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Production of Ac-225 from Th-229 for Targeted α Therapy

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This work describes a method for the separation and purification of Ac-225 from a Th-229 source. The procedure is based on the combination of ion exchange and extraction chromatographic methods in nitric acid media and allows the preparation of carrier-free, clinical grade Ac-225 with an overall yield exceeding 95%. Quality control of the product is performed using radiometric (α, γ spectrometry) and mass spectrometric methods. The Ac-225 product can be loaded on a radionuclide generator for the preparation of Bi-213 for preclinical and clinical studies of targeted α therapy of cancer and infectious diseases.

The principle of targeted α therapy (TAT) of cancer is based on the coupling of α-particle-emitting radionuclides to tumor-selective carrier molecules, e.g., monoclonal antibodies or peptides, serving as vehicles for the specific targeting of cancer cells. Due to the high linear energy transfer of α particles and, consequently, their short path length in human tissue (<100 μm), a highly cytotoxic radiation dose can be delivered to targeted cancer cells, while damage to surrounding healthy tissue is limited. As a consequence of the short range of α particles in human tissue, TAT is particularly promising for the treatment of single-cell cancers, small-cell clusters, and minimal residual disease following surgery or chemotherapy. The potential of TAT for the treatment of a variety of cancer types has been shown in several preclinical and clinical studies, e.g, refs 1−6 and has recently been demonstrated also for therapy of infectious diseases.[7]

Several α-emitting nuclides are under consideration for TAT, including Ac-225, Ra-224, Ra-223, Bi-213, Bi-212, At-211, and Tb-149. Studies on the use of Bi-213 ($T_{1/2}$ = 46 min) that can be made available to hospitals via a radionuclide generator loaded with its parent nuclide Ac-225 ($T_{1/2}$ = 10 days) are presently the most advanced. Bi-213 is a mixed α/β emitter that mainly decays to the pure α emitter Po-213 with a branching ratio of 98% (Figure 1). Due to the very short half-life of the daughter nuclide Po-213 ($T_{1/2}$ = 4.2 μs), Bi-213 itself can be practically considered an α emitter. The supply of Bi-213 through production of its parent nuclide Ac-225, however, is limited as Ac-225 can currently only be obtained by radiochemical separation from Th-229 sources. Today only two Th-229 sources for the production of clinically relevant amounts of Ac-225 exist worldwide, located at the Institute for Transuranium Elements (ITU) in Karlsruhe, Germany, and Oak Ridge National Laboratory, Oak Ridge, TN.[8−10] Alternative ways of producing Ac-225 are being investigated, mainly based on the irradiation of Ra-226 targets using protons, neutrons, or

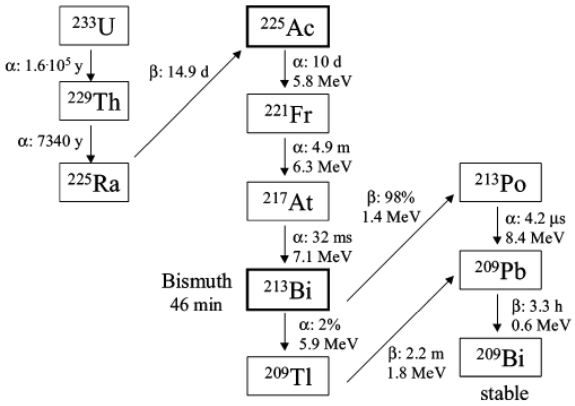

Figure 1. Decay chain of U-233.

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γ-rays,[11,12] but have so far not resulted in the routine production of clinically useful amounts of Ac-225/Bi-213.

In this work, we report on a method for the separation and purification of Ac-225 from Th-229 that has been developed and put to routine use over the last years in ITU. It is based on the combination of ion exchange and novel extraction chromatographic methods in nitric acid media. The procedure is rapid and yields a carrier-free, clinical grade Ac-225 product with an overall yield exceeding 95%, thereby minimizing loss of the rare isotope. Quality control of the product includes radiometric (α, γ spectrometry) and mass spectrometric (ICPMS) methods.

EXPERIMENTAL DETAILS

Ion Exchange and Extraction Chromatography. Solutions of nitric acid, hydrochloric acid, sodium acetate, and sodium iodide were prepared from Suprapur grade reagents (Merck). Water was obtained from a Milli-Q water purification system. All other chemicals were reagent grade and were used as received. Dowex 1×8, 100−200-mesh anion exchange resin (Serva) was obtained in Cl^- form and washed using 8 M HNO_3 until chlorine free. Dowex AG-MP-50, 100−200-mesh cation exchange resin in hydrogen form (Bio-Rad) was used as received. UTEVA (100−150 μm) and RE (on silica, 90−130 μm) extraction chromatographic resins and prefilter material (all Eichrom Inc.) were used as purchased without further purification. The extraction chromatographic resins as well as the prefilter material were packed in plastic columns (Bio-Rad), making up bed volumes of 1.0 or 1.6 mL. Average flow rates were ~0.2−0.4 mL/min.

Radiometric Analysis. α spectra were measured using a Soloist α spectrometry system (EG&G Ortec). The efficiency of the α detector was calibrated using a mixed Pu-239/Am-241/Cm-244 standard (AMR43, Amersham). γ spectra were measured using a HpGe well detector connected to a 92X spectrum master multichannel analyzer (EG&G Ortec). The efficiency of the detector was calibrated using standard radiotracer solutions.

ICPMS Measurements. ICPMS measurements were performed on a Finnigan Element 2 (Thermo Electron) installed in a glovebox. Standards were obtained as certified reference solutions (Agilent) and diluted by weight. Acids used were Suprapure grade (Merck). Ultrapure water was produced in an UHQ water purifier (USF Elga). The quantification was done by external calibration according to ITU standard operating procedures. A sequence of measurements was created including an instrument blank, the standards, process blank, the unknown samples, and finally a quality control sample. Each sample was measured in triplicate. The uncertainty stated is the total expanded uncertainty calculated according to *ISO Guide to the Uncertainty in Measurement (GUM)* with a coverage factor 2.

Analytical Approach. Preparation of the Th-229 Source. The Th-229 source was isolated from mixed waste material originating from the U-233 production at ORNL using ion exchange procedures as described in ref 13. Briefly, 0.5 kg of solid waste material (broken glass, tissues, etc.), containing ~9.5 g of U-233, 215 mg of Th-229, and 32 g of Th-232, was repeatedly heated under reflux in 2 L of 8 M HNO_3 and filtered after cooling. The filtrate containing uranium and thorium was reduced in volume by evaporation, subsequently adjusted to ~8 M HNO_3 by addition of water, and filtered again (2×). The resulting solution was added onto an anion exchange column (Dowex 1×8, 100−200 mesh, column volume 1 L). Washing with 15 L of 8 M HNO_3 removed U-233, Ra-225, Ac-225, and its daughter products, while Th-229/Th-232 was eluted using 4 L of 0.1 M HNO_3. After reduction of the volume of the thorium eluate by evaporation and adjustment to 500 mL of 8 M HNO_3, the anion exchange procedure was repeated to remove residual uranium. The final thorium eluate was again adjusted to 500 mL of 8 M HNO_3, and 500 mL of preequilibrated anion exchange resin (Dowex 1×8, 100−200 mesh) was added in portions of ~50 mL while continuously stirring the mixture for 5 h to prepare the final thorium batch. To increase sorption of thorium to the anion exchange material and to obtain a homogeneous distribution of the activity, the supernatant solution was pumped through the batch for 5 h using a peristaltic pump. This thorium batch has been in use for ~1 decade for continuous production of Ra-225/Ac-225. The radiolytic damage was found to be limited so far, mainly due to the presence of an ~150-fold excess of natural Th-232 over Th-229. α and γ spectra of the Th-229/Th-232 stock in equilibrium with its daughter nuclides are shown in the upper spectra of Figures 4 and 5.

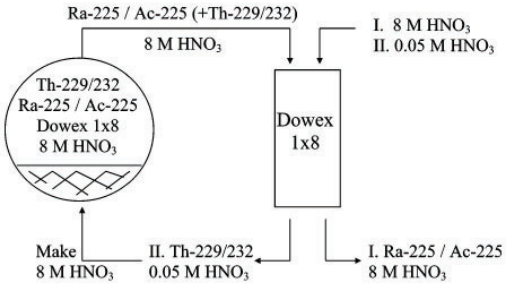

Figure 2. Anion exchange procedure for separation of Ra-225 and Ac-225 from a Th-229 source.

Separation of Ra-225/Ac-225 from Th-229. The Th-229 source available at ITU contains 214.5 mg of Th-229 ($T_{1/2}$ = 7340 years), corresponding to 45.6 mCi. Taking into account the half-lives of the daughter products Ra-225 ($T_{1/2}$ = 14.8 days) and Ac-225 ($T_{1/2}$ = 10 days) (decay chain of U-233 is shown in Figure 1, decay data are taken from[14]), 43.2 mCi of Ra-225 and 39.4 mCi of Ac-225 can be isolated from the thorium source every 9 weeks. This time interval is typically chosen to make a clinically relevant activity of Ac-225 available for each generator shipment. The ion exchange procedure used for the separation of Ra-225/Ac-225 from Th-229 is shown in Figure 2. The thorium batch, consisting of 500 mL of Dowex 1×8, 100−200-mesh anion-exchange resin equilibrated with 8 M HNO_3, is washed 5−6 times using 100 mL of 8 M HNO_3 each time. The combined washing solutions, containing Ra-225, Ac-225, and ~3−5% of Th-229/Th-232, are evaporated to near dryness, taken up in 20 mL of 8 M HNO_3 and filtered through a prefilter column (1.0 mL volume) to remove organic impurities. Subsequently, the filtrate is loaded onto an

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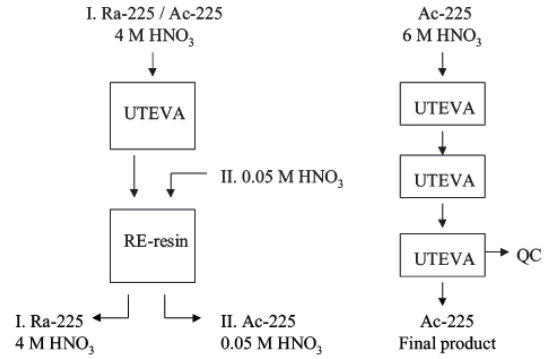

Figure 3. Extraction chromatographic procedure for Ra-225/Ac-225 separation and Ac-225 purification.

anion exchange column (Dowex 1×8, 100−200 mesh, column volume 80 mL). Ra-225 and Ac-225 are eluted by washing with 250−300 mL of 8 M HNO_3, and the eluate is evaporated to near dryness and taken up in ~10 mL of 4 M HNO_3 for subsequent radium/actinium separation by extraction chromatography. Thorium is recovered from the anion exchange column by elution with 500 mL of 0.05 M HNO_3. The thorium eluate is evaporated to near dryness, taken up in 8 M HNO_3 and added back to the thorium batch.

Separation of Ac-225 from Ra-225 by Extraction Chromatography. The separation scheme used for the separation of Ac-225 from Ra-225 is shown in Figure 3. It is based on the multiple use of UTEVA extraction chromatographic columns for the removal of any residual traces of thorium combined with the use of RE-resin for the separation of actinium from radium in nitric acid media. A main advantage of using RE-resin is that it is commercially available on silica gel support with enhanced radiation resistance compared to resins based on organic support materials. The Ra-225/Ac-225 product from the anion exchange separation is loaded in 4 M HNO_3 onto a UTEVA/RE-resin column cascade (column volume 1.6 mL each). Under these conditions, residual thorium is extracted on the UTEVA column. Ac-225 is retained on RE-resin from the 4 M HNO_3 load solution, while Ra-225 can be washed through the column with up to 20 mL of 4 M HNO_3. Subsequently, Ac-225 is stripped of the Re-resin using 8 mL of 0.05 M HNO_3, and the eluate is evaporated to near-dryness and taken up in 2 mL of 6 M HNO_3. The Ra-225 eluate is evaporated to reduce its volume, taken up in few milliliters of 4 M HNO_3, and stored for 17 to 18 days. After this time interval, the maximum amount of Ac-225, corresponding to 44.3% of the initial Ra-225 activity, has grown in and can additionally be isolated.

Final Purification of Ac-225 and Product Quality Control. For final purification, the Ac-225 product in 6 M HNO_3 is loaded onto a triple UTEVA cascade (column volume 1.0 mL each) to remove any residual traces of thorium or other metal impurities. Ac-225 is washed through the cascade, evaporated to near-dryness, and taken up in 2 mL of 4 M HNO_3 for subsequent loading of the Ac-225/Bi-213 radionuclide generator. For quality control purposes, the last UTEVA column is stripped using 0.4% ammonium oxalate solution. The eluate is evaporated, taken up in 6 M HNO_3, and analyzed by α spectrometry. No radionuclide impurities may be detected in this fraction. Quality control further includes α-, γ, and ICP-mass spectrometric analysis of the final Ac-225 product.

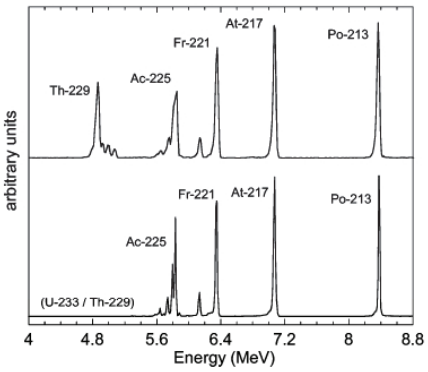

Figure 4. α spectrum of Th-229 source (top) and purified Ac-225 product (bottom).

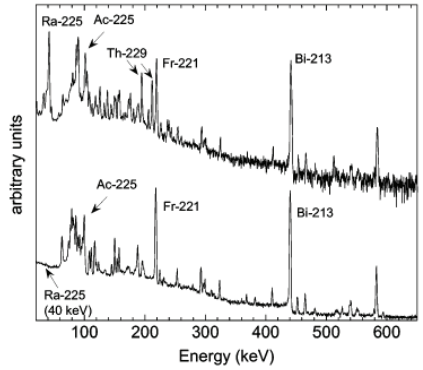

Figure 5. γ spectrum of Th-229 source (top) and purified Ac-225 product (bottom).

Table 1. Main Impurities Typically Found in the Final, Clinical Grade Ac-225 Product

	Ac-225 product (ng/g)	blank (ng/g)
Ac	1.93 ± 0.39	nd[a]
Al	10.0 ± 1.2	1.93 ± 0.23
B	12.7 ± 1.5	3.79 ± 0.46
Ca	26.7 ± 3.2	3.11 ± 0.37
Fe	105 ± 13	10.33 ± 1.24
K	32 ± 4	10.22 ± 1.23
Mg	9.5 ± 1.1	0.928 ± 0.111
Na	72 ± 9	16.21 ± 1.95
P	88 ± 11	0.626 ± 0.075
Si	8.7 ± 1.0	nd
Th-229	0.001 ± 0.001	nd
Th-232	0.086 ± 0.010	0.002 ± 0.001
Ti	6.8 ± 0.8	0.037 ± 0.004
U-233	nd	nd
Zn	14.9 ± 1.8	2.98 ± 0.36

[a] nd, not detectable.

α and γ spectra of a typical Ac-225 product are shown in the bottom spectra of Figures 4 and 5. To pass quality control, the combined activity of Th-229 and U-233 measured in the α spectrum in the energy range from 4.7 to 4.9 MeV is required to be less than 0.009% of the Ac-225 activity (limit of detection, 1800 s counting time). The γ spectrum is used to control the absence of Ra-225 contamination that has to be less than 0.002% of the Ac-225 activity (limit of detection, 300 s counting time). Table 1 summarizes the main impurities found in the ICPMS spectrum

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Table 2. Overview of ITU's Partners in the Field of Targeted α Therapy

partner	cancer type or collaboration project
Technical University of Munich, Nuclear Medicine Department (D)	gastric cancer
University of Düsseldorf, Department of Hematology, Oncology and Clinical Hematology (D)	non-Hodgkin's lymphoma (NHL)
German Cancer Research Center (DKFZ) (D)	non-Hodgkin's lymphoma (NHL)
University of Ghent, Department of Hematology (B)	non-Hodgkin's lymphoma (NHL)
Virga Jesse Hospital, Hasselt (B)	non-Hodgkin's lymphoma (NHL)
INSERM, Nantes (F)	multiple myeloma
Clinic of Neurosurgery, Kantonsspital Basel (CH)	glioma, glioblastoma
St. George Cancer Care Center & Medical Scitec (Australia)	melanoma and prostate, pancreas, breast, ovarian cancer
Memorial Sloan-Kettering Cancer Center (USA)	acute myelogenous leukemia (AML)
Albert Einstein College of Medicine (USA)	antimicrobial therapy
Johns Hopkins Medicine (USA)	breast cancer

of the Ac-225 product. The most prominent impurities are iron and phosphorus. U-233 is not detectable; concentrations of Th-232 and Th-229 are in the low ppt range, corresponding to a decontamination factor of the separation process exceeding 10^9. The low concentrations of metal impurities in the Ac-225 product can be expected to be further reduced during the selective elution of Bi-213 from the Ac-225/Bi-213 radionclide generator and the radiolabeling procedure used for the preparation of the radiopharmacon (see below).

Preparation of Radionuclide Generators and Radiolabeling Procedure. ITU's Ac-225/Bi-213 radionuclide generator consists of a cation exchange column (Dowex AG-MP-50, 100−200 mesh) with a total volume of 330 μL that can be washed and eluted at a constant rate by means of a peristaltic pump connected via Teflon tubing. The purified Ac-225 product is loaded onto the generator in 2 mL of 4 M HNO_3, followed by 2 mL of fresh 4 M HNO_3. Subsequently, the generator is converted into Cl^- form by sequential washes using 2 mL of 0.05 M HNO_3, 2 mL of 2 M HCl, and 2 mL of 0.01 M HCl before it is ready for use. Using this loading technique from 4 M HNO_3 media, a homogeneous distribution of Ac-225 over the first ~60% of the cation exchange resin can be obtained. In this manner, the radiolytic degradation of the resin can be significantly minimized and the performance of the generator can be stabilized over several weeks. Previously, Ac-225 had been loaded on the generator from dilute hydrochloric acid media, leading to the deposition of radioactivity in a narrow band at the inlet of the generator. This often led to decreasing generator performance after extended time periods when the generator was not in use, e.g., during overseas transport.

For labeling of antibodies, Bi-213 is eluted from the generator with ~90% yield using 600 μL of 0.1 M NaI/0.1 M HCl, where Bi is expected to be present as BiI_4^-/BiI_5^{2-} species.[15] The eluate is buffered to pH 5.5 by addition of 130 μL of 4 M sodium acetate and 50 μL of 20% ascorbic acid solution as radioprotectant. Typically, 0.1−1 mg of antibody, chelated with DTPA or a derivative thereof, are added, and the mixture is incubated for 5 min to allow for Bi complexation. Subsequently, the reaction is quenched by addition of 10 μL of 1.5 mg/mL DTPA solution; the Bi-213-labeled antibody is immediately purified from free bismuth by size exclusion chromatography and sterilized by filtration. Labeling yields typically exceed 85%. Due to the relatively short half-life of Bi-213, the labeling and purification procedure should be performed as rapidly as possible, which is possible for trained personnel within ~16−18 min. Consequently, the activity of antibody-bound Bi-213 that can be made available from one generator elution corresponds to ~60% of the activity of Ac-225 loaded on the radionuclide generator.

Over the past decade, more than 180 shipments of purified, clinical grade Ac-225, corresponding to an excess of 3.5 Ci of Ac-225, have been sent from ITU to its various partners at hospitals and cancer research centers. An overview of selected collaborations in the field of targeted α therapy of cancer and infectious diseases is given in Table 2.

CONCLUSIONS

The procedure presented in this work for the separation and purification of the relatively short-lived α emitter Ac-225 is robust and rapid and can be performed by trained personnel within 2−3 working days. Since the method is mainly based on column processes, necessary manipulations are relatively simple and can easily be performed in a shielded glovebox environment. All chemical separations are performed in nitric acid media of varying molarity. Thus, the use of corrosive reagents, in particular hydrochloric acid, required for conventional thorium/actinium separation procedures based on ion exchange[16] can be reduced to a minimum. Due to the time efficiency and high overall yield of >95% of this process, the loss of the highly valuable and rare material Ac-225 is minimized. The resulting Ac-225 product is of high purity and continues to be used in clinical trials of targeted α therapy.

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The authors dedicate this work to Prof. Basil Kanellakopoulos on the occasion of his 70th birthday.

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