A new method for separation of ^{97}Ru from irradiated by α-particles molybdenum for nuclear medicine

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A new method for separation of ^{97}Ru radionuclide from irradiated by α-particles molybdenum, which potentially could be applied in nuclear medicine, is proposed. Carrier-free ruthenium-97 was separated from macroamounts of molybdenum and trace amounts of technetium by extraction chromatography on a commercial DGA sorbent. The distribution of ^{97}Ru in the body organs of mice was studied.

Key words: ruthenium-97, extraction chromatography, *in vivo* mice model.

The success of cisplatin as a promising anticancer agent inspired researchers to pay careful attention to a possible role of group VIII metals as components of new medicines.[1,2] In addition, the searches for their radionuclides suitable for the design of radiopharmaceuticals were started. Ruthenium, which is capable of different-type complexations and possesses less toxic properties compared to platinum, is of special interest.[3] Moreover, ruthenium can bind to albumin, transferrin, and thiol-bearing biomolecules, which improves its bioavailability.[4]

Ruthenium-97 ($T_{1/2}$ = 2.9 days) has long been considered as a promising isotope for application in nuclear medicine,[5] where it can serves as a more efficient analog of ^{67}Ga, ^{99m}Tc, and ^{111}In.[6] Methods for its preparation from different targets by their irradiation with protons, neutrons, and helium nuclei in accordance with the reactions ^{96}Ru(n,γ)^{97}Ru,[7] ^{nat}Mo(^{3}He,n)^{97}Ru and ^{nat}Mo(α,n)^{97}Ru,[8] ^{99}Tc(p,3n)^{97}Ru,[9] and ^{nat}Rh(p,2p5n)^{97}Ru were proposed.[10] Also, the use of heavy ion bombardment of niobium and yttrium targets according to the reactions ^{93}Nb(^{7}Li,3n)^{97}Ru (see Ref. 11) and ^{89}Y(^{12}C,p3n)^{97}Ru (see Ref. 12) was studied. Despite a variety of approaches, optimum methods for the synthesis of ^{97}Ru have not been found until now. Irradiation of technetium-99 by protons or of rhodium targets by high-energy protons is considered as the most promising approach; however, the former requires targets made of rare and radioactive technetium and the latter requires high-power accelerators.[10] Irradiation of natural molybdenum targets is accompanied by the formation of a long-lived ^{103}Ru impurity, which is disadvantageous for medical application. However, this can be avoided if targets enriched by light molybdenum nuclei (^{94}Mo, ^{95}Mo) are used in order to exclude the reaction ^{100}Mo(α,n)^{103}Ru. Various methods for separation of ^{97}Ru from irradiated targets, for example, distillation of $^{97}RuO_4$, anion-exchange chromatography, and extraction,[12] have been described; however, these procedures are needed to be simplified and made more rapid.

In the present work, we propose a new original method for separation of ^{97}Ru from irradiated molybdenum by extraction chromatography and considered the *in vivo* distribution of its simple chemical form (chloride) on a murine model.

Experimental

Irradiation of targets and registering of γ-spectra. In the present work, two double-folded targets made of rectangular-shape molybdenum foil (60 and 70 mg, 99.9% purity) with di-

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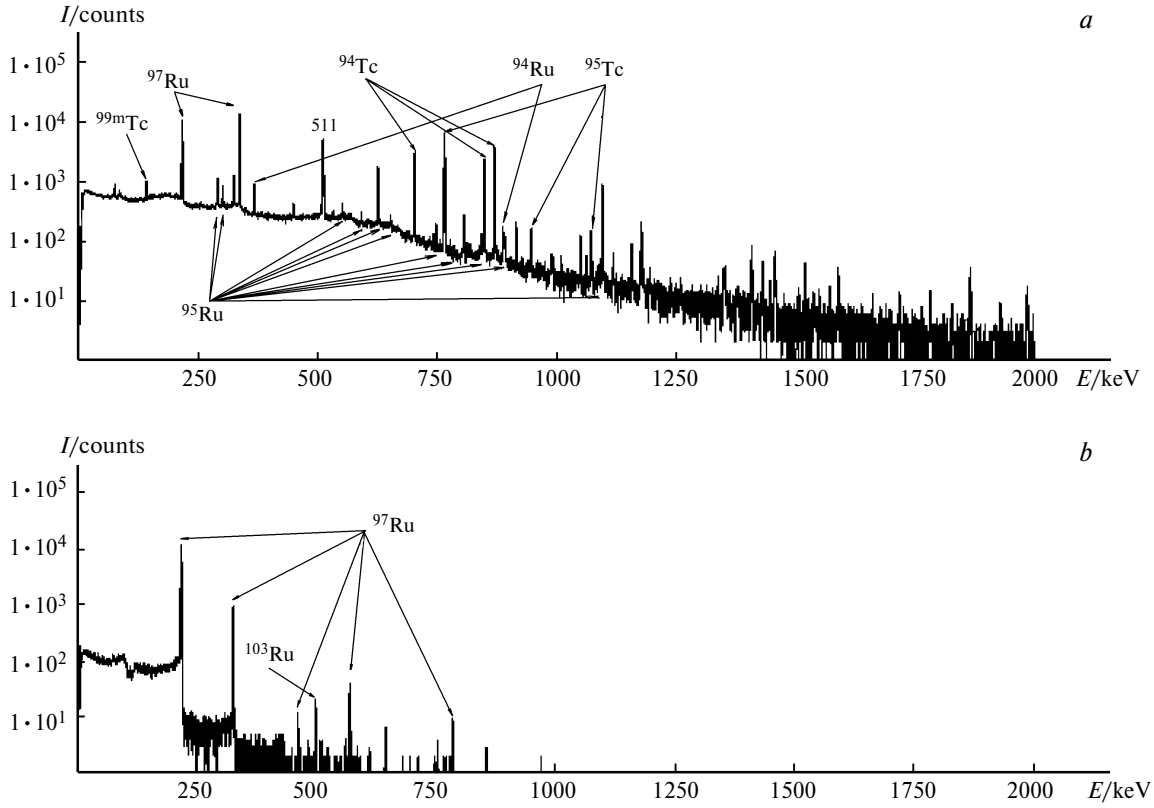

Fig. 1. γ-Spectra of the irradiated target 4 h after EOB (*a*) and fractions of the purified ruthenium for *in vivo* experiments after irradiation for 4 days (*b*).

mensions of 0.8×2 cm and a thickness of 50 μm were irradiated. The irradiation was performed with 30 MeV beams of α-particles at a current of 2.5 μA for 1.5 h. The targets were cooled for 4 h and their γ-spectra were registered to determine the radionuclide composition (Fig. 1, *a*). γ-Spectra were registered on a spectrometer equipped with a GR 3818 high-purity germanium detector (Canberra Inc., USA). The detector was calibrated using certified reference sources (^{22}Na, ^{60}Co, ^{241}Am, and ^{137}Cs) and a solution of ^{152}Eu with known activity.

Separation of ruthenium by extraction chromatography. Ruthenium isotopes were separated by extraction chromatography on commercial sorbents, DGA (based on *N*,*N*,*N*′,*N*′-tetrakis-2-ethylhexylglycolamide, 100—150 mesh) and TEVA (based on quaternary ammonium salts, 100—150 mesh) (Triskem, France). The sorbents were preliminary kept in 0.01 *M* HCl for at least 1 h. Foil pieces (4—20 mg) were cut from the targets and dissolved in 40% H_2O_2 (1—3 mL) with moderate heating. The solution was evaporated and the residue was dissolved in HCl (1, 2 or 3 mol L^{-1}, 200 μL). A polypropylene column with a volume of 3 mL, a length of 7 cm, and a diameter of 0.7 cm was packed with the sorbent and washed with HCl (1, 2 or 3 mol L^{-1}, 10 mL). The solution (1—3 mL) obtained upon dissolution of the target was applied onto the column. The eluate was collected portionwise (3 mL each) registering their γ-spectra to determine the contents of ruthenium and technetium.

Spectrophotometry. The possible oxidation state of ruthenium after extraction chromatography was estimated by spectrophotometry of ruthenium macroamounts on a Shimadzu UV-1280 spectrophotometer (Japan) using the starting solution of Ru^{IV} in HCl, which was applied onto a sorbent-packed column passing through this column solutions of the same media where ^{97}Ru was separated. To determine the content of molybdenum in the ^{97}Ru eluate, the fractions were studied by spectrophotometry. The search was performed for the peaks of MoO_4^{2-} and $Mo(OH)_6$ in a region of 219 nm.[13] For this purpose, aliquots (1.5 mL each) (3 *M* HCl) were sampled from each fraction after separation on the column and 4 *M* NaOH (1.5 mL) was added. The execution of measurements in the alkaline medium was due to the need for removing many forms of polymolybdate, a complex equilibrium of which exists in acidic media.

***in vivo* Experiments.** Animal experiments were performed in accordance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Prior to experiments, mice lived for a 12-h cycle under standard conditions. Laboratory white normal female mice with a body weight of 28—33 g were used. The ^{97}Ru eluate obtained after separation on DGA was evaporated to dryness and dissolved in distilled water with a slight amount of sodium carbonate being added until pH 7. Aliquots of this solution were administered intraperitoneally to mice. The activity of ^{97}Ru in the aliquot was ~1500 Bq. The animals were sacrificed after 15, 30, and 60 min (four mice in each time) by cerebral dislocation. The organs were extracted and additionally washed with water. Their radioactivity was measured on a γ-spectrometer by ^{97}Ru (the line at 215.7 keV).

Results and Discussion

Radionuclide composition of the irradiated molybdenum targets. Table 1 gives the composition of one of the targets (the activity was calculated at the end of bombardment (EOB)). The γ-spectrum is shown in Fig. 1, *a*. The earlier measurements display no peaks of ^{96}Tc and ^{103}Ru due to a very low activity; however, after irradiation for several days the above-mentioned peaks could be observed. Using a thin foil, we achieved a ^{97}Ru activity of ~60 kBq, which seems to be promising, since the nuclear medicine contemplates the use of thick targets. As noted above, the disadvantage is the formation of impurity amount of ruthenium-103, which can be avoided in medical experiments. In our study, the *in vivo* activity of ^{103}Ru was <5 Bq per mouse and the radiation exposure was insignificant.

Extraction chromatography on DGA and TEVA. For separation of ^{97}Ru upon *in vivo* experimentation, HCl is a preffered medium, which decreases the number of steps required upon extraction of the radionuclide for biological tests. Molybdenum, ruthenium, and technetium have a wide range of oxidation states. The use of hydrogen peroxide favors the formation of highest-oxidation-state anions of these elements. For the DGA resin, the distribution coefficients (K_d) of molybdenum and ruthenium in HCl are known;[14] however, the oxidation state of ruthenium in Ref. 14 was not determined. Molybdenum(VI) strongly binds to DGA at HCl concentrations higher than 2 mol L^{-1}. Under these conditions, technetium is also strongly retained by the sorbent. However, the K_d values for ruthenium are low and, therefore, ruthenium can be eluted using 3 *M* HCl without risk of Mo and Tc elution. The curve of elution with 3 *M* HCl are shown in Fig. 2. The γ-spectra showed the absence of Tc isotopes in ruthenium fractions. After 40 mL of the solution has been eluted,

Table 1. Radionuclide composition of the irradiated target

Nuclide	Main reaction	$T_{1/2}$	E_γ[a]/keV	A_{EOB}[b]/kBq
^{94}Tc	^{92}Mo(α,pn)	4.9 h	702.6 (99.6), 849.7 (95.7), 871.1 (100)	114±23
^{95}Tc	^{92}Mo(α,p)	20 h	765.1 (93.8), 947.7 (1.95), 1073.7 (3.74)	163±14
^{96}Tc	^{94}Mo(α,pn)	4.28 days	778.2 (100), 1127.0 (15.2)	0.5±0.1
^{99m}Tc	^{96}Mo(α,p)	6 h	140.5 (89)	3.7±0.2
^{94}Ru	^{92}Mo(α,2n)	52 min	366.9 (75), 891.7 (25)	201±33
^{95}Ru	^{92}Mo(α,n)	1.64 h	290.48 (4), 336.43 (7), 1096.76 (21)	777±172
^{97}Ru	^{94}Mo(α,n)	2.9 days	215.7 (86), 324.5 (11)	62±6
^{103}Ru	^{100}Mo(α,n)	39.26 days	497.1 (90.8)	0.10±0.02

[a] Energy of γ-quanta used to determine corresponding peaks; the probability (%) of emission of corresponding quantum by nucleus is given in parentheses.
[b] Activity produced at the end of bombardment.

toluene was passed through the column (see Fig. 2) to remove the sorbent from the support. The toluene eluate contained 100% of Tc and 5% of Ru. Then, long-term measurements of γ-spectra for 0—40 mL fractions were performed and these fractions were studied by spectrophotometry in order to determine Tc and Mo tracers. After continuous measurement for 2.5 days, γ-peaks of technetium were detected and its amount after column decreased by five orders of magnitude. Upon spectrophotmetric study of the same fraction, no peaks of molybdenum were detected and the limit of detection was determined by the

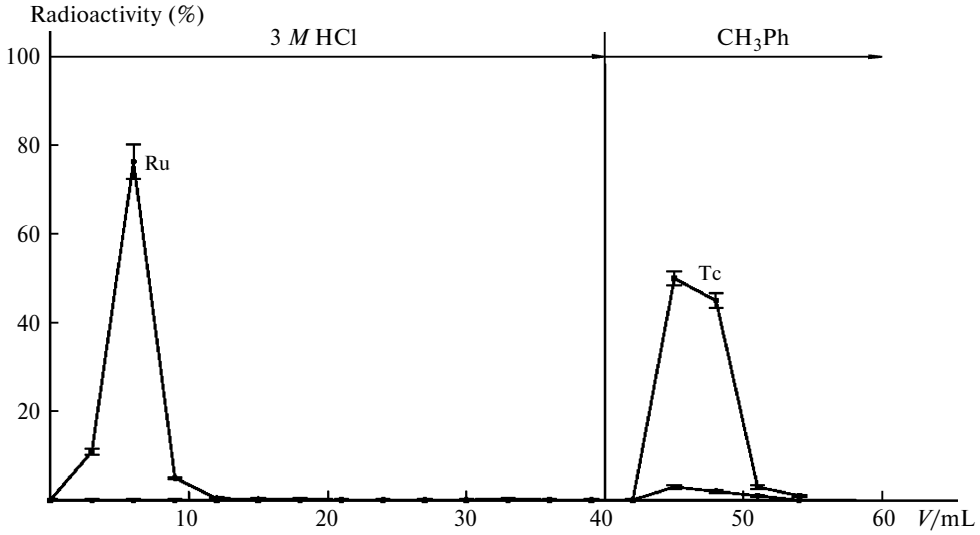

Fig. 2. Elution curves of Ru and Tc on DGA resin. The radioactivity (%) is given based on the initial activity prior to separation.

Table 2. Distribution of ruthenium-97 specific activity (%) in the organs of mice

Time of ^{97}Ru exposure in mouse/min	Distribution of ^{97}Ru specific activity (%) in the organs of mice				
	lungs	liver	kidneys	heart	spleen
15	1.1±0.2	1.07±0.04	5.7±0.4	1.02±0.09	0.84±0.08
30	0.89±0.16	3.2±0.2	3.4±0.3	0.88±0.12	2.6±0.3
60	1.3±0.2	1.42±0.12	3.4±0.3	0.8±0.2	0.45±0.07

calibration curve to be $9.37\cdot10^{-5}$ mol L^{-1} (9.0 μg mL^{-1}). Thus, the total content of molybdenum in each collected fraction (3 mL each) was at most 36 μg and, consequently, the partition coefficient of ruthenium and molbydenum was at least 550. This purity of the final solution of ruthenium-97 is sufficient for the requirements of nuclear medicine. If thick targets require higher purity of eluate, it is recommended to use a second DGA-packed column and to carry out additional purification under the same conditions.

It was expected that the TEVA resin will also be applied for separation of ruthenium from Tc and Mo;[15] however, this sorbent fell short of expectations: 12 mL of the ruthenium eluate contained a considerable amount of technetium, up to 20% of the column-loaded activity. The increase in the HCl concentration (from 1 to 2 mol L^{-1}) did not help to achieve better results.

Spectrophotometry of stable ruthenium. To assess in which form ruthenium-97 leaves from the DGA column, solutions of stable ruthenium were used. The anionic $[RuCl_6]^{2-}$ forms with absorption maximum at $\lambda = 485$ nm (Fig. 3, *a*) were detected in solution with hydrogen peroxide and 3 *M* HCl, which corresponds to the procedure for dissolution of the target. This agrees with the literature data.[16—18] Consequently, the $[^{97}RuCl_6]^{2-}$ form is the most probable state of ruthenium-97 extracted from the target. Upon subsequent pH increase as a result of dilution of the solution, this form transforms into different aquacomplexes,[19,20] such as

$$[Ru(H_2O)Cl_5]^- \rightleftharpoons [Ru(OH)Cl_5]^{2-} \rightleftharpoons [Ru_2OCl_{10}]^{2-},$$

with peaks appearing in a region of 320, 380, and 450 nm, which were actually detected in the spectra (Fig. 3, *b*). Therefore, we can state that ^{97}Ru was introduced to *in vivo* experiments in the form of different chloride aquacomplexes.

***in vivo* Experiments.** The γ-radiation spectrum of the solution used for injections is shown in Fig. 1, *b*. The administered activity of ^{97}Ru was 1.5 kBq per mouse and the activity of ^{103}Ru impurity was less than 5 Bq. As it is seen from the γ-spectrum, other radionuclides were absent. The distribution of ruthenium-97 in the body organs of mouse is given in Table 2. After exposition of the drug for 30 min, its metabolism significantly changes, which follows from the distribution of ruthenium in organs. After 60 min exposure, the murine organs, especially kidneys, still contained a considerable concentration of the radionuclide. This is likely due to a long-term clearance; however, the above-mentioned fact requires further studies. Changes in the organ distribution of ruthenium chloride with time can be due to redox processes involving biological components of the body under conditions of its active transport in blood with the help of albumin, transferring, and metallothionein.

Thus, a simple and convenient method is proposed for the preparation and separation of ^{97}Ru radionuclide for nuclear medicine using commercially available sorbents. Enriched molybdenum targets are preferred for irradiation, which will make it possible to avoid the formation of long-lived ^{103}Ru impurity; however, in this experiment the

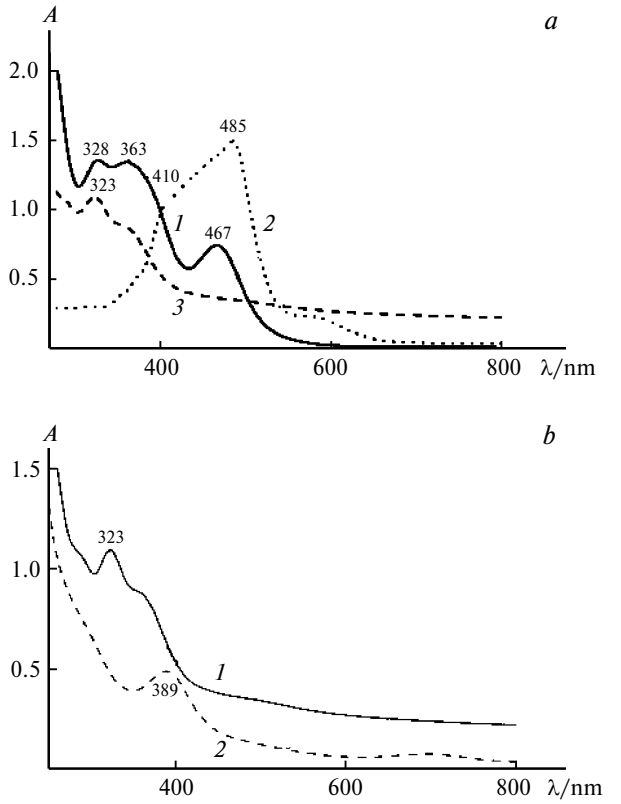

Fig. 3. Absorption spectra of stable ruthenium: (*a*) in 3 *M* HCl (*1*), H_2O_2 + 3 *M* HCl (*2*), and HCl at pH 1 (*3*); (*b*) at pH 4.5 (*1*) and 7 (*2*).

percentage of ^{103}Ru was insignificant for radiation burden upon *in vivo* experiments. The organ distribution of ^{97}Ru suggests that the isotope is not removed rapidly from the body even in a simple chloride form. This offers a hope not only for its diagnostic potential, but also for its therapeutic efficacy.

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