Article

Quantitative Analyses of Urinary Uranium by μ -PIXEAkihiro Uehara , Masakazu Oikawa, Izumi Tanaka, Hiroshi Ishihara and Shino Homma-Takeda

National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology 4-9-1 Anagawa, Inage, Chiba 263-8555, Japan; oikawa.masakazu@qst.go.jp (M.O.); tanaka.izumi@qst.go.jp (I.T.); ishihara.hiroshi@qst.go.jp (H.I.); takeda.shino@qst.go.jp (S.H.-T.)

* Correspondence: uehara.akihiro@qst.go.jp; Tel.: +81-43-382-5511

Abstract: Simple methods for the determination of elements in biological fluids have been developed. It is important to quantify the accidental incorporation of radionuclides during the decommissioning work at nuclear power plants. Herein, we proposed the simple preparations and determination methods of uranium concentrations in urine for microbeam scanning particle induced X-ray emission (μ -PIXE) analysis in a rat model. A droplet (1 μ L) of mixed solution of urine treated with a five-fold amount of concentrated nitric acid was placed on polypropylene film coated with perfluoroalkoxy alkanes (PFA) and dried at room temperature. The μ -PIXE imaging analysis revealed that successful condensation with homogeneous distribution of uranium in the specimen was achieved using by PFA coating. Uranium concentrations in the urine collected from uranium-injected rats were quantified. The obtained results were consistent with those determined by inductively coupled plasma mass spectrometry.

Keywords: uranium; internal exposure; simple and rapid quantification; urine; μ -PIXE

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1. Introduction

Fundamental knowledge is required to estimate the accidental exposure of nuclear fuels, including uranium (U) and fission products, during the decommissioning work at the Fukushima Daiichi Nuclear Power Plants [1]. Uranium is considered to be a chemical toxic and radiological toxic element that accumulates in the kidneys [2] and bones [3] after the exposure, where it has the potential to subsequently impact the metabolism. Urine is one of the best body fluids for monitoring the U exposure, because the quantification of urinary U reflects the amount of U taken into the body and/or the amount of remaining U. Various methods for the detection of urinary uranium with a small volume by a simple sample preparation have been developed [1,4,5], including alpha spectrometry [6], neutron activation analysis (NAA) [7], fission-track analysis (FTA) [8], total reflection X-ray fluorescence (TXRF) [9], and inductively coupled plasma mass spectrometry (ICP-MS) [10–13]. The limit of detection by alpha spectrometry is approximately 1 mBq of ^{238}U (80 ng) in urine sample, although a counting time of approximately one day is required to achieve this sensitivity [14]. Higher sensitivity is attained by NAA using a neutron source such as a nuclear reactor for the sample irradiation. FTA and TXRF are used as rather simple analytical methods when preconcentration of sample solutions is sufficient. Currently, concentrations in the parts per billion range have been achieved by ICP-MS [15]. However, sophisticated methodologies are required for sample purification to separate U from inorganic and organic matrix for these analytical methods. As a result, it takes hours to yield better detection limits.

Particle induced X-ray emission (PIXE) analysis is suitable for small amounts of biological samples [16–18]. Trace elements in liquid samples such as drinking water, river water, blood, and body fluids have been measured dropped on analytical film and dried up [19,20]. Combined with microbeam scanning to PIXE (μ -PIXE), local quantification of thin section standard sample has been performed with spatial resolution at the μm

scale [21–24]. Recently, U accumulated in the micro region of rat kidney tubules was quantified [24]. μ -PIXE analysis of the dropped specimen shows that the diameter of the drop trace and the homogeneity of the elements depend on the composition and physical properties of the drop solution. In our previous study, yttrium (Y), used as a simulated U, was added in the urine, and the concentration of Y was measured based on the count of Y-K α line. The specimen was prepared by using solution in the mixture of urine and acid [25]. By applying the μ -PIXE analysis to the quantification of U in urine, the homogeneity of U in the specimen can be evaluated, and U concentration can be measured based on the X-ray fluorescence spectroscopy.

Herein, the properties of the droplets of the urine drop specimen were evaluated to simply and quickly quantify the U in biological fluids using μ -PIXE. Uranium in urine sample collected by administering U to rats was quantified based on the calibration line of urine sample. Obtained values were compared with those measured using ICP-MS.

2. Materials and Methods

μ -PIXE measurements using two kinds of specimens were performed, i.e., a specimen based on 0.1 M HNO₃ containing no urine, and that of concentrated HNO₃ containing 20% urine to evaluate the uniformity of U in the specimens and to quantify the U.

2.1. Preparation for Uranium Specimens Using 0.1 M Nitric Acid

Specimens containing a known concentration of U were prepared using 0.1 M nitric acid (HNO₃). The 0.1 M HNO₃ was prepared from concentrated HNO₃ (68%, ultrapure analytical grade, Tama Chemicals Co. Ltd, Kawasaki, Japan). Uranium acetate (depleted uranium, Wako Pure Chemicals Industries, Tokyo, Japan) was dissolved into 0.1 M HNO₃ as a stock solution (100 ppm). The solutions of 1, 5, 10, and 50 μ g/g U in 0.1 M HNO₃ were prepared using 0.1 M HNO₃ and U stock solution. One μ L of U standard solution was dropped on polypropylene film (RIGAKU Co. Ltd, Tokyo, Japan) of 6 μ m thickness. At least three specimens were prepared at each standard solution. Tips of the micro-pipet were changed each time after the dropping. The droplet was dried in the desiccator with silica gel for one night.

2.2. Preparation for Uranium Specimens Using Mixed Solutions of Urine with Nitric Acid

Uranium specimens of the urine and concentrated HNO₃ mixtures were prepared. Uranium acetate was dissolved into concentrated HNO₃ as a stock solution (100 ppm). The standard solutions of 0, 1, 5, 10, and 50 μ g/g U were prepared using urine of untreated rats (see Section 2.3), concentrated HNO₃, and uranium stock solution for the calibration curve. Here, volume of urine was 20% in concentrated HNO₃. The standard solutions were maintained at room temperature for 24 h before the preparation of the droplet on the sheet. The droplet was dried in the desiccator with silica gel for a night. One μ L of U standard solutions containing urine were dropped on polypropylene film coated tetrafluoroethylene perfluoroalkyl vinyl ether copolymer (PFA, FC-115, Fine Chemical Japan Co. Ltd, Tokyo, Japan). The PFA coating was performed 12 h prior to its use. At least three specimens were prepared at each standard sample. Tips of the micro-pipet were changed each time subsequent to the dropping. The sample solution was dropped on the sheet coated via PFA owing to its high-water repellency. Two or five μ L droplets containing U were also prepared to study the effect of counting on the volume.

Uranium specimen of urine samples collected by administering U to rats was treated with five-fold HNO₃ to be 20% urine in concentrated HNO₃. Concentration of uranium in urine was also determined by ICP-MS (Agilent 7500a, Yokogawa Analytical Systems, Inc., Tokyo, Japan). For the ICP-MS measurement, the urine of 0.1 mL was digested with 0.5 mL of concentrated HNO₃ at 90 °C for 30 min using a microwave oven. Each resulting specimen was diluted with ultrapure water, and the U concentration was determined [26].

2.3. Animal Experiments

Urine samples were collected from Wistar male rats (10 weeks old; CLEA Japan). The animals were acclimated to the controlled temperature (22 ± 2 °C), humidity ($50 \pm 10\%$), and day/night cycle environment (light 7:00–19:00) for a week prior initiating the study. As a control urine sample, urine was obtained from the rats in metabolic cages for 24 h. As a toxic animal model early after exposure [27], uranyl acetate was dissolved in saline and administered to the animals through subcutaneous injection at 0.5 and 4 mg kg^{−1} body weight. Urine was obtained from four animals per group in a metabolic cage for five hours after the administration. Collected urine samples were stored in the freezer at -80 °C. All experimental procedures were approved by the Animal Care Committee of the National Institutes for Quantum and Radiological Science and Technology (No. 18-0005, May 2018).

2.4. μ -PIXE Measurement

μ -PIXE analysis was performed at National Institute of Radiation Science, Japan, using a Model OM-2000 microbeam scanning PIXE system (Oxford Microbeams Ltd., UK) with a CdTe detector (XR-100TCdTe, Amptek, active area: 25 mm², USA) and Si(Li) detector (GRESHAM Sirius80, active area: 80 mm², UK) [28]. Energy resolution of Si(Li) and CdTe detectors is 256 and 438 eV (full-width half maximum: FWHM), respectively, at 13.6 keV U L α X-ray. X-ray spectrum, μ -PIXE images, and calculation of total counts of X-ray intensity for target element using a Gaussian fitting were performed using the OMDAQ data acquisition system (Oxford Microbeams Ltd., UK). Elemental images were constructed using the intensity data of the U-L α line (12.88–14.21 keV) and U-L β 1 line (16.83–17.77 keV) through a CdTe detector. The data was obtained by scanning the specimens under the following conditions: proton energy, 3.0 MeV; integrated current, 0.2 μ C; spatial resolution, 1×1 μ m.

3. Results and Discussion

3.1. Uranium Distribution and Concentration Dependence of U Specimen Prepared Using HNO₃

μ -PIXE measurements were performed to evaluate the uniformity of U distribution in the specimen and to quantify the U from the counts of U-L α and U-L β 1 lines. First, μ -PIXE measurements were performed on a specimen based on U standard specimen based on 0.1 M HNO₃ containing no urine. When 1 μ L of 0.1 M HNO₃ containing U was dropped on a polypropylene film, the diameter of the droplet was approximately 3 mm at the time of dropping; however, it reduced to almost 0.1 mm at the time of drying. Curve 1 in Figure 1a shows the μ -PIXE spectra of 0.1 M HNO₃ solution containing 50 μ g/g of U. Here, the intensity in Figure 1a represents the sum of the counts of the droplet measured on 256×256 pixel. The X-ray fluorescence peaks corresponding to U-M α , U-L1, U-L α , U-L β 2, U-L β 1, and U-L γ 1 lines at 3.17, 11.62, 13.61, 16.42, 17.22, and 20.25 keV, respectively, were observed. There were no significant intensities observed at U-L α and U-L β 1 lines in the absence of U (curve 2). Figure 1b shows the images obtained from U-L α and U-L β 1 lines. The elemental distribution was not uniform, and U was found to be close to the center. It is believed that this occurred when the droplets dried, the droplet began to dry from the water around the droplets and U was concentrated in the center. The X-ray intensity was calculated using area irradiated and net count results of plotting the U-L α and U-L β 1 lines against the concentration of 1–100 μ g/g (Figure 2 and Table 1); the slopes of the regression lines are 98.0 ($R^2 = 0.998$) and 23.2 ($R^2 = 0.997$), respectively. Here, U-L β 2 line at 16.42 keV overlapping with zirconium (Zr)-K α line at 15.78 keV was not employed to measure the U concentration in this study, because Zr was one of the materials in the metallic sample holder.

Figure 1. (a) μ -PIXE spectra of 1 μ L of 50 μ g/g U in 0.1M HNO_3 for curve 1 and that of blank for curve 2. (b) U images at U-L α and U-L β 1 lines.

Figure 2. Calibration lines of 1–100 μ g/g U in 0.1 M HNO_3 . Horizontal axis: U concentration (μ g/g); vertical axis: X-ray intensity in the analyzed area of (a) U-L α (12.8–14.2 keV) and (b) U-L β 1 (16.8–17.8 keV) lines. Each point represents the mean and standard deviation of data from three specimens.

Table 1. X-ray intensity of U-L α and U-L β 1 lines in 1 μ L of U standard in 0.1 M HNO $_3$.

U Concentration * (μ g/g)	U-L α line		U-L β 1 line	
	Intensity (Counts $\pm$ SD)	RSD (%)	Intensity (Counts $\pm$ SD)	RSD (%)
1	103 $\pm$ 5	5	19 $\pm$ 4	21
5	579 $\pm$ 40	7	148 $\pm$ 21	14
10	1191 $\pm$ 47	4	286 $\pm$ 44	15
50	5322 $\pm$ 515	10	1294 $\pm$ 110	9
100	9811 $\pm$ 482	5	2321 $\pm$ 151	7

* μ -PIXE measurements were performed on three specimens for one concentration. Each point represents the mean and standard deviation of data from three specimens.

3.2. Quantification of U in Urine Collected from U Injected Rats

μ -PIXE measurement was performed for the measurement of U in urine. When 1 μ L urine without HNO $_3$ dropped onto the non PFA-coated polypropylene film and then dried, the shape of the drop was completely different from that in 0.1 M HNO $_3$. The surface of the drop was rough, and its diameter was approximately 1.5 mm [25]. It was considered that the surface tension of the droplets decreased owing to urea and organic substances in urine. When urine was diluted five-fold with concentrated HNO $_3$, the drop size was reduced to a diameter of approximately 0.7 mm. This was because the organic components contained in urine were decomposed by the HNO $_3$. Furthermore, when samples were dropped on a PFA-coated film, the diameter of the dried drop reduced to approximately 0.5 mm. Curve 1 in Figure 3a is the μ -PIXE spectrum of the specimen containing 50 μ g/g U. In addition of U-L α , U-L β 1, and U-L β 2 lines, Br-K α line at 11.92 keV was observed as endogenous elements in the urine. Note that U-L α line overlaps with Br-K β line at 13.29 keV, which was observed in urine specimen without U (curve 2 in Figure 3a). On the other hand, rubidium (Rb) was supposed to be another element that Rb-K α line at 13.39 keV could overlap with U-L α line; however, it was thought that counts of Rb are not significant, because no Rb-K β line at 14.96 keV was also observed. It is reported that concentration of Br in urine is 3–6 μ g/g, which is about five to ten times higher than that of Rb [19,29,30]. Imagings of U-L α and U-L β 1 lines measured with CdTe detector in Figure 3a and K-K β line measured with Si(Li) detector in Figure A1 (see Appendix A) of the specimen containing 50 μ g/g uranium were shown in Figure 3b1. Here, the K-K β line was shown as an example of the main elements of urine. From the imaging results, U appears to be almost uniformly distributed without any extreme bias in element distribution. It is considered that this is owing to the water being vaporized and the reduction in droplets size due to the PFA coating. When the droplets reached the saturated solubility of the matrix including organic and inorganic components, they virtually dried up at the same time. These mechanisms are different from the droplet in the lower concentration of matrix, such as 0.1 M HNO $_3$ (Figure 1b). Some counts corresponding to the Br-K β line were observed in the image of U-L α line in the absence of U (Figure 3b2), although the counts at U-L β 1 line were negligible. Figure 4 and Table 2 show the counts of detected U-L α and U-L β 1 lines against the amount of U in urine. The slope and intercept of the regression lines of U-L α and U-L β 1 lines were 65.9 and 285 ($R^2 = 0.999$) and 14.9 and 12.6 ($R^2 = 0.999$), respectively. A linear relationship was observed between 0 and 50 μ g/g; however, counts at the U-L α line were significant at 0 μ g/g U owing to the presence of Br in urine.

Uranium in urine sample obtained by exposure of rat to U was quantified. Figure 5a is the μ -PIXE spectrum obtained from the urine of the 4 mg/kg U. Imaging of U-L β 1 line was clearly observed. It was confirmed that the homogeneity of uranium in the image of Figure 5b was similar with that of standard specimens in Figure 3b. The U concentrations in one urine sample selected from each group (0.5 and 4 mg/kg U) were calculated based on the calibration curves by U-L β 1 lines. For comparison, the results measured by ICP-MS are also shown in Table 3. When compared with the ICP-MS results, the values at the high and low doses determined by the U-L β 1 line were close to those determined by ICP-MS

within the margin of error. It was confirmed that the other elements did not disturb the intensity of the U-L β 1 line by the U administration to rats.

To improve the accuracy of uranium determination, the effect of counting on the volume of droplets was investigated. As a result of measurement of 1, 2, and 5 μ L droplets containing 50 μ g/g U, which were prepared using urine and concentrated HNO₃ mixture, the counts increased linearly depending on the volume dropped. Detection of lower concentration of U in urine is to be attained by using 5 μ L droplets.

Figure 3. (a) μ -PIXE spectra of specimens prepared using 1 μ L droplet of mixed solutions of urine treated with five-fold HNO₃ containing 50 μ g/g U for curve 1 and that without U for curve 2. (b) X-ray imaging of K-K β by Si(Li) detector, U-L α , and U-L β 1 lines by CdTe detector in the presence (1) and absence (2) of U. *Imaging of U-L α line expresses sum of the counts at U-L α and Br-K β lines.

Figure 4. Calibration lines of 1–50 $\mu\text{g/g}$ U in mixed solutions of urine treated with five-fold HNO_3 . Horizontal axis, U concentration ($\mu\text{g/g}$); vertical axis, X-ray intensity of (a) U-L α (12.8–14.2 keV) and (b) U-L β 1 (16.8–17.8 keV) lines. Each point is mean and error of data from three positions per specimen. Note that count of U-L α (12.8–14.2 keV) line overlaps with counts at Br-K β line.

Figure 5. (a) $\mu\text{-PIXE}$ spectra of specimens prepared using 1 μL droplet of mixed solutions of urine treated with five-fold HNO_3 . The urine was collected for five hours after 4 mg/kg U administration to rats. (b) X-ray imaging of K-K α line by Si(Li), U-L α , and U-L β 1 lines by CdTe detector. *Imaging of U-L α line expresses sum of the counts at U-L α and Br-K β lines.

Table 2. X-ray intensity of U-L α and U-L β 1 lines in 1 μ L of mixed solutions of urine treated five-fold HNO₃.

U Concentration * (μ g/g)	U-L α line		U-L β 1 line	
	Intensity (Counts $\pm$ SD)	RSD (%)	Intensity (Counts $\pm$ SD)	RSD (%)
0	311 $\pm$ 21	7	0	0
1	375 $\pm$ 95	25	23 $\pm$ 18	78
5	589 $\pm$ 31	5	97 $\pm$ 64	66
10	913 $\pm$ 62	7	172 $\pm$ 79	46
50	3589 $\pm$ 869	24	753 $\pm$ 85	11

* μ -PIXE measurements were performed on three specimens for one concentration. Each point represents the mean and standard deviation of data from three specimens.

Table 3. Uranium concentration in urine administration specimens determined by calibration line of U-L β 1 line.

Administration U (mg/kg)	U Concentration (μ g/g)	
	U-L β 1 Line *	ICP-MS
0.5	18.5 $\pm$ 12.5	14.1 **
4	50.7 $\pm$ 9.0	53.9 **

* PIXE measurements were performed on three specimens for one concentration. ** values determined by ICP-MS.

4. Conclusions

Uranium concentration in the urine samples ranging in several μ L was determined with a simple method using μ -PIXE analysis. Concentrated HNO₃ and film coated by PFA were employed to decompose organic matrices and increase the hydrophobicity of the film, resulting in a decreased area of the droplet specimen. Based on the calibration curve of U-L β 1 line, U in urine sample collected from U administered rats was quantified without chemical separation of elements contained in urine. It was noted that Br should be considered when U concentration is determined using the U-L α line owing to the overlapping with U-L α and Br-K β lines. The method could be applied to preliminary bio-monitoring of multi elements including U in liquid samples such as urine and blood serum in acute internal exposure to nuclear accidents.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Figure A1. μ -PIXE spectra of specimens prepared using 1 μ L droplet of mixed solutions of urine treated with five-fold by HNO_3 containing 50 $\mu\text{g/g}$ U for curve 1 (black line) and that without U for curve 2 (red line). Si(Li) detector was used for the measurements.

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