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TRLFS Studies on Indirect Determination of U(IV) in Biological Samples Containing Mixed U(IV/VI) Species

Wansik Cha[a*], Seung-Yeop Lee[b], Hye-Ryun Cho[a], Euo Chang Jung[a], Min-Hoon Baik[b]
Nuclear Chemistry Research Division[a], Radwaste Disposal Research Division[b]
Korea Atomic Energy Research Institute, 1045 Daedeok-daero, Yuseong, Daejeon 305-353, Korea
[*]Corresponding author: wscha@kaeri.re.kr

1. Introduction

The microbial-mediated metal reduction process has been viewed as a long-term remedial approach for in situ immobilization of radionuclide under anaerobic conditions in aquifers and saturated soils. Such approach depends on the fact that many radionuclides of concern including uranium are redox active and less soluble in their reduced form. Thus, the interaction of uranium with microorganisms has been the subject of considerable effort over the past decade [1].

Uranium reduction occurs as a result of dynamic metabolic activity of microbials and biochemical reactions. The typical incubation system of uranium reducing bacteria such as sulfate-reducing *Desulfovibrionales* contains various geochemical components including electron donors (e.g., acetate, lactate, ethanol, thiol or glucose), anionic electron acceptors (sulfate and nitrate) and metal ions (Fe(II/III), Mn, Ni, etc.). The anoxic growth of bacteria results in uranium reduction by forming U(IV) aggregates or colloids [1].

In most studies the isolation of U(IV) from U(VI) largely depends on filtration of precipitates and centrifugal separation of colloidal particles. Such U(IV) species were subsequently analyzed via ICP-MS for elemental uranium or kinetic phosphorescence analysis (KPA) in form of U(VI) although the relative proportion of U(IV) and U(VI) in the frozen samples can be estimated using X-ray absorption spectroscopy. However, recent studies report the formation of extracellular uranite nanoparticles, which may suspend in the resultant filtrate and supernatant solutions. Thus, the development of an analytical technique capable of estimating the dissolved or suspended U(IV) species is further required for better understanding of the bacterial-mediated uranium sequestration and dissolution process.

In this study we explore the possibility of using TRLFS (time-resolved laser-induced fluorescence spectroscopy) to indirectly measure the concentration of U(IV) as well as that of U(VI) in bacterial culture media. The strategy is based on the measurement of U(VI) luminescence (LM) signals and the comparison of calculated U(VI) concentration before and after the oxidation of U(IV) species present in sample solution. Preliminary results using a standard addition method showed that the higher concentration of U(VI) after H_2O_2 oxidation was estimated in the given media samples indicating the presence of U(IV) before oxidation (see below). In addition, the influence of individual chemical component in the culture media on the LM intensity and decay lifetime was examined in detail.

2. Experimental

The microbial (*Desulfovibrio desulfuricans*) incubation was conducted as described elsewhere [1,3]. Briefly, the initial composition of culture media is as follows: U(VI) (50 µM); sodium bicarbonate (30 mM); sodium lactate (10 mM); $FeSO_4$ (2 mM); cysteine (1.6 mM); $MnCl_2$ (0.2 mM) or $NiCl_2$ (0.2 mM). After two weeks of anaerobic incubation the culture media were filtered through a membrane filter of 0.2-µm pore size and used in a few hours for spectroscopic measurement.

The stock solution of uranium(VI) perchlorate was prepared from uranium dioxide. Cysteine, sodium lactate, sodium bicarbonate, hydrogen peroxide, ferric and ferrous sulfate were used as received for preparation of biologic and non-biologic reagent mixtures. All solutions including culture media samples were handled in an Ar-filled globe box. Finally, each sample solution was 1:1 (v/v) mixed with a home-made luminescence enhancing agent (LEA, 50 mM $Na_4P_2O_7$, 0.4 M H_3PO_4, pH 2.0) and transferred into a 1-cm quartz cuvette cell (Starna Scientific, Essex, UK). The final pH of the solution mixture was slightly elevated, but less than 2.1.

To collect LM spectra of U(VI) a gated ICCD-based TRLFS system was used as described elsewhere [4], with a gate delay time of 250 ns and a gate width of 10 µs. LM lifetime was measured separately by analyzing the LM decay profile recorded at 520 nm using an oscilloscope connected to a monochromator-PMT system.

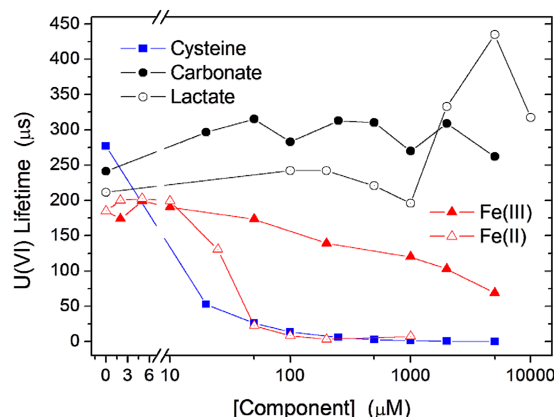

Figure 1. Influence of various media components on the LM lifetime of U(VI) complexed with LEA in non-media solutions of $[U(VI)_{total}]$, 50 µM and 100 µM for cysteine/carbonate/lactate and Fe(II)/Fe(II), respectively.

3. Results and Discussion

3.1. Influence on U(VI) LM Lifetime by Reagents in Bacterial Culture Media

In KPA, the use of LEA is known to prolong U(VI) LM lifetime up to 300 µs as shown in Fig. 1. Originally, this technique is designed to measure the LM intensity at the onset of decay (the initial LM intensity), which is the LM intensity at time zero after termination of the laser pulse used for excitation and proportional to the uranium concentration in the sample. Calibration standards of known uranium concentrations are used to construct the calibration curve between the initial LM intensity and uranium concentration. This calibration curve is used to determine the uranium concentration of unknown samples from their initial LM intensity [2]. The underlying assumption of KPA is that the collisional deactivation of excited state U(VI) population is the sole LM quenching process.

However, in preliminary TRLFS tests we found that the measured LM lifetime of U(VI) in the given bacterial culture samples was considerably shorter (0.2 – 1.0 µs) than the expected values as mentioned above. As summarized in Fig. 1 further studies revealed that cysteine and Fe(II), which act as reducing agents, are mainly responsible for such significant decrease of LM lifetime. Interestingly, carbonate and lactate act oppositely to extend the LM lifetime. Moreover, it is also found that cysteine, Fe(II) and Fe(III) considerably induce static quenching property, which make it difficult the accurate construction of KPA calibration curve based on the initial LM intensity.

3.2. Determination of U(IV) and U(VI) in Culture Media by Using Standard Addition Method

Thus, in the presence of excess amount of such LM quenchers we concluded that the monitoring of the area integration of LM spectra is more practical. Fig. 2 shows the calibration curves based on a standard addition method. The LM intensity in the absence of added U(VI), i.e., $[U_{added}] = 0$, indicates the signal from U(VI) present in the original culture media. The calibration curves appear linear, therefore the U(VI) concentration in each sample can be determined by extrapolating the plot to the zero LM intensity.

Fig. 2 also shows the standard addition curves of each sample containing Mn or Ni after oxidation by air-bubbling (1 h) and H_2O_2 incubation (10 mM, 1.5 h). The estimated U(VI) concentrations after such oxidation processes were higher than those from the intact (non-oxidized) samples. We suppose that this increase of the estimated total U(VI) concentration after oxidation is due to the oxidation of U(IV) that turns into U(VI). Then, the difference between the measured U(VI) concentrations before and after oxidation should represent U(IV) concentration in the original sample. Table 1 summarizes the results of the U(VI) and U(IV) concentration estimation in two media samples.

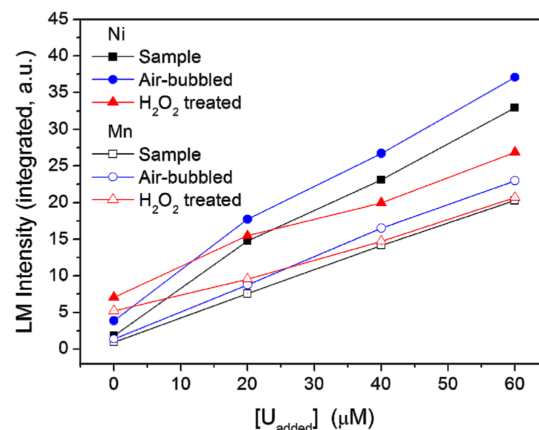

Figure 2. LM intensity change upon addition of known amount of U(VI) to the culture media samples (standard addition method). LM intensity was calculated by area-integration of LM spectra.

Table 1. Estimated U(VI) and U(IV) concentration in the media samples from the results in Fig. 2 (units in µM)

Metal added	[U(VI)]	[U(IV)]	
		Air	H_2O_2
Mn	5.6	3.7	18.7
Ni	3.1	1.0	15.5

4. Conclusion

In this study, TRLFS technique was applied to measure U(IV) and U(VI) concentration in biological media samples. The effect of individual component that commonly presents in uranium-reducing bacteria culture, such as, cysteine, Fe(II) and Fe(III), was examined. Due to the short lifetime of U(VI) complexed with LEA the whole LM signal integration was used to determine U(VI) concentration via standard addition method. Finally, U(IV) concentration was assessed indirectly by comparing U(VI) concentrations measured before and after media oxidation process. Merits and limits of this analysis and a potential corrective procedure using TRLFS lifetime data will be presented in detail.

Acknowledgements

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