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Sequential Injection Approach for Simultaneous Determination of Ultratrace Plutonium and Neptunium in Urine with Accelerator Mass Spectrometry

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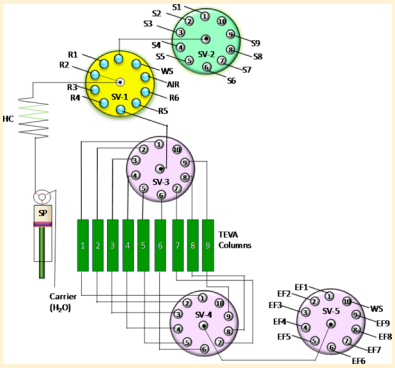
ABSTRACT: An analytical method was developed for simultaneous determination of ultratrace level plutonium (Pu) and neptunium (Np) using iron hydroxide coprecipitation in combination with automated sequential injection extraction chromatography separation and accelerator mass spectrometry (AMS) measurement. Several experimental parameters affecting the analytical performance were investigated and compared including sample preboiling operation, aging time, amount of coprecipitating reagent, reagent for pH adjustment, sedimentation time, and organic matter decomposition approach. The overall analytical results show that preboiling and aging are important for obtaining high chemical yields for both Pu and Np, which is possibly related to the aggregation and adsorption behavior of organic substances contained in urine. Although the optimal condition for Np and Pu simultaneous determination requires 5-day aging time, an immediate coprecipitation without preboiling and aging could also provide fairly satisfactory chemical yields for both Np and Pu (50−60%) with high sample throughput (4 h/sample). Within the developed method, ^{242}Pu was exploited as chemical yield tracer for both Pu and Np isotopes. ^{242}Pu was also used as a spike in the AMS measurement for quantification of ^{239}Pu and ^{237}Np concentrations. The results show that, under the optimal experimental condition, the chemical yields of ^{237}Np and ^{242}Pu are nearly identical, indicating the high feasibility of ^{242}Pu as a nonisotopic tracer for ^{237}Np determination in real urine samples. The analytical method was validated by analysis of a number of urine samples spiked with different levels of ^{237}Np and ^{239}Pu. The measured values of ^{237}Np and ^{239}Pu by AMS exhibit good agreement ($R^2 \geq 0.955$) with the spiked ones confirming the reliability of the proposed method.



Np and Pu are regarded as highly radiological and biological toxic radionuclides due to the alpha emission of their dominant isotopes (^{237}Np: 2 144 000 yr; ^{239}Pu: 24 110 yr; ^{240}Pu: 6561 yr) and accumulation in liver and/or bones once introduced into the human body.[1] Whenever people are exposed to Np and Pu contaminated circumstances occupationally or after a radiological/nuclear incident, assessment of the exposure level to Np and Pu is required for radiation protection and medical intervention. For this purpose, accurate and rapid quantification of Np and Pu in biological samples is imperative in health risk assessment and emergency situations.

Urine bioassay is the most commonly used method for internal dosimetry of Np and Pu because of the easy collection and availability of samples from the affected population. The International Commission on Radiological Protection (ICRP) has recommended an annual limit of dose equivalent of 1 mSv for the general public.[2] Considering the relative long residence times of Pu and Np in human body and therefore slow excretion of Pu and Np in urine, a highly sensitive and accurate method capable for measuring ^{237}Np and Pu isotopes at a concentration of ca. 1 fg/L in urine sample is needed to meet these regulatory requirements. To this point, radiometric methods (alpha spectrometry) and conventional ICPMS cannot meet this requirement. Thermal ionization mass spectrometry (TIMS) has been used to measure low level Pu in urine for some years due to its high sensitivity.[3,4] The major drawback of TIMS hampering its analytical capacity is the time-consuming postseparation process operation (taking some 4−8 h per sample), wherein the analytes purified and concentrated from a large sample (e.g, 0.5−1 L urine) to a very small volume of solution (<10 μL) should be deposited on a filament surface manually.[5] Accelerator mass spectrometry (AMS) is the technique of choice to cope with the requirement in precise and accurate determination of Np and Pu at sub-fg levels because of its high abundance sensitivity and exclusion to molecular ion interferences. In recent years, this technique has been applied for the measurement of Pu isotopes in urine.[6,7] However, the measurement of Np in urine has not been

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Table 1. Coprecipitation Behavior of Iron Hydroxide

		chemical yield of ^{59}Fe, %			chemical yield of ^{239}Np, %	
group no.	concentration of stable Fe, mmol/L	fresh urine	aged urine	seawater	fresh urine	aged urine
1	0.3		76.3 ± 0.2	79.5 ± 6.0		21.8 ± 0.4
2	0.9		65.1 ± 0.0	99.7 ± 0.1		22.4 ± 0.3
3	9	92.9 ± 1.8	82.1 ± 5.0	101.9 ± 0.2	89.8 ± 2.3	78.4 ± 7.3

Table 2. Investigation on Parameters Affecting the Iron Hydroxide Co-Precipitation Performance

	experimental condition									
group number	preboil time, h	aging time, day	amount of precipitator, mmol	sedimentation time	organic matter decomposition	pH adjustor	total analytical time, h	chemical yield of ^{242}Pu	chemical yield of ^{237}Np, %	ratio of ^{237}Np/^{242}Pu
1-1	0	0	6	0	none	$NH_3{\cdot}H_2O$	2	2.7 ± 1.7	0.9 ± 0.8	0.31
1-2	0	0	3	0	acid digestion	$NH_3{\cdot}H_2O$	4	51.3 ± 0.2	57.5 ± 8.8	1.12
1-3	0	0	3	0	acid digestion	NaOH	4	6.6 ± 5.2	13.7 ± 14.0	2.08
1-4	0	0	3	overnight	acid digestion	$NH_3{\cdot}H_2O$	12	23.0 ± 11.0	35.7 ± 24.4	1.55
1-5	0	0	3	overnight	acid digestion	NaOH	12	1.2 ± 0.1	0.2 ± 0.1	0.17
2-1	2	0	6	0	none	$NH_3{\cdot}H_2O$	6	1.2 ± 0.1	0.2 ± 0.1	0.17
2-2	2	0	18	0	acid digestion	$NH_3{\cdot}H_2O$	8	1.2 ± 0.3	5.9 ± 4.1	4.92
2-3	2	0	3	overnight	acid digestion	$NH_3{\cdot}H_2O$	16	66.7 ± 0.7	49.4 ± 0.5	0.74
3-1	2	1	3	0	acid digestion	$NH_3{\cdot}H_2O$	30	9.4 ± 0.4	4.2 ± 0.5	0.44
3-2	2	1	3	overnight	acid digestion	$NH_3{\cdot}H_2O$	38	21.4 ± 3.4	22.7 ± 0.7	1.06
3-3	2	2	3	overnight	acid digestion	$NH_3{\cdot}H_2O$	62	30.7 ± 11.5	11.5 ± 1.6	0.38
3-4	2	5	3	overnight	acid digestion	$NH_3{\cdot}H_2O$	134	84.0 ± 8.1	83.0 ± 4.6	0.99
3-5	2	5	18	overnight	dry and ash	$NH_3{\cdot}H_2O$	134	84.3 ± 15.6	73.3 ± 33.0	0.87
3-6	2	10	3	overnight	acid digestion	$NH_3{\cdot}H_2O$	254	25.0 ± 9.4	48.4 ± 4.6	1.94

reported yet. Although AMS is featured for its high rejections of molecular isobaric interferences and low susceptibility to matrix effects, a thorough chemical purification is still needed to concentrate and separate Np and Pu from large volumes (>1 L) of urine samples to obtain the desirable signal in the AMS measurement.

Among the vast analytical methods developed for Np and Pu determination in human urine,[8−15] several coprecipitation techniques have been exploited for preconcentration of target analytes, including calcium phosphate, calcium bismuth, and hydrogen titanium oxide coprecipitation. Iron hydroxide coprecipitation is a traditional method of choice for determination of trace levels Np and Pu. Due to its simplicity and cost-effectiveness, iron hydroxide coprecipitation has been extensively used to isolate Np and Pu from environmental samples with different matrix composition including fresh water, seawater, soil, sediment, seaweed, etc.[16−21] However, to the best of our knowledge, works reported on the usage of iron hydroxide coprecipitation for biological sample analysis are very rare.[22] This might be a consequence of the complicated and variable composition of urine matrix which might influence the analytical performance of iron hydroxide coprecipitation.

In this work, we aimed to exploit and improve the utility of iron hydroxide coprecipitation for simultaneous determination of ultratrace levels of Np and Pu in urine by coupling it with sequential injection extraction chromatographic separation and mass spectrometric measurement (ICPMS and AMS). ^{242}Pu was used as chemical yield tracer for ^{239}Pu and ^{237}Np, and also as a spike for their quantification in the AMS measurement. Experimental parameters governing the analytical performance including sample preboiling operation, aging time, amount of Fe addition, reagent for pH adjustment, sedimentation time, and organic matter decomposition approach were studied and discussed.

EXPERIMENTAL SECTION

Reagents and Samples. Nitric acid (65%), hydrochloric acid (37%), ammonia (25%), hydrogen peroxide (30%), ferric chloride, potassium disulfite, and sodium hydroxide were analytical grade reagents, and all solutions were prepared with ultrapure water (18 MΩ·cm). Solutions of ^{242}Pu (0.1037 Bq/g in 2 mol/L HNO_3) diluted from NBL-CRM 130 purchased from New Brunswick Laboratory (Argonne, IL) was used as a chemical yield tracer of Np and Pu. A ^{237}Np solution of 0.01175 Bq g^{-1} in 2 mol/L HNO_3 was diluted from NIST-SRM-4341 (National Institute of Standard and Technology, Gaithersburg, MD, USA). An in-house ^{239}Pu standard solution of 0.100 Bq/g in 2 mol/L HNO_3 was supplied by Center for Nuclear Technologies, Technical University of Denmark (Roskilde, Denmark). TEVA extraction chromatographic resin (100−150 μm particle size) was purchased from TRISKEM International (Bruz, France).

Pooled human urine samples were collected from 15 Danish healthy residents and preserved in clean and sealed polyethylene barrels under 5 °C. Unless otherwise stated, 1 L of urine spiked with known amounts of ^{237}Np (ca. 20 ng, corresponding to 0.5 mBq) and ^{239}Pu (ca. 2 ng, corresponding to 5mBq) was used as a sample for method development.

Investigation of Coprecipitation Behavior of Iron Hydroxide. To investigate the precipitation behavior of iron, 200 mL of fresh/aged human urine or seawater aliquot was acidified with 20 mL of conc. HNO_3 and then was spiked with a known amount of ^{59}Fe; 0.02−0.6 mL (depending on the experimental condition; see the details in Table 1) of 0.3 mol/L Fe (in the form of $FeCl_3$) solution was added, and then, conc. $NH_3{\cdot}H_2O$ was added to adjust the pH to 8−9 to form iron hydroxide precipitation. The sample was transferred to a 250 mL centrifuge tube and centrifuged at 4000 rpm for 10 min. The supernatant was discarded and the precipitate was

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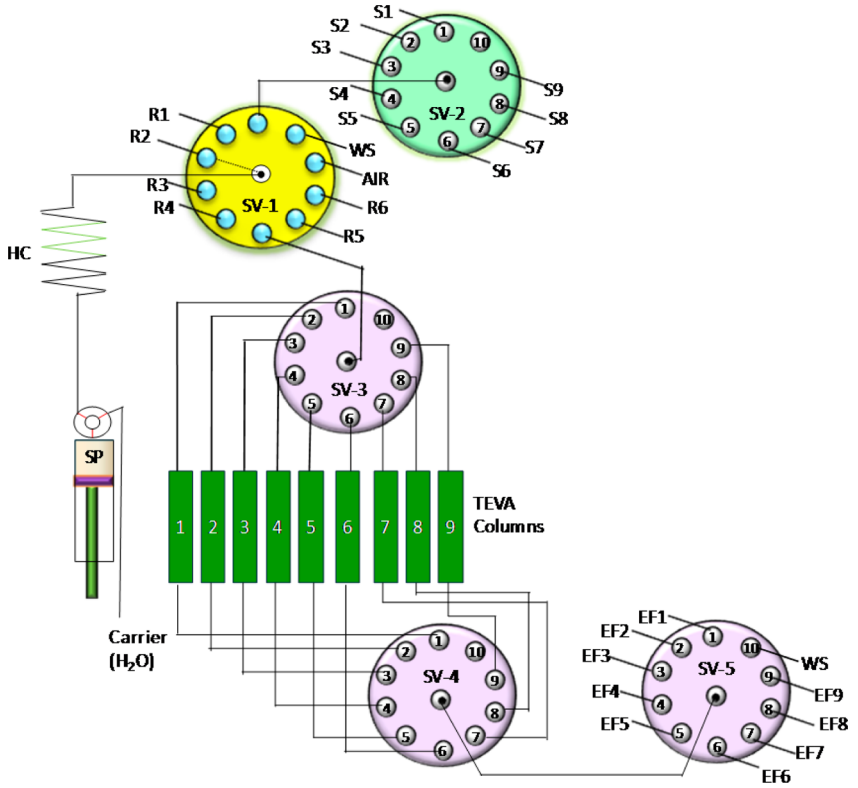

Figure 1. Schematic demonstration of the SI-EC system used in this work for Np and Pu determination (SP: syringe pump; HC: holding coil; S1−S9: ports for sample loading; EF1−EF9: ports for eluate collection; WS: waste; SV1−SV5: selection valves; R1−R6: reagents for column separation).

dissolved with 10 mL of 2 mol/L HCl and transferred to a 20 mL plastic vial for ^{59}Fe quantification with gamma spectrometry. To confirm the applicability of iron hydroxide coprecipitation for Pu and Np preconcentration, 200 mL of fresh or aged urine was spiked with a certain amount (ca. 1−3 Bq) of ^{239}Np and subjected to the same precipitation process as described above (see the details in Table 1).

Optimization of Sample Pretreatment. *Iron Hydroxides Coprecipitation.* To 1 L of human urine aliquot spiked with certain amounts of ^{237}Np and ^{239}Pu, conc. HCl was added to acidify the sample to pH = 1, and then, a known amount (ca.35 ng, corresponding to 5mBq) of ^{242}Pu was added as a chemical yield tracer for both Np and Pu (Figure 2). The sample solution was boiled on a hot plate at 200 °C for 0−2 h and then stored for 0−5 days depending on the experimental condition (see the details in Table 2). One to six milliliters of 3 mol/L Fe (in the form of $FeCl_3$) solution was added, and conc. $NH_3{\cdot}H_2O$ was added to adjust the pH to 8−9. The sample was transferred to four 250 mL centrifuge tubes and centrifuged at 4000 rpm for 10 min. The supernatant was discarded, and the precipitate was subjected to organic matter decomposition.

Decomposition of Organic Matter. Dry ashing or acid digestion was exploited in order to decompose the organic matter. In the dry ashing process, the separated precipitate was transferred to a beaker with water and evaporated to dryness on a hot plate at 150 °C and then ashed at 550 °C overnight in a muffle furnace. In the acid digestion process, the precipitate was dissolved and transferred to a beaker with 20 mL of conc HNO_3, and the sample was digested at 250 °C for 2 h with occasional addition of hydrogen peroxide in a total volume of 10 mL. The digested solution was evaporated to dryness.

Valence Adjustment. The ash or residue after evaporation was dissolved with 30 mL of 0.5 mol/L HCl. The sample solution was filtrated through a GF/A filter, and the filtrate was collected to a centrifuge tube; 30 mL of 0.5 mol/L HCl was used to wash the beaker and filter, which was combined with the filtrate. $K_2S_2O_5$ (300 mg) was added to the centrifuge tube, and the sample was stirred for 20 min to reduce overall Np to Np(IV) and Pu to Pu(III), respectively. NaOH (6 mol/L) was added to adjust the pH to 9−10, and the supernatant was discarded after centrifugation. The precipitate was dissolved with 5 mL of conc HNO_3, and the sample solution was finally adjusted to a concentration of 4−5 mol/L HNO_3 for column separation using a sequential injection extraction chromatography (SI-EC) system (Figure 1).

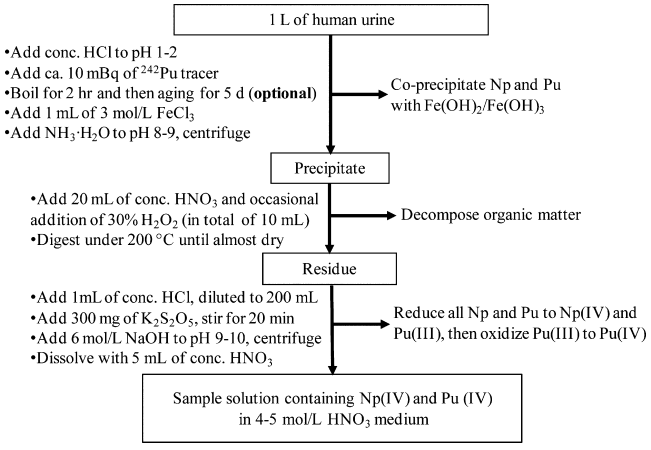

Figure 2. Optimized iron hydroxide coprecipitation procedure for simultaneous determination of Np and Pu in human urine.

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Automated SI-EC Column Separation. The automated column separation was performed with a SI-EC system developed previously in our lab, in which nine samples can be sequentially processed.[23] Nine Econo-Column columns (Bio-Rad Laboratories Inc., Hercules, CA; 5 mm inner diameter and 50 mm length, volume ca. 1 mL) packed with TEVA resin were assembled in the SI-EC system. The column separation was composed of the following steps: (1) cleaning the holding coil and the inlet and outlet of all tubing and preconditioning the TEVA column with 20 mL of 4 mol/L HNO_3; (2) loading sample solution onto the column; (3) rinsing the column with 20 mL of 1 mol/L HNO_3 followed by 10 mL of 9 mol/L HCl; (4) eluting ^{237}Np (along with ^{242}Pu) with 10 mL of 0.1 mol/L NH_2OH·HCl-2 mol/L HCl. The flow rate for the entire chemical separation was controlled at 1.0 mL/min.

Detection of Np and Pu with ICPMS and AMS. During the method development, the detection of ^{237}Np, ^{239}Pu, and ^{242}Pu was performed with ICPMS (X Series^{II}, Thermo Fisher Scientific, Waltham, MA). The eluate was evaporated to dryness and transferred to a 10 mL centrifuge tube with 0.5 mol/L HNO_3; 100 μg/L of In^{3+} solution was added to a final concentration of 1 μg/L. The ICPMS instrument was equipped with an Xs-skimmer cone and a concentric plastic nebulizer under hot plasma conditions. A least-squares regression line was used for quantification of Np and Pu over the range of 0.01 to 100 ng/L. Prior to detection, the instrumental parameters were adjusted for Np and Pu using 4.2 ng/L ^{237}Np and 3.9 ng/L ^{242}Pu solutions to optimal detection efficiency. The typical operational conditions of the instrument have been reported elsewhere.[21] Typical sensitivities of the instrument for Np and Pu ranged from 1×10^5 to 5×10^5 cps per μg/L.

The AMS measurement of Np and Pu was implemented with the compact (0.6 MV) AMS system Tandy at ETH Zurich, Switzerland. To the Pu/Np eluate which was previously evaporated to dryness and reconstituted in 0.5 mol/L HNO_3, ca. 1 mg of Fe (as $FeCl_3$ solution) was added, and the sample was neutralized with ammonia to pH > 9 to coprecipitate Pu and Np. The supernatant was discarded after centrifugation; Pu and Np along with iron hydroxide coprecipitate was dried in an oven under 100 °C. The dried sample was baked in a furnace for 2−3 h at 650 °C to obtain Fe_2O_3 and oxides of Pu and Np. This material was then grounded to fine powder, mixed with 4−5 mg of niobium powder, and finally pressed into a Ti target holder for the AMS measurement. The general AMS instrumental condition for actinide isotopes has been reported elsewhere.[24] PuO^- and NpO^- ions were extracted from a modified NEC MC-SNICS ion source. Here, element specific yields for the formation of the negative molecular ions may be present. The isotopes ^{237}Np, ^{239}Pu, and ^{242}Pu were injected sequentially into the accelerator by means of a fast switching system. The cycle time amounted to 8 s for the spiked isotope ^{242}Pu and to 15 s for the target isotopes ^{237}Np and ^{239}Pu. The Tandy was running at terminal voltages of 311 kV (^{242}Pu), 315 kV (^{239}Pu), and 318 kV (^{237}Np). In the interaction with helium stripper gas at the terminal of the accelerator, the molecules are broken up and positively charged ions are created. Triply charged Pu and Np ions are chosen for detection on the high-energy side of the system, because this charge state has the highest yield for actinides in the energy range.[25]

Molecular background from surviving UH_3^+ molecules is suppressed by adjusting the density of the stripper medium.[26] The ions are identified in a gas ionization chamber, which suppresses m/q interferences such as $^{79}Br^+$, $^{158}Dy^{2+}$, or $^{158}Gd^{2+}$. In the fast switching between the isotopes, the voltages of the beam injection pulsing system, the terminal, and the electrostatic analyzer on the high-energy side had to be changed. This sequence was repeated five times on each target. The isotope ratios are calculated for such a run from the average counting rates. The AMS targets were measured in this way 7 to 10 times. The total measurement time on each target was 20 to 30 min. The final result then is calculated as the error weighted mean of the single runs. Differences between the nuclides might be caused by several processes: (1) The tuning of the beam is different for each nuclide. This might lead to a deviation in efficiency of few percentage for the various nuclides. (2) The formation of negative ions is element dependent; i.e., it is different for ^{237}Np and $^{239, 242}$Pu.[27] (3) Slight differences (probably again only in the few percentage range) might be present in the charge state yields of Np and Pu after the stripping process. All these effects are corrected via a series of standards, which is prepared and measured in parallel to the real samples.

Gamma Spectrometry for ^{59}Fe and ^{239}Np Measurement. In the investigation of the precipitation behavior of iron in urine, ^{59}Fe was utilized as a radioactive tracer of iron and measured with a HPGe (high pure germanium) gamma detector system at 1091−1108 keV and 1284−1302 keV, respectively. ^{239}Np was measured with NaI well-shape gamma spectrometer at 32−92 keV.

RESULTS AND DISCUSSION

Precipitation Behavior of Iron in Urine. Iron hydroxide coprecipitation is a classic technique widely used as a preconcentration step for Pu and Np determination in environmental samples. However, previous reports involving the application of iron hydroxide coprecipitation are rarely devoted to the Pu and Np urine assay. This might be based on the assumption that the complex organic content of urine (e.g., vitamins, hormones, organic acids, miscellaneous organic compounds) would deteriorate the coprecipitation efficiency of iron hydroxide. To clarify the applicability of iron hydroxide coprecipitation in the Pu and Np urine assay, as well as to reveal the effect of urine matrix on the coprecipitation efficiency, ^{59}Fe was spiked into a batch of fresh or aged urine and seawater aliquots and precipitated with the addition of different amounts of stable iron. It can be seen that chemical yields of ^{59}Fe are consistently lower in urine compared with the corresponding values in seawater, indicating the negative effect of urine matrix on the precipitation efficiency of iron (Table 1). However, with the increase of iron concentration in urine ranging from 0.3 to 9 mmol/L, the chemical yield of ^{59}Fe is improved up to 82.1%. The relatively low precipitation efficiency of Fe in urine might be related to the complexation of Fe with the organic substances contained in the sample (e.g., ascorbic acid), and practically, some very fresh urine samples were observed to turn red colored when Fe was added, which could confirm somehow the complex reaction.

Moreover, even using the same batch of sample, the composition of urine matrix may also change with time, which is reflected by the difference of ^{59}Fe chemical yields between fresh urine and aged urine under the same experimental condition (see group 3, Table 1). This also reveals that the sample storage is important to ensure analytical repeatability, and thus, it is recommended to refrigerate or freeze urine samples after collection. Notwithstanding the fact that the urine matrix imposes some deterioration to the

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scavenging of ^{239}Np and the precipitation efficiency of Fe itself, with the sufficient addition of stable iron, iron hydroxide coprecipitation is still applicable for Pu and Np preconcentration from urine matrix, as indicated by the satisfactory chemical yields of ^{239}Np under these conditions (see group 3, Table 1).

Crucial Parameters in Iron Hydroxide Coprecipitation. In the iron hydroxide coprecipitation, the basic principle is ion exchange adsorption; that is, under alkaline condition (e.g., pH= 8−10), functional groups (−OH) of iron hydroxide dissociate hydrogen and the generated $-O^-$ groups bind Np and Pu cations onto the precipitate.[5] Many parameters may influence the coprecipitation performance, such as the competitive adsorption of organic compounds in urine and adsorption kinetics of Np and Pu onto iron hydroxide particles. Therefore, to establish an effective analytical procedure, several experimental parameters including sample preboiling treatment, aging time, amount of coprecipitant, alkaline reagent for pH adjustment, and sedimentation time of the precipitate were investigated and compared in detail. The overall analytical results are summarized in Table 2, and relevant interpretations are discussed below.

Preboiling and Aging. The purpose of preboiling was to decompose the organic matter contained in urine. Because in the coprecipitation process these soluble organic substances may form complexes with Np and Pu or adsorb Np and Pu onto their surface and remain in the supernatant, they thereafter induce a loss of Np and Pu in the iron hydroxide coprecipitation. Since adsorption is a surface reaction, the larger the surface area of the sorbent substance, the greater is the adsorption.

The analytical results show that preboiling could somehow decompose organic substances contained in urine and thus enhance the chemical yields of Np and Pu; e.g., when preboiling was not performed, the chemical yields of Np and Pu were 35.7 ± 24.4% and 23.0 ± 11.0% (see group 1-4 in Table 2), respectively. However, with the same experimental operation but with preboiling for 2 h, the chemical yields of Np and Pu increased up to ca. 67% and ca. 49% (see group 2-3 in Table 2), respectively.

It is interesting that high chemical yields (ca. 80%) could only be obtained when aging the urine sample for 5 days after preboiling (see groups 3-4 and 3-5 of Table 2). This might be attributed to two factors: (1) the adsorption or bonding kinetics of Np and Pu onto organic substance and (2) aggregation or flocculation characters of organic matter contained in urine. Presumably, the organic substances contained in urine can be broken down into entities with very fine particle size during the preboiling, while the uptake of Np and Pu by these organic substances would be increased with reaction time. Within less than the 5-day aging period, the aggregation or flocculation of the fine organic particles could not reach large enough precipitation seeds to settle down. Therefore, Np and Pu bound with these organic substances would remain in the supernatant, therefore diminishing their distribution into iron hydroxide coprecipitation (see groups 3-1 to 3-3 in Table 2). When the aging time accumulated to 5 days, adsorption of Np and Pu onto organic matter may reach a maximum equilibrium plateau, while organic substances aggregate to suitable sizes that can be adsorbed or coprecipitated by iron hydroxide particles when sample pH is increased to 9−10, thus being contributive to the coprecipitation efficiency of iron hydroxide.[28]

The low chemical yields of Np and Pu obtained after a 10 day aging might be a consequence of the undue aggregation or flocculation of organic matter that form fluffy suspending substances with low density which cannot settle down in the precipitate. Instead, these aggregated or flocculated organic substances could possibly enwrap iron hydroxide into the supernatant and therefore induce decreased coprecipitation efficiency and chemical yields of Np (ca. 25%) and Pu (48%); see the results in group 3-6 of Table 2.

Although the optimal condition of 5 day aging prolongs the analytical procedure, an immediate coprecipitation without preboiling and aging could also be performed to obtain acceptable chemical yields for both Np (57.5 ± 8.8%) and Pu (51.3 ± 0.2%) (see group 1-2 in Table 2). Most importantly, under this condition, Np behaved consistently with Pu along the whole analytical procedure as indicated by the Np/Pu chemical yield ratio (1.12), revealing ^{242}Pu functioned well as a nonisotopic tracer for Np determination.

Amount of Fe Addition. Sufficient addition of Fe would provide effective coprecipitation of Np and Pu and thus ensure high chemical yield for both radionuclides. However, extra addition of Fe may increase the final sample volume and thus give rise to difficulties in the following column separation step. Moreover, bulky sample matrix may also deteriorate the adsorption of Np and Pu onto the extraction column.

In order to optimize the amount of precipitating reagent, different amounts of $FeCl_3$ (from 0.5 to 3 g) were added to a 1 L urine sample for coprecipitation of Np and Pu. The results (Table 2) show that addition of 0.5 g of $FeCl_3$ is adequate to obtain sufficiently high chemical yields (see groups 3-4 and 3-5). Extra addition of Fe up to 3 g of $FeCl_3$ could not provide higher chemical yields for Np and Pu due to the competitive adsorption of Fe onto the chromatographic column. Therefore, 0.5 g of $FeCl_3$ was finally selected as the precipitating reagent in the following experiment.

Reagent for pH Adjustment. To test the effect of different reagents for pH adjustment on the coprecipitation efficiency, two alkaline solutions including NaOH and $NH_3{\cdot}H_2O$ were investigated. Surprisingly, an extremely poor analytical performance with respect to the chemical yields (<5%) of Np and Pu was observed when using NaOH for pH value adjustment (groups 1-3 and 1-5 in Table 2). It is more likely that, with the use of ammonia, iron hydroxide precipitates more homogeneously forming fluffy particles with larger surface area, which could lead to better flocculation or aggregation. Consequently, higher Np and Pu chemical yields can be obtained when exploiting $NH_3{\cdot}H_2O$ as a reagent for pH adjustment.

Sedimentation Time. Theoretically, a longer sedimentation time is favorable for the adsorption of Np and Pu, thus obtaining better coprecipitation efficiency and repeatability. However, over a long term, sedimentation time will prolong the analytical procedure and thus impair the sample throughput. Practically, we observed that without preboiling and aging, overnight sedimentation was not favorable to improve the chemical yields of Np and Pu (see group 1-4 in Table 2). In contrast, immediate separation of the precipitation from the supernatant provided much higher chemical yields (see group 1-2 in Table 2). Differently, for samples with preboiling and aging, overnight sedimentation was necessary (see groups 3-1 and 3-2) to achieve desirable analytical performance.

This might be caused by the fact that the spiked Np and Pu existed as free ions without preboiling and aging and could quickly access the surface of iron hydroxide precipitate during

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Table 3. Analytical Results for Validation Tests Using Urine Samples Spiked with Different Amounts of ^{237}Np and ^{239}Pu

	^{239}Pu			^{237}Np		
sample ID	spiked [fg][a]	measured [fg]	Bri [%]	spiked [fg][a]	measured [fg]	Bri [%]
TP0299	0.00	1.46 ± 0.23		0.00	21.17 ± 0.88	
TP0300	0.00	5.08 ± 0.39		0.00	21.18 ± 0.91	
TP0301	1.12	1.23 ± 0.28	10.11	0.99	24.60 ± 1.63	2393.20
TP0302	1.12	2.50 ± 0.42	123.63	0.99	26.26 ± 1.88	2557.46
TP0303	10.84	10.46 ± 0.59	−3.51	9.74	15.30 ± 1.02	57.14
TP0304	11.17	10.69 ± 0.60	−4.30	8.79	12.49 ± 0.91	42.14
TP0305	49.67	44.63 ± 1.78	−10.13	48.54	58.24 ± 2.12	20.0
TP0306	50.00	47.38 ± 1.78	−5.24	49.90	63.55 ± 5.09	27.37
TP0307	101.92	98.39 ± 2.70	−3.46	93.44	92.15 ± 2.97	−1.39
TP0308	101.67	99.25 ± 2.89	−2.38	107.96	105.01 ± 6.10	−2.73
B_r [%][b]			−2.70	B_r [%][b]		−23.76
S_B [%][b]			6.19	S_B [%][b]		23.72

[a]Uncertainties for all values are less than 5%. [b]For calculating B_r and S_B of ^{239}Pu, sample TP302 is excluded. For calculating B_r and S_B of ^{237}Np, samples TP301 and TP302 are excluded.

its formation. In contrast, after boiling and aging processes, the spiked Np and Pu no longer exist as free ions but have reached chemical equilibrium with the urine matrix via ion exchange or complexation. Thus, before being adsorbed onto iron hydroxide, Np and Pu need to be liberated from the electron hole or complexing bond. Therefore, longer reaction time is required.

However, undue long sedimentation time (e.g., >12 h) is inadvisable, since the evaporation of $NH_3{\cdot}H_2O$ during the sedimentation will cause the decrease of pH and thus induce desorption of Np and Pu from iron hydroxide. Besides, in alkaline solution, negatively charged iron hydroxide may also be adsorbed or trapped by positively charged organic collides via electro-static force; thus, Np and Pu previously adsorbed onto iron hydroxide will then enter into the aqueous phase and give rise to low distribution in the precipitate. Therefore, in our work, when preboiling was not conducted, immediate separation of the precipitation with centrifugation was selected as the optimal condition for achieving higher chemical yields for Np and Pu, and when preboiling and aging was performed, overnight sedimentation was preferred.

Decomposition Approach of Organic Matter. For decomposition of organic matter after the coprecipitation, acid digestion and dry ashing were investigated. In the acid digestion, a mixture of HNO_3−H_2O_2 was used to form Fenton's reagent wherein iron acts as a catalyst (see eqs 1 and 2) to produce hydroxyl and peroxide radicals, thus accelerating the decomposition of organic matter.[29]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^- \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH\cdot + H^+ \quad (2)$$

The results (Table 2) indicate that acid digestion functions equally effective as dry ashing for organic matter decomposition (see groups 3-4 and 3-5 in Table 2). A slightly lower chemical yield of Np is noticed when samples were dry ashed (see group 3-5 in Table 2), which might be due to the Np loss in the column separation caused by the competitive sorption of Fe since 3 g of $FeCl_3$ was used under this condition. However, considering the operational time consumed in both approaches, dry ashing (ca. 12 h) takes much longer than acid digestion (ca. 3 h). Therefore, acid digestion was selected as the method for decomposing organic matter in this work.

Analytical Time and Sample Throughput. With the merit of the automated SI-EC system, the flow rate for the chromatographic separation is consistently controllable and the entire chromatographic separation could be completed within 1 h for a single sample. Whereas in traditional methods, in which the chromatographic separations are typically performed in a manual fashion, the high matrix content in the sample loading solution often makes the flow rate unstable and even causes the blockage of the column. In these cases, the turnover analytical time for a single column separation may take up to 1−2 days. To this point, the application of the SI automation technique notably saves analytical time, improves the analytical efficiency, and reduces the labor intensity compared with traditional methods.

On the basis of the investigation for sample pretreatment as stated above, two analytical procedures can be selected for options according to analytical purpose. In case that high chemical yields and analytical reliability are imperative, preboiling and 5 day aging followed by overnight sedimentation (corresponding to the experiment conditions in group 3-4 in Table 2) can be selected as the method of choice. Although a long analytical time (134 h) is needed for a single sample under this experimental condition, a large number (e.g., 20−30) of samples can be processed in a batch-wise manner. Thus, a theoretical sample throughput of 4−6 samples/day can be achieved.

In emergency or other similar situations, immediate coprecipitation after adding the precipitating reagent can be the alternative (corresponding to the experiment conditions in group 1-2 in Table 2) to cope with the requirement of rapid Np and Pu urine bioassay. However, it should be noted that an underestimation of the Pu/Np values with the use of immediate coprecipitation is possible, since the isotopic equilibrium between the ^{242}Pu tracer and the intrinsic Pu and Np in urine may not be reached in this very short time frame without preboiling. However, for screening purposes, this expedited method might be very valuable in emergency situations to estimate the contamination levels of Np and Pu.

Method Validation. To assess the trueness of the proposed analytical method, a set of 10 samples (200 mL of urine of each) spiked with different amounts of ^{237}Np and ^{239}Pu (up to ca. 100 fg, corresponding to 2.6 μBq of ^{237}Np and 230 μBq of ^{239}Pu, respectively) was processed through the entire analytical

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procedure. The measurements of these low concentrations were performed by means of AMS and resulted in a good confirmation of the expected values for ^{239}Pu down to the single fg level and showed a reliable agreement for ^{237}Np in the range above 10 fg (Table 3). Linear fits ($y = a*x + b$) result in parameters of $a = 0.80 \pm 0.06$ and $b = 18 \pm 3$ for ^{237}Np and $a = 0.95 \pm 0.02$ and $b = 1.0 \pm 0.9$ for ^{239}Pu, respectively (Figure 3). The quality of the linear fits, with correlation coefficients (R^2) of 0.955 and 0.997 for ^{237}Np and ^{239}Pu, respectively, documents the reliability of the proposed method.

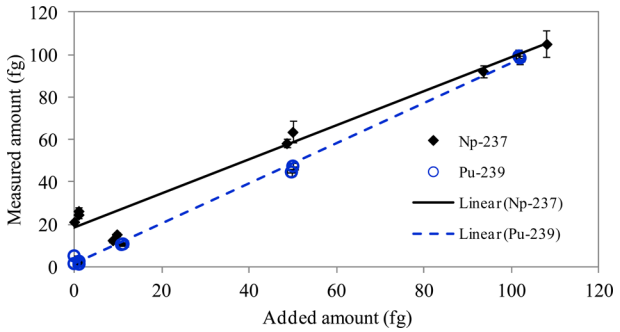

Figure 3. Measured ^{237}Np and ^{239}Pu amounts are plotted versus the added quantities in 200 mL urine samples.

In the samples spiked with less than 10 fg of ^{237}Np, significantly increased values (up to 25 fg) were measured, pointing to an average contamination of these samples with ca. 20 fg of ^{237}Np. This might be a consequence of contaminations with residual ^{237}Np from the SI system, lab wares, chemicals, and fume cupboard. Since the linear fit to the ^{237}Np data in Figure 3 includes all samples, the offset of 18 ± 3 fg also reflects this contamination. Theoretically, such contaminations might be eliminated by carefully cleaning all possible residual ^{237}Np. However, we practically encountered difficulties in reducing the background level of ^{237}Np down to femtograms, since method development studies for ^{237}Np have been frequently carried out in our laboratory with spiking 2−20 ng of ^{237}Np standard in each sample to obtain sufficient signal in ICPMS measurements. Therefore, to achieve a nearly "^{237}Np free" circumstance, a new "clean" lab might be needed in the future to improve the detection limit of ^{237}Np in the AMS measurement.

A limit of detection can be calculated for a standardized normal distribution of the procedural blanks as $L_d = 2*1.645*\sigma_0$.[30] Using the uncertainties (corresponding to 3 and 0.9 for ^{237}Np and ^{239}Pu, respectively) of the offset b from the linear fits as σ_0, detection limits of 10 fg and 3 fg are calculated for ^{237}Np and ^{239}Pu, respectively. This probably slightly underestimates the true detection limit for ^{237}Np in the current preparation, as the samples containing 10 fg of ^{237}Np are measured significantly below the average blank level. From the current data, one would rather expect a ^{237}Np detection limit of ca. 20 fg. In any case, the detection limits reached with the merit of AMS for real urine bioassays in this study are still much lower than the detection limit of the ICPMS (30−100 fg for both ^{237}Np and Pu isotopes in our work) used for the above ^{237}Np and ^{239}Pu determinations.

The relative bias and relative precision for the spiked urine samples were also assessed on the basis of the guidance provided in ANSI N13.30.[31] The relative bias for B_{ri} individual measurement and the relative precision S_B were calculated according to eqs 3 and 4, respectively.

$$B_{ri} = \frac{A_i - A_{ei}}{A_{ei}} \times 100\% \quad (3)$$

$$S_B = \sqrt{\frac{\sum_{i=1}^{N} (B_{ri} - B_r)^2}{N - 1}} \times 100\% \quad (4)$$

where A_{ei} and A_i are the expected and measured amounts for an individual sample, respectively, B_r is the mean relative bias, and N is the number of replicate measurements.

Apart from the values of ^{237}Np in samples TP301-303 and ^{239}Pu in TP302, the expected and measured ^{237}Np and ^{239}Pu amounts (Table 3) deviated within ranges of −10.13% to +42.14%; the mean relative biases were −23.76% for ^{237}Np and −2.70% for ^{239}Pu, and the relative precisions were 23.72% for ^{237}Np and 6.19% for ^{239}Pu. For those samples with >10 fg of ^{237}Np and ^{239}Pu spikes, the validation test passed the ANSI N13.30 performance criteria for both relative bias (−25% to +50%) and relative precision (±40%) by a very substantial margin. As mentioned above, improvements are still needed in our future work in the quantification of relatively low concentrations of ^{237}Np and ^{239}Pu ranging in 0−10 fg.

CONCLUSIONS

In this study, an SI-EC method was developed for simultaneous determination of Np and Pu in large volume urine samples in the connection with ICPMS and AMS quantification. A classic iron hydroxide coprecipitation technique was exploited in the preconcentration step, wherein crucial experimental parameters affecting the analytical performance such as the preboiling treatment, aging time, pH value adjustment reagent, sedimentation time, and decomposition approach of organic matter contained in urine were investigated in detail for the first time. Our results reveal that the preboiling process and aging time play important roles to obtain optimal analytical results. Equally high chemical yields (80%) for Np and Pu could only be achieved when aging the sample for 5 days after preboiling. The reagent used for pH adjustment is also crucial since low chemical yields of Np and Pu were encountered when using NaOH for the pH adjustment, while $NH_3 \cdot H_2O$ is favorable for getting better coprecipitation performance. The developed method is simple and robust and provides a relatively high sample throughput and separation capacity. In the connection with the AMS measurement, the low detection limit could meet the regulatory requirement for a reportable dose of 1 mSv for occupational Np and Pu exposures.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Qiao, J.; Hou, X.; Miró, M.; Roos, P. *Anal. Chim. Acta* **2009**, *652*, 66−84.

(2) International Commission on Radiological Protection (ICRP). *The 2007 Recommendations of the International Commision on Radiological Protection*; JAICRP 37, ICRP Publication; ICRP: Ottawa, 2007.
(3) LaMont, S. P.; Shick, C. R.; Cable-Dunlap, P.; Fauth, D. J.; LaBone, T. R. *J. Radioanal. Nucl. Chem.* **2005**, *263*, 477−481.
(4) Elliot, N.; Bickel, G.; Linauskas, S.; Paterson, L. *J. Radioanal. Nucl. Chem.* **2006**, *267*, 637−650.
(5) Lehto, J.; Hou, X. *Chemistry and Analysis of Radionuclides*, 1st ed.; Wiley-VCH: Weinheim, 2010.
(6) Dai, X.; Christl, M.; Kramer-Tremblay, S.; Synal, H. *J. Anal. At. Spectrom.* **2012**, *27*, 126−130.
(7) Hernandez-Mendoza, H.; Chamizo, E.; Yllera, A.; Garcia-Leon, M.; Delgado, A. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2010**, *268*, 1331−1333.
(8) Dai, X.; Kramer-Tremblay, S. *Health Phys.* **2011**, *101*, 144−147.
(9) Hang, W.; Zhu, L. W.; Zhong, W. W.; Mahan, C. *J. Anal. At. Spectrom.* **2004**, *19*, 966−972.
(10) Elias, G. *Radioact. Radiochem.* **1997**, *8*, 20−24.
(11) Li, C.; Vlahovich, S.; Dai, X.; Richardson, R. B.; Daka, J. N.; Kramer, G. H. *Health Phys.* **2010**, *99*, 702−707.
(12) Sawant, P. D.; Prabhu, S.; Kalsi, P. C.; Pendharkar, K. A. *J. Radioanal. Nucl. Chem.* **2009**, *279*, 179−183.
(13) Bores, N. E.; Schultz, M. K.; Rankin, J. M.; Denton, A. J.; Payne, G. F. *J. Radioanal. Nucl. Chem.* **2008**, *277*, 111−116.
(14) Li, C.; Lariviere, D.; Kiser, S.; Moodie, G.; Falcomer, R.; Elliot, N.; Burchart, L.; Paterson, L.; Epov, V.; Evans, D.; Pappas, S.; Smith, J.; Cornett, J. *J. Anal. At. Spectrom.* **2008**, *23*, 521−526.
(15) Ridone, S.; Arginelli, D.; Berton, G.; Bortoluzzi, S.; Canuto, G.; Montalto, M.; Nocente, M.; Vegro, M. *Czech. J. Phys.* **2006**, *56*, D195−D201.
(16) Chen, Q. J.; Dahlgaard, H.; Nielsen, S. P.; Aarkrog, A.; Christensen, I.; Jensen, A. *J. Radioanal. Nucl. Chem.* **2001**, *249*, 527−533.
(17) Chen, Q. J.; Dahlgaard, H.; Nielsen, S. P.; Aarkrog, A. *J. Radioanal. Nucl. Chem.* **2002**, *253*, 451−458.
(18) Montero, M. P. R.; Sánchez, A. M.; Vázquez, M.T.C.; Murillo, J. L. G. *Appl. Radiat. Isot.* **2000**, *53*, 259−264.
(19) Kim, G.; Burnett, W. C.; Horwitz., E. P. *Anal. Chem.* **2000**, *72*, 4882−4887.
(20) Qiao, J. X.; Hou, X. L.; Roos, P.; Miró, M. *Talanta* **2011**, *84*, 494−500.
(21) Qiao, J. X.; Hou, X. L.; Roos, P.; Miró, M. *J. Anal. At. Spectrom.* **2010**, *25*, 1769−1779.
(22) Singh, N. P.; Zimmerman, C. J.; Lewis, L. L.; Wrenn, M. E. *Radiochim. Acta* **1984**, *35*, 219−222.
(23) Qiao, J. X.; Hou, X. L.; Roos, P.; Miró, M. *Anal. Chem.* **2011**, *83*, 374−381.
(24) Christl, M.; Vockenhuber, C.; Kubik, P. W.; Wacker, L.; Lachner, J.; Alfimov, V.; Synal, H. A. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2013**, *294*, 29−38.
(25) Vockenhuber, C.; Alfimov, V.; Christl, M.; Lachner, J.; Schulze-König, T.; Suter, M.; Synal, H. A. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2013**, *294*, 382−386.
(26) Lachner, J.; Christl, M.; Vockenhuber, C.; Synal, H. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2013**, *294*, 364−368.
(27) Fifield, L. K.; Clacher, A. P.; Morris, K.; King, S. J.; Cresswell, R. G.; Day, J. P.; Livens, F. R. *Nucl. Instrum. Methods Phys. Res., Sect. B* **1997**, *123*, 400−404.
(28) Andersson, P. S. Personal Communication, 2012.
(29) http://en.wikipedia.org/wiki/Fenton%27s_reagent.
(30) Currie, L. A. *Anal. Chim. Acta* **1999**, *391*, 127−134.
(31) Health Physics Society (HPS). *Performance Criteria for Radiobioassay: an American National Standard*; ANSI/HPS N13.30; American National Standard Institute: New York, 1996.