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A technique for the determination of ruthenium stable isotopes in urine samples

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Proton activation analysis has been recently applied for the determination of stable isotopes of trace metals in blood plasma samples taken from volunteers during tracer kinetic studies. The very low values of intestinal uptake for some elements, like ruthenium, make the kinetics of the excretion crucial for interpreting the bioassay data. Therefore, a procedure has been developed to process urine samples in order to have proper targets for the activation with protons. Preliminary tests with Ru-doped samples, conducted using the MC-40 Cyclotron at JRC Ispra, has confirmed the feasibility of the method. The minimum detectable concentrations, in the current operating conditions, are 16 ng $^{99}Ru{\cdot}ml^{-1}$ and 0.5 ng $^{101}Ru{\cdot}ml^{-1}$.

Introduction

Tracer kinetic studies conducted using stable isotopes as tracers represent an ethically justifiable methodology to investigate the uptake, bio-distribution and excretion of trace metals and of their radionuclides in healthy volunteers without exposing them to undue risks. In recent years, a series of investigations has been performed in order to collect biokinetic information for elements like molybdenum, ruthenium, and zirconium,[1–3] for which data were up to now limited basically to animal experiments. This information can be obviously extended to the radionuclides of these elements, and enables the development of more realistic compartmental models[4,5] that can be used for more reliable estimates of the internal dose coefficients and for a correct interpretation of bioassay measurements in contaminated subjects.

The simultaneous administration of two different isotopes of the same element as tracers (the so called double tracer technique) requires sophisticated analytical techniques, like nuclear activation or mass spectrometry, for the identification and determination of both tracers in the same sample, usually a complex biological matrix. These techniques must be employed on external samples like body fluids, excreta, etc. Proton activation analysis has been applied for the determination of stable isotopes of iron,[6] molybdenum,[1] ruthenium,[7] and zirconium[5] in blood plasma samples taken from volunteers during the tracer kinetic studies. Thermal ionization mass spectrometry (TIMS), fast atom bombardment mass spectrometry (FABMS) and inductively coupled plasma mass spectrometry (ICPMS) have also been used for the measurements of iron,[8] calcium,[9] strontium[10] tellurium[11] and molybdenum[12,13] isotopes in blood and urine samples.

The very low values of intestinal intake for some of these elements, like ruthenium and zirconium, make the kinetics of excretion crucial for interpreting the bioassay data.[14] However, mass spectrometric techniques like TIMS and ICPMS are currently not accurate enough for single isotope determination of these elements, in a complex biological matrix. Moreover, the procedure used for the preparation of blood plasma samples for the activation experiments was not applicable to urine. The technique of activation analysis with charged particles has indeed the advantage of presenting a large number of reaction channels, thus allowing the simultaneous determination of different isotopes of the same element. However, it can be hardly used with liquid samples when high currents (some μA's) are used. The dissipation of the beam energy leads to dramatic temperature increase, with potential risks of evaporation, which could be particularly critical in the case of biological samples, so solid samples are preferred. Therefore, a procedure to process urine samples has been developed in order to produce proper solid targets for the activation with protons. Preliminary tests with Ru-doped samples have been conducted using the MC-40 Cyclotron at JRC Ispra to assess linearity, sensitivity and feasibility of the technique.

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Experimental

Reagents

Water desalination cartridge (Zefa-Laborservice, Harthausen, Germany) and MilliQ (Millipore) system (Membrapur, Bodenheim, Germany) were used for the deionization and further purification of water.

Nitric acid (HNO_3, 65%, suprapure grade, Merck, Darmstadt, Germany) purified by sub-boiling distillation in PTFE stills; HCl (30%, suprapure grade, Merck, Darmstadt, Germany); KOH (pellets, purest grade, Merck, Darmstadt, Germany), and KNO_3 (suprapure grade Merck, Darmstadt, Germany) were applied as reagents.

Urine

Urine samples were collected from healthy volunteers in polyethylene bottles (Sarstedt, Nümbrecht, Germany). Aliquots of 100 ml of urine were mixed with 5 ml of concentrated nitric acid and kept frozen at –20 °C until analysis.

Ruthenium stable isotopes

Ruthenium-99 and -101 metal powders were purchased (Chemotrade, Dusseldorf, Germany and Campro Scientific, Veenendaal, The Netherlands). Isotopic enrichments are shown in Table 1 and compared to the natural isotopic composition. Enriched ^{99}Ru and ^{101}Ru solutions were prepared weighing 10 mg of metal powder in a Ni crucible with an oxidizing alkaline mixture of about 1.4 g KOH and 0.14 g KNO_3, and heating for 45 minutes at 520 °C in a muffle furnace. The cooled melt was then dissolved at room temperature in 40 ml of HCl/HNO_3 mixture, and evaporated to dryness. Finally the residue was diluted in deionized water to the desired concentration.

Internal standard

Vanadium isotope: ^{51}V stock solution (Aldrich, Milwaukee, Wisconsin, USA) was used as internal standard.

Preparation of urine samples

Several methods were tested and compared in order to find the proper conditions for processing urine samples. These methods were based on the wet-chemical sample decomposition with nitric acid in closed quartz vessels, which is one of the most efficient methods for trace element analysis in biological samples, like urine, with low contamination levels from the container and decomposition reagents. In order to guarantee the complete decomposition of organic matter with nitric acid, temperatures up to 300 °C and high pressures of up to 80 bars are required. A pressurized microwave decomposition (PMD) system by Kürner Analysentechnik (Rosenheim, Germany) was used for the digestion of the samples.

The tested methods differed with regard to the amount of urine and/or reagents used, and to the duration of the various steps. As a result of the optimization process, following operational conditions were chosen.

Ten ml of urine was mixed with 4 ml of HNO_3 (sub-boiling distilled) in a teflon beaker. A known amount of either ^{99}Ru- or ^{101}Ru-enriched solution was added. Additionally, a known amount of ^{51}V, used as internal standard (reference isotope) was added. The teflon beaker was covered with a watch glass and the solution was simmered on a heated plate at 250 °C for about 0.5 hour. After the heating period, the watch glass was removed and the solution was evaporated to about 2 ml. This residue was taken and transferred into quartz glass vessels of the microwave digestion system. The teflon beaker was rinsed with fresh 2 ml of sub-boiled HNO_3, which was also transferred to the quartz glass vessel. The digestion program of the PMD system, which was connected to a cooling system, was run with following operating conditions: temperature 260 °C, pressure 80 bar, heating time 10 minutes, cooling time 10 minutes. After the digestion each solution was decanted in a 10 ml teflon beaker and evaporated to dryness in a controlled atmosphere at 140 °C. Then, 2 ml of deionized water was added and the samples were dried again. The dry residue, showing a whitish color, was then pressed in a moulder to form a self-supporting round tablet.

Sample irradiation and analysis

Each tablet was put into a proper water-cooled aluminum frame, with 1 mm cover in front of the tablet, and then mounted in an irradiation chamber, positioned on the beam line of the Scanditronix MC-40 Cyclotron at Joint Research Center of the European Community in Ispra (Italy). The reactions considered were those previously employed in the analysis of blood plasma samples, i.e., $^{99}Ru(p,n)^{99}Rh$ and $^{101}Ru(p,n)^{101m}Rh$ for the Ru isotopes and $^{51}V(p,n)^{51}Cr$ for the internal standard.[15] The characteristic physical parameters of the chosen reactions and of the radioactive products are summarized in Table 2. Each sample was irradiated singularly, with 19 MeV protons and a beam current of approx. 300 nA. Irradiation times ranged between 0.5 and 6 hours, according to the amount of the isotope in the sample.

Table 1. Isotopic composition (in atom%) of natural ruthenium and of the enriched powders used in this work

Powder	^{96}Ru	^{98}Ru	^{99}Ru	^{100}Ru	^{101}Ru	^{102}Ru	^{104}Ru
Natural composition	5.51	1.87	12.72	12.62	17.07	31.64	18.58
^{99}Ru enriched	0.12	0.12	97.69	0.74	0.48	0.58	0.27
^{101}Ru enriched	0.07	0.07	0.15	0.32	97.82	1.35	0.21

Table 2. Characteristics of the reactions used and of the reaction products[16]

Reaction	Threshold energy, MeV	Product half-life, days	Main gamma-emissions, keV	Abundance, %
^{99}Ru(p,n)^{99}Rh	2.9	16.1	528.2	38.0
			353.0	34.6
			89.7	33.4
^{101}Ru(p,n)^{101m}Rh	1.4	4.34	306.9	81
			545.1	4.3
^{51}V(p,n)^{51}Cr	1.6	27.7	320.1	10

At the end of the irradiation, after a proper cooling time, related to the decay of the short-lived radionuclides produced in the sample matrix, the irradiated sample was transferred into a clean plastic frame, and its gamma-spectrum was collected using a HP Ge detector (32% relative efficiency, 1.67 keV resolution at 1.33 MeV line of ^{60}Co) connected to a PC through a multichannel buffer card (EG&G Ortec, Model 916A MCB). ^{99}Rh, ^{101m}Rh and ^{51}Cr were identified through their gamma-emissions at 353.0 keV, 306.9 keV and 320.1 keV, respectively. In order to compare the results from different samples irradiated and measured under different experimental conditions, the intensities of the gamma lines were corrected in order to correspond to a standard situation with fixed irradiation, cooling and measurement times.

Results and discussion

The urine samples, prepared according to the procedure described above, proved to be perfectly suitable for the activation experiments. The water-cooled sample holder were able to prevent damage to the samples, that showed a brownish color due to the temperature increase but remained compact.

Figure 1a shows a comparison of the gamma-spectrum measured in a blank sample (pure urine, without addition of any of the enriched solutions of interest) with those collected in samples containing a known amount of ^{99}Ru (Fig. 1b) and of ^{101}Ru (Fig. 1c). The doped samples contained also a given amount of ^{51}V. Only the portions of the spectra relative to the energy of interests are shown. It can be clearly seen that the blank sample presented only one peak at the energy of 300 keV, corresponding to the emission of ^{67}Ga produced in the urine matrix through a (p,n) reaction on intrinsic Zn (^{67}Zn: 4.1% isotopic abundance). No interferences were seen in correspondence with the emissions at 306.9 keV (^{101m}Rh), 320 keV (^{51}Cr) and 353 keV (^{99}Rh). The interference observed in blood plasma at 306.5 keV,[7] and originating from ^{79}Kr produced on intrinsic bromine present in plasma, was not present in the urine samples. In view of this result, therefore, it was not required to introduce corrections in the evaluation of the gamma-line intensities, as it was done with blood plasma samples.

Figure 2 shows the decrease of the intensity of the gamma-emission at 306.9 keV of ^{101m}Rh in a sample containing approx. 5 μg ^{101}Ru measured at different waiting times after irradiation. The dashed line represents a mono-exponential fit to the experimental data. The half-life of ^{101m}Rh as derived from the fit is equal to 4.2±0.1 d, to be compared with the expected value of 4.34 d.[16] Analogously, the half-life of ^{99}Rh was estimated to be 19±6 d, to be compared with the expected value of 16 d.

The linearity of the technique was positively verified by irradiating samples containing increasing amounts of Ru, ranging from 0.1 μg to 7 μg. Finally, minimum detectable concentrations (MDC) were also estimated on the basis of the intensities of the gamma-emissions. Minimum detectable concentration for ^{101}Ru in the urine samples, in the operating conditions used (6-hour irradiation at 300 nA, 2.5-day waiting time, 16-hour measurements) is 0.5 ng·ml^{-1}. MDC for ^{99}Ru in the urine samples, in the current operating conditions (6-hour irradiation at 300 nA, 3-day waiting time, 20-hour measurements) is 16 ng·ml^{-1}. It must be taken into account that in these preliminary tests no optimization of the operational conditions with regard to MDC was performed. This is particularly the case for ^{99}Rh, as can also be seen from the relatively low peak at 353 keV in the spectrum presented in Fig. 1. Increasing the irradiation time up to 10 hours, the beam current up

to a few μA (which can be made possible by using a rotating multi-sample disc for simultaneous irradiation of several samples, like the ones previously used at JRC Ispra[17] and at the Paul Scherrer Institut)[7] and choosing proper cooling and measurement times, the MDC could be realistically improved down to 2 ng·ml^{-1} for ^{99}Ru and to 0.1 ng·ml^{-1} for ^{101}Ru.

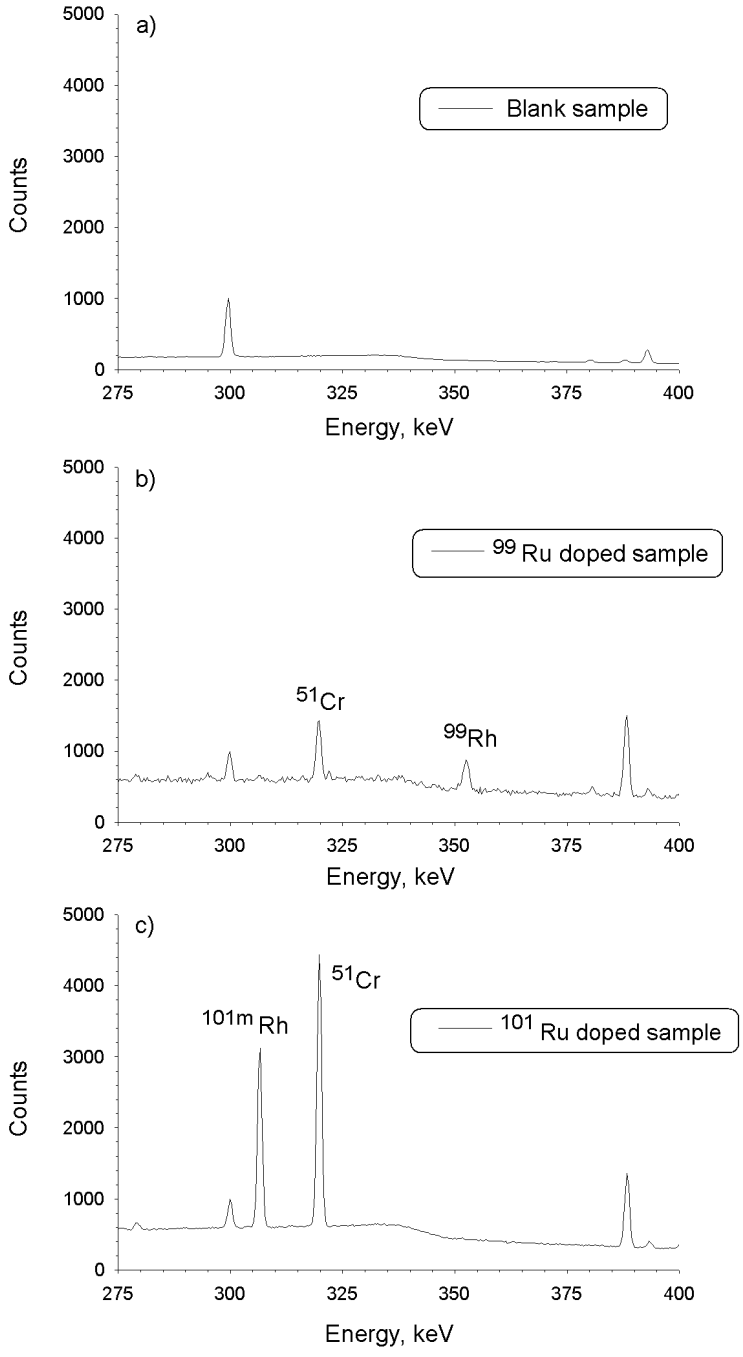

Fig. 1. Gamma-spectra after irradiation of a blank urine sample (a), of a urine sample containing ^{99}Ru and ^{51}V (b) and of a urine sample containing ^{101}Ru and ^{51}V (c)

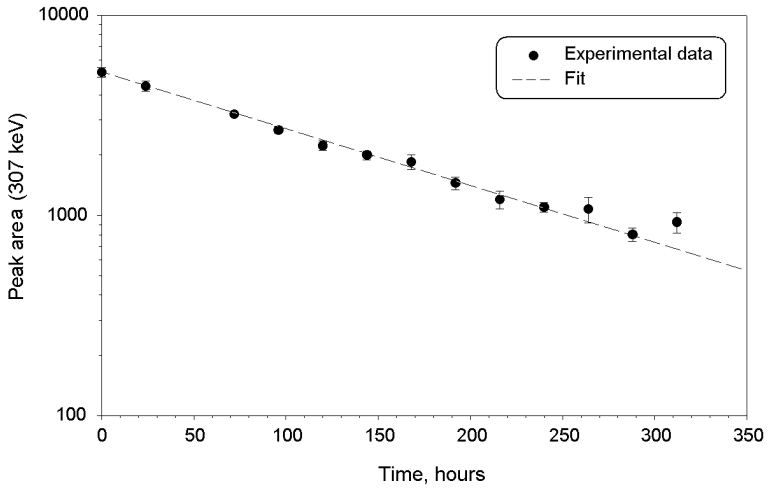

Fig. 2. Decay of the intensity of the gamma-line at 306.9 keV of ^{99}Rh with time. The dashed line is a mono-exponential fit to the measured data

Conclusions

A methodology has been developed for the preparation of urine samples for proton beam irradiation. This methodology is based on the wet-chemical sample decomposition with nitric acid in quartz vessels, with temperatures of 260 °C and pressures of 80 bar. Preliminary tests have been conducted to prove the feasibility of this procedure in the case of determination of the stable isotopes ^{99}Ru and ^{101}Ru by means of the reactions ^{99}Ru(p,n)^{99}Rh and ^{101}Ru(p,n)^{101m}Rh, respectively. These tests have included checks for interfering signals, control measurements of the half-lives of the activation products and verification of the linearity and sensitivity. The technique can now be applied for the analysis of urine samples collected in tracer kinetic studies, in order to properly characterize the excretion kinetics of Ru radionuclides and gain the information required for the definition of a reliable biokinetic model and for the correct interpretation of bioassay measurements.

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