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Improved procedures of $Sc(OH)_3$ precipitation and UTEVA extraction for ^{44}Sc separation

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ABSTRACT

BACKGROUND: ^{44}Sc is becoming attractive as a PET radionuclide due to its decay characteristics. It can be produced from ^{44}Ca present in natural calcium with 2.08% abundance.

MATERIALS AND METHODS: The targets were mostly prepared from natural $CaCO_3$ or metallic calcium in the form of pellets. After irradiation they were dissolved in 3 M hydrochloric acid and ^{44}Sc was separated from excess of calcium by precipitation of scandium hydroxide using ammonia. Alternatively, targets were dissolved in 11 M hydrochloric acid and ^{44}Sc was separated by extraction chromatography on UTEVA resin. As the next step, in both processes ^{44}Sc was further purified on a cation exchange resin. Initially, the separation procedures were developed with ^{46}Sc as a tracer. Gamma spectrometry with a high purity germanium detector was used to determine the separation efficiency. Finally, the $CaCO_3$ pellet with 99.2% enrichment in ^{44}Ca was activated with protons via $^{44}Ca(p,n)^{44}Sc$ nuclear reaction.

RESULTS: Altogether twenty two irradiations and separations were performed. The working procedures were developed and the quality of separated ^{44}Sc solution was confirmed by radiolabeling of DOTATATE. The chemical purity of the product was sufficient for preclinical experiments. At the end of around 1 hour proton beam irradiation of $CaCO_3$ pellet with 99.2% enrichment in ^{44}Ca the obtained radioactivity of ^{44}Sc was more than 4.8 GBq.

CONCLUSION: ^{44}Sc can be produced inexpensively with adequate yields and radionuclidic purity via $^{44}Ca(p,n)^{44}Sc$ nuclear reaction in small cyclotrons. The recovery yield in both investigated separation methods was comparable and amounted above 90%. The obtained ^{44}Sc was pure in terms of radionuclide and chemical purity, as shown by the results of peptide radiolabeling.

KEY WORDS: ^{44}Sc production, cyclotron, natural calcium, $Sc(OH)_3$ precipitation, UTEVA extraction

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Introduction

Scandium-44 ($T_{1/2}$ = 3.93 h) is practically a pure β^+ emitter having also a long-lived isomeric state ^{44m}Sc ($T_{1/2}$ = 2.44 d), and accompanying intense gamma radiation of 1.157 MeV. The relatively long half-life of ^{44}Sc is of advantage in comparison with that of ^{68}Ga ($T_{1/2}$ = 67.7 min). This longer half-life allows transporting ^{44}Sc from the cyclotron to nearby places of its processing. Moreover, it gives time for the separation after target irradiation. In PET imaging it allows later acquisitions up to 24 h post injection. Potential drawback of ^{44}Sc as PET imaging agent is the presence of high-energy gamma lines, which may create hazard for personnel. However, in the visual comparison of ^{68}Ga and ^{44}Sc radiolabeled PSMA-617 in patients as well as in the calculation of the signal to noise-ratio, no significant differences were found [1]. Relatively simple route of ^{44}Sc production uses ^{44}Ca present in natural calcium in 2.08% abundance. Target materials with high enrichment are also available so the production of ^{44}Sc in $^{44}Ca(p,n)^{44}Sc$ reaction in gigabecquerel quantities can easily be achieved.

The aim of this study was to develop a working procedure for ^{44}Sc production of sufficient purity and specific activity to be used for medical needs. A number of papers were published in recent years on the separation of ^{44}Sc for medical applications [2–8]. In this work, the two methods of ^{44}Sc separation from calcium targets were compared: precipitation of $Sc(OH)_3$ and extraction chromatography with UTEVA resin. Both methods are attractive due to their relative simplicity. The detailed studies of these methods have been undertaken with the purpose to optimize the separation yield of ^{44}Sc and processing time after target dissolution. Natural calcium in the form of $CaCO_3$ or dendritic metal chunks pressed into pellets was used for preparation of irradiation targets. One

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enriched [^{44}Ca]$CaCO_3$ target was also irradiated and processed. Since Sc forms stable complexes with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), the somatostatin analog DOTA-Tyr^3-Thr^8-octreotide (DOTATATE) has been used for tests of radiolabeling with obtained ^{44}Sc solution.

Materials and methods

Chemicals and equipment

Natural calcium carbonate $CaCO_3$, ACS reagent, chelometric standard, 99.95–100.05% dry basis, and natural metallic calcium dendritic chunks 99.99% purity, Sigma Aldrich, as well as [^{44}Ca]$CaCO_3$, 99.2 ± 0.1% enriched, Isoflex, USA, were used for target preparation. The UTEVA extraction resin (coated with dipentyl pentylphosphonate, 100–150 mesh), Eichrom, USA, was used for the separation of Sc(III) from Ca(II). In addition, scandium (III) was concentrated and purified on a cation exchange resin AG® 50W-X4 hydrogen form, 100–200 mesh, BIO-RAD, Denmark. The flow rate of all liquids was 0.5 mL/min. Solutions were delivered on the columns and filters using peristaltic pump MasterFlex, Cole Palmer. For scandium separation by hydroxide precipitation a syringe filter PTFE, 13 mm, 0.45 μm, Cronus, was used. The HPLC grade water, hydrochloric acid ACS Reagent, 37%, sodium acetate used in chemical separation were from TraceSELECT™ Honeywell, Fluka™ and ammonia solution 25% NH_4OH ACS reagent grade, was from Merck, Germany. Radio TLC was performed using glass microfiber chromatography paper impregnated with silica-gel (iTLC-SG, Agilent Technologies). The distribution of radioactivity on radiochromatograms was determined using Cyclone®Plus (PerkinElmer). DOTATATE from the freeze dried kit, NCBJ OR POLATOM, Poland, was used for radiolabeling. The sample aliquots of all radioactive solutions were measured by gamma spectrometry, with high purity germanium (HPGe) detectors type GX1520 (Canberra) and GEM20P4 (Ortec). The pellets were pressed with hydraulic press PLH-25. Proton irradiations were performed in GE PETtrace 840 cyclotron (at Heavy Ion Laboratory, University of Warsaw).

Cyclotron targetry and irradiations

Calcium targets were prepared by pressing either 0.2 g natural calcium carbonate powder into pellets of 10 mm diameter or 0.15 g natural metallic calcium dendritic chunks into pellets of 8 mm diameter. The vacuum pressing was used for $CaCO_3$ compacting. Pellets were pressed into titanium backing of 12 mm external and 10 mm internal diameter and 2 mm deep cavity. Metallic calcium pellets were pressed into titanium ring instead of titanium backing to make the target dissolution easier after irradiation. For irradiation with proton beam the targets were covered with 5 μm thick aluminum foil and loaded into aluminum holder which was mounted in cyclotron external target station. All irradiations were performed in GE PETtrace cyclotron using 16 MeV protons. Depending on the type of targets ($^{nat}CaCO_3$, metallic ^{nat}Ca or enriched [^{44}Ca]$CaCO_3$) the different irradiation time and beam current were used. Typical irradiation conditions for natural calcium carbonate targets were: 1.5 to 3 h irradiation at 13 to 23 μA of the proton beam intensity/current. The front side of the target was immersed in a helium environment, while the back side was cooled by water through an aluminum holder. After irradiation the calcium targets were removed from the holder and dissolved in HCl of required concentration.

The enriched [^{44}Ca]$CaCO_3$ target contained 86 mg of calcium carbonate powder pressed into pellet of 6 mm diameter. It was placed in the graphite bed of 10 mm external diameter with cavity of 6 mm in diameter in the center [9]. The activation time was 45 min with 15 μA of the proton beam intensity.

Dissolution of targets

Depending on the separation method the target dissolution was performed as follows:

For precipitation of scandium hydroxide. After irradiation the $CaCO_3$ dismantled target was placed in 10 mL vial and 3 M HCl was added to the vial in portions of 1.5 up to 3.5 mL, depending on the speed of dissolution. The Ti backing was removed. Finally, water was added to bring the final volume of solution to 5.5 mL.

For extraction chromatography on UTEVA. The irradiated $CaCO_3$ target was dissolved in 7.2 mL of concentrated HCl with addition of 0.5 mL of water to maintain the H^+ concentration of about 11 M. As soon as $CaCO_3$ was dissolved the Ti backing was removed. The same procedure was used to dissolve the enriched [^{44}Ca]$CaCO_3$. Targets in the form of Ca metal pellet, after dismantling from the holding ring, were placed in a plastic vial and water was added in 500 μL portions to complete dissolution, then the concentrated HCl was added to final 11 M concentration. This solution was filtered before separation of ^{44}Sc.

Radiochemical separation

Separation of ^{44}Sc by precipitation of hydroxide

To the dissolved target 2.0 to 3.0 mL of 25% NH_4OH solution was added to set the pH value between 8.2 and 9.0. In next step the solution was filtered through a syringe filter with a hydrophilic membrane of 13 mm diameter and 0.45 μm pore size. Filter was rinsed with 2 mL of 1.5 M NH_4OH and then with 2 mL of water. ^{44}Sc precipitate was dissolved in 5.0 mL of 0.5 M HCl and solution was transferred to the column filled with AG50W-X4 resin. The column was rinsed with 2mL 0.1M HCl and then 2 mL of water. ^{44}Sc was eluted with 1 M sodium acetate pH 4.0 in 1 mL fractions. The scheme of separation process is shown in Figure 1.

Separation of ^{44}Sc using UTEVA resin

Before use, UTEVA resin was soaked in 0.1 M HCl for 24 h. The PEEK column of 50 x 2.1 mm dimension was filled with soaked resin of about 70 mg dry mass. Before use the columns were conditioned with 10 mL 11 M HCl solution. The dissolved target solution was preliminarily filtered and then transferred onto the column. After rinsing the column with 2 mL 11 M HCl the ^{44}Sc was eluted with 5 mL of water and the eluate was transferred on the column filled with AG50W-X4 resin to remove the excess of HCl. The column was rinsed with 2 mL 0.1 M HCl and then 2 mL of water. Finally, ^{44}Sc was eluted using 1 M sodium acetate pH 4.0 in 1 mL fractions. The schematic presentation of this process is shown in Figure 2.

The radioactivity of ^{44}Sc and its radionuclidic impurities was measured with use of gamma spectrometers with high purity germanium (HPGe) detectors. Detectors were calibrated using multigamma standard sources with activities traceable to the National Standard of Radionuclides Activity in Poland. Samples for measurement were prepared by adding 25 μL of tested ^{44}Sc solution

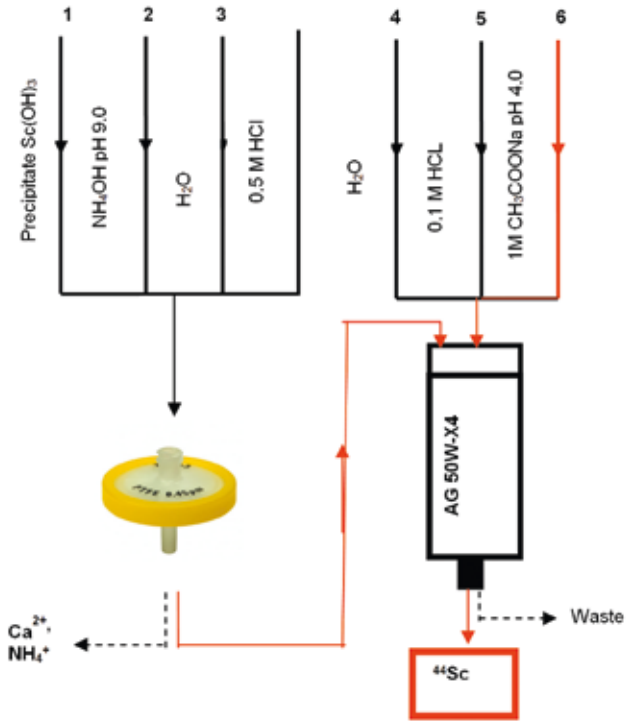

Figure 1. Scheme of the separation of ^{44}Sc by precipitation of hydroxide

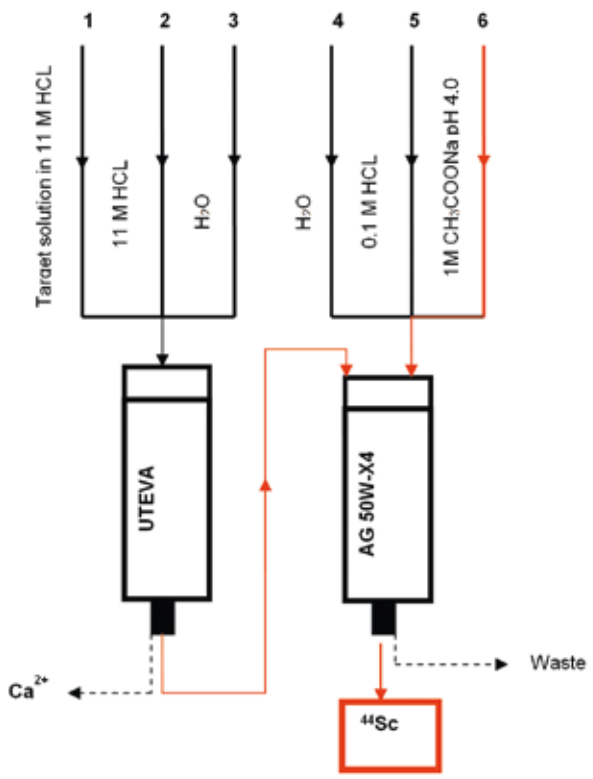

Figure 2. Scheme of the ^{44}Sc separation with UTEVA resin

to 10 mL glass vials and filling up with 0.1 M HCl to 1 mL to maintain calibration geometry. Gamma spectra of radionuclides were evaluated with Genie2k software (Canberra).

DOTATATE radiolabeling for quality control of ^{44}Sc

The freeze-dried kits containing 100 μg of DOTATATE were used to prepare the peptide aliquots for ^{44}Sc radiolabeling. The content of the freeze-dried kit was dissolved in 250 μL of 1M CH_3COONa, pH = 4, the appropriate volumes of this solution with 6.25, 12.5, 25 or 50 μg of the peptide (4.4; 8.8; 17.5; 35 nmol respectively) were transferred to another vial and filled up to 250 μL with 1M CH_3COONa, pH = 4, followed by 200–500 μL of ^{44}Sc solution. The reaction mixture was incubated at 90°C for 30 min. The labeling yield was assessed by thin layer chromatography using silica gel plates (ITLC-SG) with 0.1 M sodium citrate (pH = 5) or 1 M NH_4OAc/Methanol, 50/50 (v/v) as the mobile phase. In the first system the radiolabeled peptide remained at the start while the free radionuclide migrated with the front of the solvent (Rf = 0.7–0.8). In the second system the radiolabeled peptide migrated with the front of the solvent (Rf = 0.7–0.8) while the non-bound ^{44}Sc remained at the start.

Results and discussion

Preparation of thermally stable targets from $CaCO_3$ powder was technically demanding as $CaCO_3$ powder is an insulator [10]. Although stable pellets were prepared, almost all of them fractured during proton irradiation due to local overheating and gas release. $CaCO_3$ traces were also present on the Al foil cover of the targets. There were also some losses due to sticking to the Ti backing. Also, some irradiated targets required longer time for dissolution. All these problems dissappeared with calcium in metal form.

More than 90% of $CaCO_3$ targets were dissolved in 3 to 5 minutes. Pellets containing from 86 mg to 200 mg of natural $CaCO_3$ were irradiated and ^{44}Sc separated by precipitation of hydroxide. The ^{44}Sc activity after target dissolution in 3 M HCL ranged from 162 to 365 MBq. The average recovery of the final product was 89 ± 9% (n = 6). Although precipitation method used for ^{44}Sc separation from excess of calcium seemed to be simple and effective [2], it was not very reproducible in our experiments.

In separation based on UTEVA resin the extraction yield of scandium strongly depended on the concentration of HCl. Three targets of calcium metal were activated and dissolved in 9, 10 or 11 M HCl. When 11 M HCl was used the ^{44}Sc recovery efficiency was 93% while for 10 M and 9 M it reached only 84% and 61%, respectively.

Natural calcium has 6 stable isotopes, the main constituent being ^{40}Ca (97%). These other than ^{44}Ca isotopes present in target material (natural and enriched) are sources of other radioactive scandium isotopes produced in nuclear reactions with protons. Their contribution to activity of ^{44}Sc varied and is presented in Table 1.

Although at the end of irradiation the radioactivity of enriched target was more than 4.8 GBq, at the time of this target dissolution the activity of ^{44}Sc obtained was about 3 GBq. The overall separation efficiency was close to 91% with about 4% losses on UTEVA column and about 1.4% on cation exchange column. In ^{44}Ca-enriched target the only significant impurity was ^{44m}Sc and its content was low, at the level of 0.01%. Irradiation of enriched target allowed decreasing the content of other scandium nuclides to a negligible level (not detectable after 30 min long gamma spectrometry measurement).

Table 1. Radionuclidic purity of ^{44}Sc

% of total activity after target separation		
Radionuclide	Target	
	$^{nat}CaCO_3$	$[^{44}Ca]CaCO_3$
^{44m}Sc	0.86 ± 0.24	< 0.01
^{43}Sc	2.95 ± 0.14	nd
^{47}Sc	0.66 ± 0.29	nd
^{48}Sc	1.25 ± 0.39	nd

The high chemical purity of the obtained scandium fraction was verified by labeling the DOTATATE. Using the ^{44}Sc solution with radioactivity from 11 to 450 MBq and the mass of peptide from 4.4 to 25 nmol the specific activity of obtained ^{44}Sc-DOTATATE was in the range from 0.3 to 102 MBq/nmol of peptide. More than 98% labeling yield was obtained for 50 μg (specific activity up to 13 MBq/nmol) which is comparable to the results reported by Domnanich KA et al. [8]. Specific activities up to 25 MBq/nmol could be obtained as well, however at the cost of radiochemical purity (> 90%).

Conclusions

Two investigated methods: precipitation and extraction chromatography on UTEVA resin can be used to effectively separate ^{44}Sc from macro amount of calcium. Both procedures use hydrochloric acid for target dissolution. For precipitation of scandium hydroxide the 3 M HCl is sufficient but for extraction with UTEVA the 11 M HCl is necessary. Therefore, to lower chemical hazard and the risk of corrosion the first one will be preferred. Our experiments delivered ^{44}Sc solution of comparable chemical and radiochemical purity suitable for medical applications. In both cases ^{44}Sc was eluted in a volume of about 1 mL with recovery yield above 90%. The irradiation of natural calcium target material led to the production of ^{44}Sc in radioactivity levels suitable for radiolabeling of biomolecules; however, to reach the radioactivity levels needed for human application the enriched target material must be used.

Conflict of interest

The authors declare that they have no conflict of interest.

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