Fe(III) concentrations by difference. This procedure has broad applicability and is independent of the presence of strong chelating ligands in the aqueous solution.

Future work is focused on extending the applicability of these procedures to complex environmental systems and developing a co-extraction approach when multivalent actinides are also present.

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