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Comparison of sample preparation methods for reliable plutonium and neptunium urinalysis using automatic extraction chromatography

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ABSTRACT

This paper describes improvement and comparison of analytical methods for simultaneous determination of trace-level plutonium and neptunium in urine samples by inductively coupled plasma mass spectrometry (ICP-MS). Four sample pre-concentration techniques, including calcium phosphate, iron hydroxide and manganese dioxide co-precipitation and evaporation were compared and the applicability of different techniques was discussed in order to evaluate and establish the optimal method for in vivo radioassay program. The analytical results indicate that the various sample pre-concentration approaches afford dissimilar method performances and care should be taken for specific experimental parameters for improving chemical yields. The best analytical performances in terms of turnaround time (6 h) and chemical yields for plutonium (88.7 ± 11.6%) and neptunium (94.2 ± 2.0%) were achieved by manganese dioxide co-precipitation. The need of drying ashing ($\geq$7 h) for calcium phosphate co-precipitation and long-term aging (5 d) for iron hydroxide co-precipitation, respectively, rendered time-consuming analytical protocols. Despite the fact that evaporation is also somewhat time-consuming (1.5 d), it endows urinalysis methods with better reliability and repeatability compared with co-precipitation techniques. In view of the applicability of different pre-concentration techniques proposed previously in the literature, the main challenge behind relevant method development is pointed to be the release of plutonium and neptunium associated with organic compounds in real urine assays. In this work, different protocols for decomposing organic matter in urine were investigated, of which potassium persulfate ($K_2S_2O_8$) treatment provided the highest chemical yield of neptunium in the iron hydroxide co-precipitation step, yet, the occurrence of sulfur compounds in the processed sample deteriorated the analytical performance of the ensuing extraction chromatographic separation with chemical yields of $\leq$50%.

1. Introduction

Due to the alpha emission with considerably long half-lives and readily enrichment in bones and livers, neptunium (namely, ^{237}Np ($t_{1/2}$=2.144 × 10^6 yr)) and plutonium isotopes (namely, ^{239}Pu ($t_{1/2}$=2.411 × 10^4 yr) and ^{240}Pu ($t_{1/2}$=6.561 × 10^3 yr)) are regarded as highly radiological and biological toxic radionuclides to human health [1]. Therefore, Np and Pu exposure assessment is imperative for radiation protection and medical intervention to workers or individuals who are exposed to Np and Pu in nuclear facilities or after a radiological/nuclear incident, respectively. Urinalysis for ^{237}Np and Pu isotopes is widely used to estimate the internal radiation dose of individuals. For this purpose, it is essential to develop reliable and effective methods for Np and Pu urine bioassays.

The International Commission on Radiological Protection (ICRP) has recommended an annual limit of dose equivalent to 1 mSv for the general public [2]. Due to the long retention time of Np and Pu in the human body and thus the very low excretion rates in urine, it is required to measure ultra-trace levels of Np and Pu to be able to meet the ICRP screen criteria of annual internal dose limit. To this point, large urine volumes (e.g., $\geq$1 L) are normally required to cope with the sensitivity demands even in the modern mass spectrometric detection techniques, e.g., accelerator mass spectrometry (AMS). Over the past few decades, a number of urine bioassay methods have been developed for actinides determination

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[3–24]; however, efforts devoted to determination of Np and Pu (especially Np) in large volume ($\geq$1 L) of urine samples are still limited to a few works [4,19,24–26].

In the case of ^{237}Np determination, the choice for tracer is very restricted. No suitable alpha-emitting Np tracer is available. Thus, the beta emitter ^{239}Np is commonly used instead, since it can be obtained from neutron irradiation of ^{238}U or by milking a sample of ^{243}Am with which it is in equilibrium as the alpha-decay daughter (^{243}Am$\rightarrow$^{239}Np+^{4}He). However, because of the short half-life of ^{239}Np (2.36 d), it needs a regular weekly preparation and standardization which is time consuming and expensive. Furthermore, ^{239}Np decays to ^{239}Pu, which increases the sample background for ^{239}Pu in cases where sequential analysis of Np and Pu is performed for the same sample. ^{235}Np can be used as a tracer because of its relatively long half-life ($t_{1/2}$=396.1 d) compared to other Np isotopic tracers, but it contains ^{237}Np as an impurity from the tracer preparation process. Another cyclotron produced isotope, ^{236}Np ($t_{1/2}=1.54\times10^{5}$ yr), could also be used as a tracer, but it is not easy to generate and still not available in a pure form to most researchers. A further option is to use a non-isotopic tracer like ^{242}Pu and to measure the mass concentrations of ^{242}Pu and ^{237}Np simultaneously by ICP-MS. ^{236}Pu can also be used as a tracer instead of ^{242}Pu since the latter tracer interferes with the ^{237}Np measurement when alpha spectrometry is employed. Several researchers have used ^{242}Pu as a tracer for Pu and ^{237}Np determination in environmental samples [27,28].

The matrix composition of urine is very complicated and unpredictable due to the large variation in diet from one individual to another and with time [29]. The changeable matrix effects pose inevitable challenges to the method development for Np and Pu urinalysis especially when handling large sample volumes. An important issue for urinalysis is the complete decomposition of organic matter or the liberation of endogenous radionuclides into free ions from organic matter associations. This is, to the best of our knowledge, still a bottleneck hampering the applicability of most developed radioassays for real urine samples, as the endogenous Np and Pu species in urine are always associated with different organic substances (e.g., nitrogenous compounds, vitamins, hormone, organic acids and miscellaneous organic compounds) to some extent [29]. This is because Pu and Np follow a tortuous metabolic system once entering the human body and react with a number of body fluids. Whenever the release of organically bound Pu and Np is not complete or the species of endogenous Pu and Np are not identical to the spiked chemical yield tracer, analytical results might lack reliability due to the isotopic disequilibrium between the intrinsic Pu/Np and the tracer. In addition, the high content of organic components in the urine could significantly deteriorate the analytical performance of further chemical purification protocols, especially for anion exchange or extraction chromatographic separations. Consequently, there is a quest for novel sample pre-concentration protocols enabling to release quantitatively the organically associated Pu and Np to ensure the accuracy of the urinalysis methods.

Evaporation and co-precipitation are the most often used pre-concentration methods for urinalysis of actinides. Phosphate co-precipitation methods, using calcium phosphate ($Ca_3(PO_4)_2$) [24], or bismuth phosphate ($BiPO_4$) [6] have been widely applied for urine bioassays of transuranics. In recently years, a co-precipitation based on titanium hydroxide (HTiO) [22,26] has been patented for pre-concentration of Pu from 1.4 L of urine, yet applied in few laboratories. Recently, manganese dioxide (MnO_2) and iron hydroxide ($Fe(OH)_3$) co-precipitation protocols have been exploited for urine Np and Pu assays [30,31]. Nevertheless, the analytical applicability of individual pre-concentration technique has not been systemically compared and little effort has been devoted to investigate the urine matrix effects on the analytical performance for actinides urinalysis.

This work aims to investigate and compare the analytical performances of different sample pre-concentration techniques and organic matter decomposition protocols for the determination of Pu and Np in urine in order to select the optimal procedure for in vivo radioassay program. Four sample pre-concentration techniques including evaporation, $Ca_3(PO_4)_2$, MnO_2 and $Fe(OH)_3$ co-precipitation for 1 L urine analysis were performed and the effects of the various parameters (e.g., valence adjustment reagents, organic matter decomposition protocol, urine matrix effect, and aging of urine) on the analytical performances with respects to the chemical yields of Pu and Np, the coherence of Np and Pu behavior and the turnaround time were evaluated. Challenges in liberating organically associated Pu and Np were also tackled by testing and comparing different approaches for the decomposition of organic matter.

2. Experimental

2.1. Reagents and samples

^{237}Np solution of 0.01175 Bq g^{-1} in 2 mol L^{-1} HNO_3 was prepared by dilution of a stock solution supplied by the Center for Nuclear Technologies, Technical University of Denmark (DTU-Nutech). ^{242}Pu standard solution (0.1037 Bq g^{-1} in 2 mol L^{-1} HNO_3) was prepared by dilution of NBL-CRM 130 (New Brunswick Laboratory, Argonne, IL). TEVA extraction chromatographic resin (100–150 μm particle size) was purchased from TRISKEM International (Bruz, France). All chemicals used in this work were analytical grade reagents, and all solutions were prepared with deionized water (18 MΩ cm). Human urine samples were collected individually or pooled together from Danish healthy residents and preserved in clean and sealed polyethylene barrels under 5 °C. Unless otherwise stated, 1 L aliquot of urine spiked with 0.5 mBq of ^{237}Np, 5 mBq of ^{239}Pu and 5 mBq of ^{242}Pu was used as a sample throughout this work. One seawater sample collected from Roskilde Fjord, Denmark (55°41′N, 12°5′E) in 2012 was used for investigating efficiency of the $Fe(OH)_3$ co-precipitation.

2.2. Sample pre-concentration

2.2.1. Co-precipitation techniques

For calcium phosphate co-precipitation, 1 mL (or 2 mL) of 1.3 mol L^{-1} $Ca(NO_3)_2$ and 2 mL (or 4 mL) of 0.65 mol L^{-1} KH_2PO_4 were added to the sample aliquot. In some cases, the sample was heated to 40–60 °C as indicated in Table 1. Conc. $NH_3\cdot H_2O$ was added until pH=9–10 to co-precipitate Pu and Np with $Ca_3(PO_4)_2$. For manganese dioxide co-precipitation, the sample pH was adjusted to 7–8 using conc. $NH_3\cdot H_2O$, and 5 mL of 0.2 mol L^{-1} $KMnO_4$ solution was slowly added while stirring. 1–2 mL of 25% $NH_3\cdot H_2O$ was then added to adjust the pH to 9–10 and the sample was stirred for 10 min to allow for the complete uptake of Pu and Np onto the formed MnO_2. For iron hydroxide co-precipitation, the sample aliquot was boiled on a hotplate at 200 °C for 2 h and then stored for 5 d. 1 mL of 3 mol L^{-1} $FeCl_3$ solution was added and conc. $NH_3\cdot H_2O$ was added to adjust the pH to 8–9.

After forming the desired precipitate, each sample was centrifuged at 4000 rpm for 10 min, the supernatant was discarded, and the precipitate was then dry ashed or wet digested to further decompose the organic matter contained. In the dry ashing approach, the precipitate was transferred to a beaker with water and heated on a hotplate to dryness and then ashed in a muffle oven at 550 °C overnight. Due to the difficulties in dissolving the MnO_2 residue after ashing, the dry ashing operation was not applied to the sample from MnO_2 co-precipitation.

Table 1
Effect of different experimental parameters on the analytical performance using $Ca_3(PO_4)_2$ co-precipitation.

Group number	Experimental conditions				Chemical yield of ^{242}Pu (%)	Chemical yield of ^{237}Np (%)
	Co-precipitation temperature (°C)	Amount of precipitation reagent	Organic matter decomposition	Valence adjustment reagents		
1-1[a]	40	1.3 mM $Ca(NO_3)_2$+1.3 mM KH_2PO_4	Dry ashing	Ascorbic acid/conc. HNO_3	84.7 ± 5.7	80.9 ± 10.7
1-2	40	1.3 mM $Ca(NO_3)_2$+1.3 mM KH_2PO_4	Acid digestion	Ascorbic acid/conc. HNO_3	35.7 ± 9.5	50.3 ± 18.2
1-3	40	1.3 mM $Ca(NO_3)_2$+1.3 mM KH_2PO_4	No treatment	Ascorbic acid/conc. HNO_3	17.7 ± 3.5	30.1 ± 16.4
2-1	25	3.9 mM $Ca(NO_3)_2$+3.9 mM KH_2PO_4	Acid digestion	Fe/$K_2S_2O_5$/conc. HNO_3	37.6 ± 1.3	21.8 ± 16.1
2-2	40	3.9 mM $Ca(NO_3)_2$+3.9 mM KH_2PO_4	Acid digestion	Fe/$K_2S_2O_5$/conc. HNO_3	46.8 ± 4.1	8.3 ± 5.4
2-3[a]	60	3.9 mM $Ca(NO_3)_2$+3.9 mM KH_2PO_4	Acid digestion	Fe/$K_2S_2O_5$/conc. HNO_3	44.2 ± 17.9	35.8 ± 8.7

[a] Results of three replicates, other experiments were done in duplicate.

In the wet digestion approach, 20 mL of aqua regia was added to each precipitate (a few drops of 30% H_2O_2 were added to the MnO_2 precipitate to prompt the dissolution). The sample was digested and evaporated to dryness on a hotplate at 200 °C with the occasional addition of 0.5 mL of 30% H_2O_2 up to 10 times. The residue was dissolved with 5 mL of conc. HCl and then the sample was diluted to 200 mL with water prior to valence adjustment and chromatographic separation as described later.

2.2.2. Evaporation technique

The sample aliquot was evaporated to dryness on a hot-plate at 200 °C and the residue was ashed in a muffle oven at 550 °C overnight. 40 mL of aqua regia was added to the residue to leach Pu and Np at 150 °C for 2 h and the leachate was filtered through a GF/A filter paper (Whatman International Ltd, Maidstone, UK) into a centrifuge tube. 1 mL of 0.5 g/ml $FeCl_3$ was added followed by addition of conc. $NH_3 \cdot H_2O$ to pH 8–9 to co-precipitate Pu and Np. The supernatant was discarded after centrifugation and the precipitate was dissolved with 20 mL of 0.2 mol L^{-1} HCl. The sample was then subject to valence adjustment and chromatographic separation.

2.3. Valence adjustment

For the valence adjustment, 300 mg of $K_2S_2O_5$ (or 300 mg of ascorbic acid) was added to the preconcentrated samples solution, which was then stirred for 20 min to reduce Np and Pu to Np(IV) and Pu(III), respectively. 6 mol L^{-1} NaOH was added to adjust the pH to 9–10 and the sample was centrifuged at 4000 rpm for 10 min. After discarding the supernatant, the precipitate was dissolved with 5 mL of 65% HNO_3. To samples from $Ca_2(PO_4)_3$ and MnO_2 co-precipitation, 1 mL of 2 mol L^{-1} of $Al(NO_3)_3$ was added to complex interfering sulfate and phosphate. The sample solution was finally adjusted to 4-5 mol L^{-1} HNO_3 for column separation (sample volumes were 15–30 mL).

2.4. Automated column separation

Miniaturized extraction chromatographic separation using renewable sorptive columns was performed within a lab-on-valve bead injection (LOV-BI) platform [32]. The flow system was composed of a multi-syringe buret (BU4S; Crison Instruments, Barcelona, Spain), and a polymethylmethacrylate LOV conduit encompassing 7 integrated micro-channels of 1.2 mm i.d./14.0 mm length and a large-sized channel of 4.0 mm i.d./14.0 mm length (to prevent valve clogging) to which a methacrylate column (5 mm i.d./50 mm length) is nested for containing of beads (see Fig. 1). There was also one external three-way solenoid valve (SV, N-Research) connected to the bottom end of the external column to divert at will (on: to eluate collector; off: to waste) and one external pinch valve (PV, PK-0305-NC, Takasago Electric, Inc., Nagoya, Japan) connected to the side port of the column assisting in the replenishment and withdrawal of beads (on: beads to waste; off: trap the inside of the column).

The automated column separation method in the LOV-BI format was composed of the following steps: 1) automatic packing of the column with ca. 300 mg of TEVA resin; 2) preconditioning the TEVA column with 20 mL of 4 mol L^{-1} HNO_3; 3) loading the sample solution (15–30 mL) onto the column; 4) rinsing the column with 20 mL of 1 mol L^{-1} HNO_3 followed by 10 mL of 9 mol L^{-1} HCl; 5) eluting Np and Pu with 20 mL of 0.025 mol L^{-1} HCl; and 6) automatic removal of TEVA beads and cleaning the flow system and the inlet tubing for sample loading with 10 mL of water. The flow rates for sample loading, column washing and plutonium elution were all fixed to 1.0 mL min^{-1}. Each eluate was evaporated to dryness and re-dissolved with 0.5 mol L^{-1} HNO_3 to 5 mL for ICP-MS detection.

2.5. ICP-MS detection

The detection of ^{237}Np, ^{239}Pu and ^{242}Pu (also ^{238}U and ^{232}Th in some cases to check the decontamination of U and Th) with ICP-MS (X Series^{II}, Thermo Fisher Scientific, Waltham, MA) was performed after the addition of In ($InCl_3$) as internal standard to a final concentration of 1 μg L^{-1}. The ICP-MS instrument operated under hot plasma conditions was equipped with a concentric plastic nebulizer, an impact bead spray chamber and an Xt-skimmer cone. The detailed operational condition of the ICP-MS instrument has been reported elsewhere [33]. ^{242}Pu was used as a standard for the quantification of both ^{239}Pu and ^{242}Pu. The detection limits calculated as three times of the standard deviation (3σ) of the processing blank were 1.0–1.5 pg L^{-1} for ^{237}Np, ^{239}Pu and ^{242}Pu. A least-squares regression line was used for quantification of Np and Pu in the range of 0.01–100 ng L^{-1}. Prior to detection, the instrumental parameters were adjusted for Np and Pu using 4.2 ng L^{-1} of ^{237}Np and 3.9 ng L^{-1} of ^{242}Pu solutions for optimal detection efficiency. Typical sensitivities of Np and Pu ranged from 1×10^5 to 5×10^5 cps per μg L^{-1}.

2.6. Decomposition of organic matter in urine

0.2 L urine spiked with ^{55}Fe (1–50 Bq), ^{152}Eu (1–5 kBq) or ^{239}Np (1–5 Bq) was utilized. After acidifying the sample with 10 mL of conc. HNO_3, an oxidizing reagent (1 g $K_2S_2O_8$, 10 mL of 30% H_2O_2 (added drop by drop), 10 mL of 14% NaClO or 1 g of $NaNO_2$) was added. The sample was then boiled on a hotplate at 200 °C for 1–14 h depending on the experimental conditions. After cooling down, 1 mL of 3 mol L^{-1} $FeCl_3$ solution was added followed by the addition of conc. $NH_3 \cdot H_2O$ to adjust the pH to 8–9. The precipitate after centrifugation was dissolved with 1 mL of conc. HCl and then diluted to 10 mL with water for ^{55}Fe, ^{152}Eu or ^{239}Np measurement.

2.7. Radiometric detection

^{59}Fe or ^{55}Fe was utilized as a radioactive tracer of iron to investigate the precipitation behavior of $Fe(OH)_3$ in urine sample. ^{59}Fe was detected using an NaI gamma detector (Canberra 20, Canberra, USA) by measuring its gamma rays of 1099 and 1291 keV while ^{55}Fe was detected with a liquid scintillation counter (Quantulus™ 1220, PerkinElmer Inc.) by counting its auger electrons after addition of 10 mL of scintillation cocktail (Ultima Gold LLT, PerkinElmer Inc.). ^{152}Eu and ^{239}Np were also measured using the NaI gamma detector (Canberra, USA) by counting their gamma rays of 89.8 keV and 106 keV, respectively.

3. Results and discussion

3.1. Pre-concentration techniques

Four commonly used techniques for pre-concentration of Pu and Np from large volume (1 L) of urine samples, including $Ca_3(PO_4)_2$, $Fe(OH)_3$ and MnO_2 co-precipitation and evaporation, were investigated in this work to explore the characteristics of each technique. The assessment of the coherence of Pu and Np separation (indicated by the agreement between the chemical yields of Pu and Np) was meanwhile performed aiming to exploit ^{242}Pu as a non-isotopic tracer for Np determination, since suitable Np isotopes (e.g., ^{239}Np, ^{235}Np or ^{236}Np) are not easily available to most researchers [30,31]. After discussing the analytical results based on this work, analytical performances of some other commonly used pre-concentration techniques reported in the literature (e.g., $BiPO_4$ and HTiO co-precipitation) are also illustrated so as to give an overview of the applicability of each technique and to pinpoint the challenge behind method development and application to Pu and Np urinalysis.

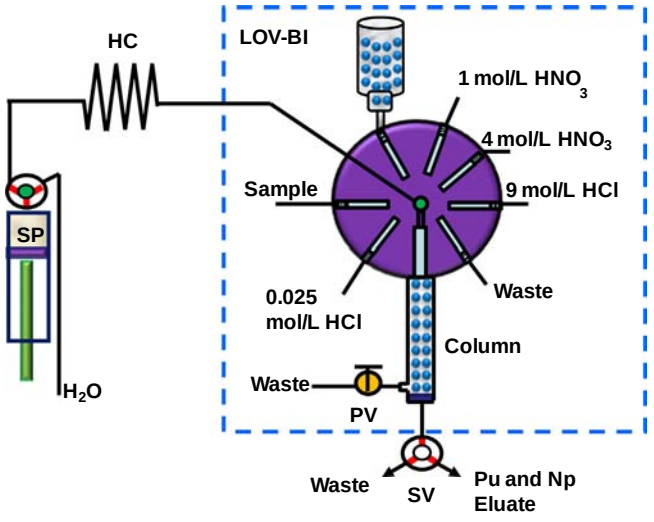

Fig. 1. Lab-on-valve bead injection system for automatic extraction chromatographic separation of Pu and Np in urine (HC: holding coil; PV: pinch valve; SV: solenoid valve).

3.1.1. Calcium phosphate co-precipitation

In the protocol using $Ca_3(PO_4)_2$ co-precipitation, different parameters including the co-precipitation temperature, the amount of precipitation reagent and technique for organic matter decomposition were studied in detail. The overall analytical results (Table 1) show that the key factor influencing the analytical performance is the procedure used to decompose organic matter after co-precipitation. Dry ashing is proven to afford satisfactory and coherent chemical yields (ca. 80%) of Pu and Np (Group 1-1, Table 1). Acid digestion using 30% H_2O_2 and conc. HNO_3 is not sufficient to completely decompose the organic matter, which possibly introduces competitive adsorption with Pu and Np onto TEVA column and gives rise to notable losses (ca. 30–50%) of Pu and Np during the sample loading sequence (Fig. 2 and Group 1-2, Table 1). The existence of organic matter in the sample solution dramatically deteriorated the analytical performance (Group 1-3, Table 1, no sample treatment). Decreased Pu and Np chemical yields (Groups 2-1 to 2-3, Table 1) were observed when employing threefold higher amount of precipitant, which might be attributed to the deteriorated separation capacity of TEVA column by the undue addition of phosphate [34] and incomplete decomposition of organic substances with acid digestion. The operational temperature for $Ca_3(PO_4)_2$ co-precipitation was selected to be 40 °C as per the plutonium results under different co-precipitation temperatures (Groups 2-1 to 2-3, Table 1) and other works reported in the literature [17].

Because tetravelent Pu and Np have the highest distribution factors onto TEVA column, thus Pu(IV) and Np(IV) valence adjustment needs to be performed prior to the chromatographic separation. For this purpose, a two-step valence adjustment operation was exploited in this work, wherein Pu and Np were first reduced to Pu(III) and Np(IV) with certain reducing reagent (as discussed below), respectively, and then Pu(III) was oxidized to Pu(IV) with the addition of a suitable oxidizer (e.g., conc. HNO_3 or $NaNO_2$)

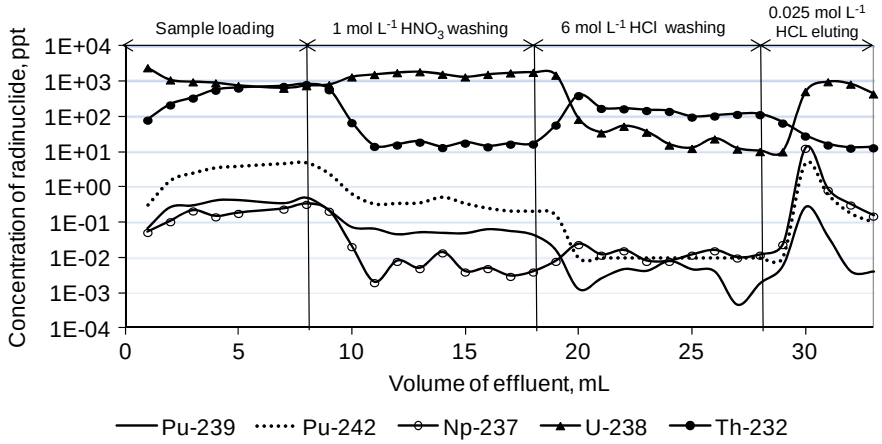

Fig. 2. Separation behavior of ^{239}Pu, ^{242}Pu, ^{237}Np, ^{238}U and ^{232}Th during TEVA chromatographic separation (sample volume: 1 L urine; sample pre-concentration protocol: $Ca_3(PO_4)_2$ co-precipitation; organic matter decomposition technique: acid digestion).

while Np(IV) was kept stable. Between the two steps of valence adjustment, $Fe(OH)_2$ co-precipitation was always performed in our operation in order to control the sample volume and further remove matrix elements. In the first valence adjustment step, with the addition of strong reducing reagents, the high valence states of Np (IV, V, VI) could possibly be reduced to Np(III) or Np (IV), and Pu(IV, V, VI)) to Pu(III) in low acidic media. Whereas in the presence of Fe(III), Np(III) would be oxidized to Np(IV) immediately. In fact, the Fe(III)/Fe(II) pair behaves in solution as a redox buffer to preserve Np(IV) from being reduced to Np(III) or oxidized to Np(V, VI). Whenever conc. HNO_3 or $NaNO_2$ is added to the sample after the $Fe(OH)_2$ co-precipitation, Pu(III) is oxidized to Pu(IV) in a few minutes. Np(IV) can be stabilized in a relatively high concentration of HNO_3 medium (e.g., 4–8 mol/L), because of the similar E_0 between HNO_3/N_2O_4 ($E_0 = +0.79$) and Np(V)/Np(IV) ($E_0 = +0.789$), and even though HNO_3 can oxidize Np(IV) to Np(V, VI), the reaction rate would be slow.

Based on our previous experience, $K_2S_2O_5$ was initially selected as reducing regent for obtaining Pu(III) and Np(IV); however, difficulties were encountered in the dissolution of the $Fe(OH)_2$ co-precipitate, which might be due to the co-existence of sulfur compounds and calcium. To tackle this problem, experiments were carried out so as to employ $NH_2OH \cdot HCl$ or ascorbic acid instead of $K_2S_2O_5$. Experimental observations indicate that ascorbic acid is more efficient than $NH_2OH \cdot HCl$ (results are not shown here). Insufficient reducing ability of $NH_2OH \cdot HCl$ in our investigation might be due to the high acidity (0.5–1 mol/L HCl) of the sample solution which hampers the dissociation of $NH_2OH \cdot HCl$. Therefore, a redox pair of ascorbic acid–conc. HNO_3 was selected for valence adjustment of Pu(IV) and Np(IV) throughout the work.

3.1.2. Iron hydroxide co-precipitation

In contrast to the calcium phosphate co-precipitation method, both acid digestion and dry ashing work equally well for organic matter decomposition after $Fe(OH)_3$ co-precipitation (Groups 2-1 and 2-2, Table 2). This might be attributed to the decomposition of organic matter prompted by iron derived Fenton's reaction as discussed elsewhere [31]. However, with $Fe(OH)_3$ co-precipitation, satisfactory chemical yields ($> 85\%$) could only be obtained when aging the urine sample for 5 d prior to co-precipitation. Although this phenomenon makes the method unattractive especially for emergency situations, it is scientifically interesting to further investigate the potential interaction of Fe with organic matter contained in the urine. Additional experiments using urine spiked with ^{59}Fe (a gamma emitter) showed that the precipitation efficiency of Fe itself is very sensitive to the urine characteristics (Fig. 3). Unlike seawater, the chemical yields of ^{59}Fe obtained for all urine samples did not demonstrate consistent correlation with the iron amount but vary significantly with the sample origin and matrix properties (fresh or aged, individual or mixed, diet and gender of the volunteer). Interestingly, both in the fresh urine 1 and 2, and aged urine 3, the lowest chemical yield of ^{59}Fe occurred when the Fe concentration was 0.9 mol L^{-1} and nearly no precipitate was obtained under this experimental condition. We were also aware that when the diet contained more dairy products, better chemical yields of ^{59}Fe were achieved as a consequence of the excretion of Ca in urine thus forming of calcium hydroxide under co-precipitation conditions. This is corroborated by the improved chemical yields of ^{242}Pu when combining $Ca(OH)_2$–$Fe(OH)_3$ co-precipitation (Group 4, Table 2) compared to solely $Fe(OH)_3$ co-precipitation (Group 2-3, Table 2) for sample pre-concentration. However, $Ca(OH)_2$–$Fe(OH)_3$ co-precipitation is not applicable for ^{237}Np determination using ^{242}Pu as a tracer because the two radionuclides behave differently from each other.

3.1.3. Other pre-concentration techniques

Other two sample pre-concentration protocols including MnO_2 co-precipitation and direct evaporation were performed in this work. The results (Table 2) show that both MnO_2 co-precipitation and evaporation could provide satisfactory chemical yields (75–90%) for Pu and Np. MnO_2 co-precipitation is the most rapid protocol among overall co-precipitation methods investigated in this work, lasting 6 h per sample (Group 3, Table 2). Nevertheless, traces of Mn were in some instances detected in the Pu and Np extraction chromatographic eluate which might pose some detection problems, such as noisy background in both alpha spectrometry and sensitive AMS measurements. Compared to MnO_2 co-precipitation, evaporation is relatively simple and straightforward, but somehow time-consuming especially when processing large volume samples (1.5 d/sample). But the outstanding advantages of evaporation are the good repeatability and robustness because the chemical yields are not prone to be influenced by the variation of urine matrix content.

In previously published papers dealing with Pu and Np urine assays, $Ca_3(PO_4)_2$ co-precipitation has been the most often used pre-concentration technique but mostly for smaller urine volumes (100–200 mL) [24,35,36]. The co-precipitation with $BiPO_4$ was a specific method performed under relatively low pH (0.3–2) [6]. Although chemical yields for Np using this technique have been reported higher than 90% for 1 L urine, evaporation was performed prior to the $BiPO_4$ co-precipitation, which is rather time-consuming [6]. An HTiO co-precipitation has also been exploited for Pu urine analysis but with chemical yields for Pu ranging within 50–65% for 200 mL of urine [26].

3.1.4. Applicability of the different pre-concentration techniques

In view of previously published Pu and Np urinalysis methods, it is worth noting that nearly all of them were developed based on the use of artificially spiked urine samples due to the limited availability of urine samples from Pu and Np exposed persons [4,6–8,13,15–19,23–26,35–38]. Under these circumstances, it can be assumed that

Table 2
Comparison of the analytical performances among different sample pre-concentration procedures.

Group no.	Pre-concentration technique	Organic matter decomposition	Valence adjustment reagents	Operational time	Chemical yield of ^{242}Pu	Chemical yield of ^{237}Np
1	$Ca_3(PO_4)_2$ co-precipitation	Dry ashing	Ascorbic acid/conc. HNO_3	13 h	84.7 ± 5.7	80.9 ± 10.7
2-1	$Fe(OH)_3$ co-precipitation	Dry ashing	$Fe/K_2S_2O_5$/conc. HNO_3	6 d	84.3 ± 15.6	73.3 ± 33.0
2-2		Acid digestion		5.5 d	80.3 ± 9.9	77.9 ± 10.9
2-3		Acid digestion		6 h	51.1 ± 0.2	51.3 ± 8.8
3	MnO_2 co-precipitation	Acid digestion	$Fe/K_2S_2O_5$/conc. HNO_3	6 h	88.7 ± 11.6	94.2 ± 2.0
4	$Ca(OH)_2/Fe(OH)_3$ co-precipitation	Acid digestion	Ascorbic acid/conc. HNO_3	6 h	87.3 ± 6.6	51.2 ± 1.6
5	Evaporation	Dry ashing + acid leaching	$Fe/K_2S_2O_5$/conc. HNO_3	1.5 d	75.5 ± 7.6	81.1 ± 8.1

All values are the average of three replicates ± standard deviation.

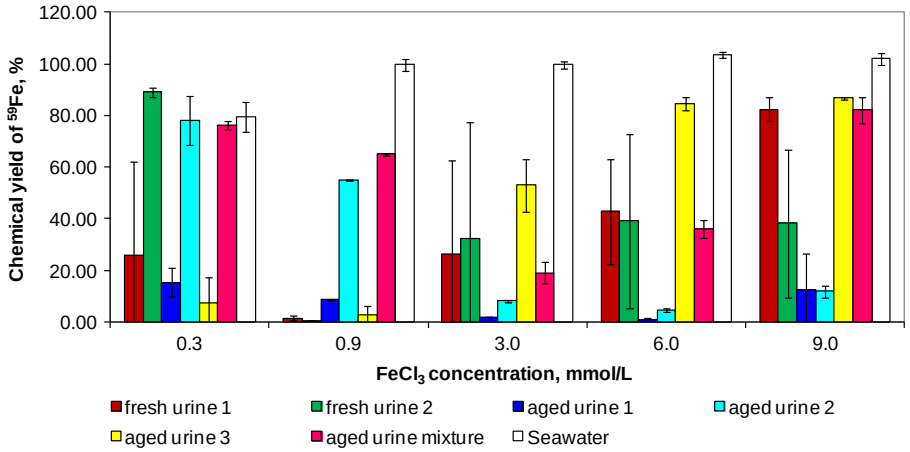

Fig. 3. Variation of chemical yields of ^{59}Fe in $Fe(OH)_3$ co-precipitation with precipitant concentration and urine properties (sample volume: 0.2 L; fresh and aged urine 1: from female volunteer 1; fresh and aged urine 2: from female volunteer 2; aged urine 3: from male volunteer 3, aged mixture: from 15 volunteers including 5 males and 10 females; the error bars refer to the standard deviation of two replicates).

Table 3
Variation of chemical yields of ^{55}Fe, ^{152}Eu and ^{239}Np using $Fe(OH)_3$ co-precipitation with different treatment approaches for decomposing organic matter in urine.

Group no.	Sample volume (L)	Treatment method	Chemical yield (%)		
			^{55}Fe	^{152}Eu	^{239}Np
1-1	0.2	No pre-concentration	82.1 ± 25.5	98.5 ± 3.2	63.9 ± 9.6
1-2	Supernatant from 1-1	No pre-concentration	–	–	5.0 ± 0.8
2-1	0.2	1 g of $K_2S_2O_8$, 200 °C, 1 h	98.6 ± 16.2	101.6 ± 0.5	101.3 ± 4.2
2-2	0.2	1 g of $K_2S_2O_8$, 200 °C, 2 h	–	101.1 ± 2.9	–
2-3	0.2	1 g of $K_2S_2O_8$, 200 °C, 3 h	–	100.9 ± 1.3	95.7 ± 9.6
2-4	0.2	1 g of $K_2S_2O_8$, 200 °C, 14 h	–	94.2 ± 0.2	–
3-1	0.2	10 mL of 30% H_2O_2, 200 °C, 2 h	96.9 ± 4.4	111.9 ± 45.0	93.4 ± 9.3
3-2	1.0	10 mL of 30% H_2O_2, 200 °C, 2 h	–	–	13.0 ± 1.1
4-1	0.2	10 mL of 14% NaClO, 200 °C, 2 h	–	–	88.8 ± 8.9
5-1	0.2	1 g of $NaNO_2$, 200 °C, 2 h	–	–	77.4 ± 7.7

All values are the average of three replicates ± standard deviation.
'–'symbols refer to experiment that were not performed under these conditions.

all the co-precipitation techniques employed immediately after sample acidification might only be sufficient for scavenging the spiked Pu and/or Np occurring as free ions but might not be able to quantitatively pre-concentrate the endogenous Np and/or Pu associated with urine organic substances. Consequently, radionuclide underestimation might be expected in urinalysis methods whenever the release of endogenous Pu and Np bound to organic matter is not complete or the species of endogenous Pu and Np are not identical to the spiked chemical yield tracer. To this point, the evaporation method in combination with dry ashing should be able to ensure the isotopic equilibrium between the endogenous Pu and Np and the spiked chemical yield tracer, providing a valuable tool to assess the reliability of other pre-concentration methods. But in most cases, evaporation is very time-consuming thus not suitable for rapid processing large volume of urine samples as per requirements in emergency situations. Eventually, aiming at applying expeditious co-precipitation techniques to achieve rapid and reliable Pu and Np urinalysis, the most challenging issue turns to be the development of protocols enabling the quantitative release of organically associated Pu and Np.

3.2. *Release of Pu and Np from organic associations in urine*

3.2.1. *Protocols for organic matter decomposition*

To tackle the above mentioned challenge, several organic matter decomposition protocols using different oxidizing reagents have been investigated in this work. Considering that the addition of oxidizing reagents might prevent the formation of MnO_2 (based on the reduction of $KMnO_4$) and the easy availability of the Fe radioactive tracer (^{55}Fe) for monitoring the co-precipitation efficiency, $Fe(OH)_3$ co-precipitation was selected instead for this investigation. To compare the co-precipitation behavior of different group elements, ^{152}Eu and ^{239}Np were also utilized in some cases as tracers. The results (Table 3) indicate that the co-precipitation efficiency of ^{239}Np is very sensitive to the content of organic matter, and the average chemical yield of ^{55}Fe is also somewhat lower but with high standard deviation when organic matter is not decomposed (Group 1-1, Table 3). Differently, ^{152}Eu is immune to the content of organic matter and shows quantitative co-precipitation efficiency under all investigated conditions. Considering the chemical yields obtained for ^{239}Np and ^{55}Fe (Group 2-1, Table 3), $K_2S_2O_8$ treatment was presumably deemed as the most effective protocol to enhance the co-precipitation efficiency of Pu and Np. To evaluate the effectiveness of $K_2S_2O_8$ treatment for liberating Pu and Np bound to organic urine ingredients, samples were prepared by spiking freshly collected urine with ^{239}Pu and ^{237}Np and keeping the samples for equilibration with the matrix over different time frames because of unavailability of urine containing endogenous Pu and Np. Prior to sample processing (organic matter decomposition), ^{242}Pu was spiked as a tracer to compare the separation behavior of ^{239}Pu and ^{237}Np with freshly added ^{242}Pu. Experimental results (Fig. 4) indicate that the chemical yields of ^{239}Pu and ^{237}Np decrease with the increase of the equilibration time, most likely as a consequence of (1) the

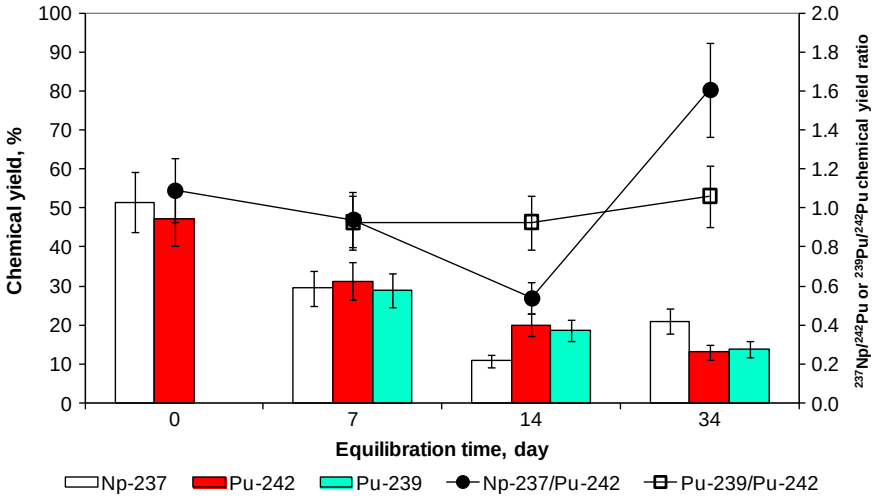

Fig. 4. The variation of chemical yields of ^{237}Np, ^{239}Pu and freshly added ^{242}Pu (tracer) with the equilibration time in urine (sample volume: 0.2 L urine; protocol used for liberating Pu and Np from organic species: $K_2S_2O_8$ treatment).

inefficiency of the oxidant for releasing the radionuclides when the concentrations of organically associated ^{239}Pu and ^{237}Np are increased, and/or (2) the pronounced matrix effect of aged urine samples in the further purification protocols. The first assumption should be excluded for Pu as per the good agreement between chemical yields of ^{239}Pu and the freshly spiked ^{242}Pu. The presence of sulfur compounds (generated by $S_2O_8^{2-}$) in the pre-treated sample solution seriously deteriorated the analytical performance of TEVA extraction chromatographic separations [34], giving chemical yields of $<50\%$ for both Pu and Np (Fig. 4). The chemical yield ratios of ^{237}Np/^{242}Pu under the investigated experimental conditions vary from 0.5 to 1.6, without exhibiting any notable correlation with the equilibrium time. The reason for this phenomena might be the consequence of different bounding behavior between Np and Pu with urine organic/inorganic matrix and need to be studied further.

3.2.2. Possible solutions and perspectives

With regards to decreasing potential interfering effects by the addition of the oxidizing reagent (e.g., $K_2S_2O_8$) during the decomposition of organic matter, the chromatographic separation procedure might need further optimization or complexing agents (e.g., $Al(NO_3)_3$) should be added to mask the competitive adsorption of sulfur compounds (e.g., SO_4^{2-}). In fact, a salt-free oxidant (e.g., H_2O_2) might be used as a possible substitute of $K_2S_2O_8$ based on our preliminary results in Group 3-1 (Table 3) without modifying the chromatographic separation. Efforts could also be devoted to develop other advanced organic matter decomposition procedures, such as photolysis, ultrasound-based or electrolytic oxidation.

4. Conclusions

In this work, several analytical methods for reliable determination of Np and Pu in large volumes of urine samples applying different sample pre-concentration techniques ($Ca_3(PO_4)_2$, $Fe(OH)_3$ and MnO_2 co-precipitation and evaporation) were undertaken and their analytical performance in terms of chemical yields, turnaround times and coherence of Np and Pu separation behavior was systematically compared. The key factor affecting the analytical performance in $Ca_3(PO_4)_2$ co-precipitation is the organic matter decomposition method. Dry ashing is proven to be more effective than wet digestion and afforded equally satisfactory chemical yields for both Pu and Np. $Fe(OH)_3$ co-precipitation is very sensitive to the variation of sample matrix and further improvement especially for emergency situations is still needed as per the requirement of long-term (5 d) aging prior to the co-precipitation step. MnO_2 co-precipitation is the most rapid procedure among the co-precipitation methods investigated in this work. However, awareness should be paid that traces of Mn might occasionally remain in the Pu and Np eluate which might pose noisy background in alpha spectrometry or AMS measurement. The evaporation technique endows urinalysis methods with good repeatability and robustness despite the fact that it is somewhat time-consuming when processing large volume samples. The investigation of the release of Pu and Np from organic urine components using different organic matter decomposition protocols indicates that even though the treatment using potassium persulfate is effective to provide relatively high $Fe(OH)_3$ co-precipitation efficiency, the analytical performance of the TEVA chromatographic separation is deteriorated (chemical yields below 50%).

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