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Separation of ^{44}Ti from proton irradiated scandium by using solid-phase extraction chromatography and design of ^{44}Ti/^{44}Sc generator system

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Abstract

Scandium-44g (half-life 3.927 h [1]) shows promise for positron emission tomography (PET) imaging of longer biological processes than that of the current gold standard, ^{18}F, due to its favorable decay parameters. One source of ^{44g}Sc is the long-lived parent nuclide ^{44}Ti (half-life 58.9 a). A ^{44}Ti /^{44g}Sc generator would have the ability to provide radionuclidically pure ^{44g}Sc on a daily basis. The production of ^{44}Ti via the ^{45}Sc(p,2n) reaction requires high proton beam currents and long irradiation times. Recovery and purification of no-carrier added (nca) ^{44}Ti from scandium metal targets involves complex separation chemistry. In this study, separation systems based on solid phase extraction chromatography were investigated, including branched

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diglycolamide (BDGA) resin and hydroxamate based ZR resin. Results indicate that ZR resin in HCl media represents an effective $^{44}Ti/^{44g}Sc$ separation system.

1. **Introduction**

Scandium-44g exhibits favorable characteristics for PET imaging applications ($E_{\beta+mean}$ 0.632 MeV, positron branching 94.27% [1]). The half-life of 3.927 h makes ^{44g}Sc suitable for the radiolabeling of small to medium biomolecules, including peptides and antibody fragments, since it matches their biological half-lives. Additionally, scandium has several other isotopes suitable for imaging and therapy, namely ^{43}Sc [2] and ^{47}Sc [3, 4], which support the potential for a theranostic system. Recently, imaging with ^{44g}Sc based radiopharmaceuticals was tested in humans and showed similarity, even slight superiority over ^{68}Ga based systems [5, 6] in terms of image quality and time for logistics and compound preparation. The DOTA chelate is often utilized in imaging studies to attach scandium to biomolecules [7-9], although several other chelates have also been tested [10, 11]. *In vitro* and *in vivo* stability studies with DOTA have shown adequate stability of ^{44g}Sc labeled compounds to perform the full course of imaging [12-14].

Several production methods have been proposed for ^{44g}Sc. The direct production via proton irradiation of natural or enriched calcium with low energy protons and deuterons via the reactions $^{44}Ca(p,n)^{44m,g}Sc$ and $^{44}Ca(d, 2n)^{44m.g}Sc$, respectively, were tested by several groups [12-16]. These production routes resulted in a relatively high production yield. However, daily irradiations and close proximity to a production site are required to maintain and use a constant supply of ^{44g}Sc. Several additional scandium isotopes are co-produced by proton irradiation of

calcium including the nuclear isomer ^{44m}Sc ($t_{1/2}$ 58.6 h), which is unavoidable. Metastable ^{44m}Sc, however, undergoes isomeric transition and converts back to the ground state ^{44g}Sc. It can thus serve as an *in vivo* generator for the shorter-lived ground state [17]. After production, ^{44m,g}Sc need to be recovered from the target material. Several recovery methods were reported based on solid phase extraction chromatography [12-16]. Reported methods resulted in sufficient yields with high degrees of radionuclidic purity, a requisite condition for the labeling of ^{44g}Sc with biomolecules.

An alternative way to obtain ^{44g}Sc is by the decay of the parent isotope ^{44}Ti ($t_{1/2}$ 58.9 a). Titanium-44, in turn, is generated by proton irradiation of natural (monoisotopic) ^{45}Sc via the ^{45}Sc(p,2n)^{44}Ti nuclear pathway. The ^{44}Ti/^{44g}Sc generator system provides a daily source of very high purity ^{44g}Sc without the need for daily irradiations. However, this system is associated with several challenges: (a.) the longer half-life of ^{44}Ti requires large quantities of integrated proton beam, i.e., high beam currents and long irradiation times, to produce sufficient activities for preclinical applications via the ^{45}Sc(p,2n)^{44}Ti reaction, (b.) the separation of ^{44}Ti from the scandium target material is not trivial, and (c.) a generator system is contingent upon the development of an efficient separation system with high ^{44g}Sc elution yields and minimal breakthrough of the parent ^{44}Ti. Such a system should remain reliable to last for many years. Several research groups reported on the production of ^{44}Ti by proton irradiation of natural (monoisotopic ^{45}Sc) scandium [18, 19]. Moreover, different concepts of radiochemical processing were proposed for the purification of ^{44}Ti product from the scandium matrix, including methods of solid-supported ion exchange [18, 19] and chelation [20].

A generator concept based on an “alternating flow” elution of an anion exchange-HCl/$H_2C_2O_4$ system was introduced [19] with subsequent (post-) purification steps [21]. A ^{44g}Sc product of high radiochemical and isotopic purity was obtained, which led to a high radiolabeling yield [9].

In this study, we evaluated two methods for the separation of ^{44}Ti from a larger scandium mass based on solid phase extraction. With large target matrix masses (grams scale), a separation approach that relies on scandium sorption on a resin or on extraction into an organic phase was not feasible. Hence, ^{44}Ti sorption/retention vs. scandium sorption came into consideration. One option pursued by our team was the anion exchange retention from high molarity HCl matrices, and the results will be published elsewhere. Alternatives include the utilization of extraction chromatography resins. Based on literature precedence [20,22], hydroxamate based “ZR resin” as well as branched diglycolamide (BDGA) resin show strong retention of Ti, which laid the groundwork for our motivation to investigate the application of these stationary phases. The equilibrium distribution coefficients of Ti/Sc for various HCl concentrations were measured to determine optimal separation conditions. Based on these results, dynamic column separations were performed. It was found that ZR resin demonstrated a higher potential for recurrent Ti/Sc separations due to its strong retention of Ti and comparatively weak sorption of Sc for all tested HCl concentrations. Therefore the potential application of ZR resin for a possible ^{44}Ti/^{44g}Sc generator was evaluated within a proof-of-principle scope.

2. Materials & Methods

2.1. Chemicals

All chemicals were used without further purification. Hydrochloric acid, Optima grade, was purchased from Fisher Scientific (Pittsburgh, PA, USA). Deionized water (≥18 MΩ cm) was prepared onsite with a Millipore water purification system. Branched DGA resin (50-100 µm) was purchased from Eichrom (Lisle, IL, USA), and Zirconium "ZR" resin (conataining hydroxamate functional groups) was provided by Triskem Intl. (Bruz, France). For thin layer chromatography analysis, silica coated Aluminum TLC plates were used (Billerica, MA, USA). Autoradiography was performed with a PerkinElmer Cyclone Plus Storage Phosphor System (Waltham, MA, USA). 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), citric acid and acetate salts for buffering were purchased from Sigma-Aldrich (St. Louis, USA). The scandium disk (Sc purity TREM>99.99%) for irradiation were manufactured to size by Symcon Inc (Charlotte, NC, USA) and was supplied as a scandium metal sputtering target. ICP-OES trace analysis [ppm]: Ti (14); Fe (190); Ni (50); Nb (10); Ta (1000).

2.2. γ-ray spectrometry

Gamma-ray spectrometry was conducted using an EG&G Ortec Model GMX-35200-S HPGe detector system in combination with a Canberra Model 35-Plus multichannel analyzer. The detector diameter was 50.0 mm; detector length 53.5 mm; Be window thickness 0.5 mm; outer dead-layer thickness 0.3 µm. Detector response function determination and evaluation were performed using standards of radionuclide mixtures containing ^{241}Am, ^{109}Cd, ^{57}Co, ^{139}Ce, ^{203}Hg, ^{113}Sn, ^{137}Cs, ^{88}Y, ^{60}Co, traceable to the National Institute of Standards and Technology (NIST) and supplied by Eckert & Ziegler, Atlanta, GA, USA. The detector was a p-type Al-windowed HPGe detector with a measured FWHM at 1333 keV of approximately 2.2 keV and a relative efficiency of about 10%. Relative total source activity uncertainties ranged from 2.6% to 3.3%.

Counting dead time was kept below 10%. Titanium-44 was detected directly and after ^{44g}Sc in-growth; directly via via γ-lines 67.875 keV (94.4%) and 78.337 keV (96%) [1]. The detected ^{44g}Sc decay emission γ-line was1157.031 (99.9%). Scandium-46 was detected via the 889.277 keV (99.984%) and 1120.545 keV (99.987 %) γ-lines.

2.3. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

Determination of scandium concentrations throughout the chemical separation was carried out via Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) using a Shimadzu ICPE-9000 instrument equipped with a 2 cm x 2 cm CCD detector calibrated on the day of use. Instrument responses were calibrated to known scandium concentrations in calibration solutions; calibration solutions were prepared from certified stocks, purchased from Fisher Scientific (Pittsburgh, PA, USA). Analyses were performed in triplicate and based on a 30 s sample exposure time. Output data were managed and analyzed using the ICPE Solutions software version 1.01 (Shimadzu Corp. 2005).

2.4. Production of ^{44}Ti

The excitation function for the reaction ^{45}Sc(p,2n) has been measured [23], and the reported experimental threshold of the reaction has been determined to be roughly 13 MeV. The experimental excitation function maximum is 42 mb at 22 MeV, which places the optimum proton energy in the lower (<40 MeV) energy range. Scandium proton irradiation by-products include directly formed ^{44mg,}Sc as well as ^{43}Sc and ^{43}K (via ^{45}Sc(p,np), (p, 2np), (p,3p), respectively). A ^{44}Ti integral yield of 1.4 MBq/C (0.136 μCi/μAh) was reported for the energy range 38 MeV → threshold [cf. Ref 23] based on measured and compiled reaction cross sections.

Based on the analysis of measured cross sections [23], a scandium metal target (27 g mass each) was irradiated in the low energy slot (≤ 35 MeV) at the Isotope Production Facility (IPF) at Los Alamos National Laboratory (LANL). The target accumulated 41.7 ± 0.8 mAh of integrated beam over a seven-day irradiation period (average beam current 230 μA). Portions of this target were used for all the radiochemical development reported in this work.

2.5. Target dissolution

The irradiated scandium target was shipped to the LANL hot cell facilities for remote-manipulation chemical processing [24]. After mechanical removal of the Inconel target shell, the target was placed in a 500 mL polypropylene bottle and portions (10-15 mL) of 6 M HCl were added until full dissolution was achieved. Complete dissolution required 380 ± 10 mL of 6 M HCl. The target dissolution solution was then passed through a cellulose nitrate filter (0.45 μm), and the filer was washed with additional 6 M HCl (25 mL).

2.6. Equilibrium distribution coefficient (K_d) study

2.6.1. Procedure for K_d determination

Equilibrium distribution coefficients were determined by batch mode according to the following procedure. For each experiment, 100 mg of resin was placed in a 2 mL microfuge tube. Then, 1 mL of liquid phase (HCl of variable concentration) and 8 μL of the scandium target solution (Section 2.5) were added. The mixtures were vigorously stirred for 2 minutes and allowed to equilibrate for 24 hours at ambient temperature. Mixtures were centrifuged, and a 700 μL aliquot of the solution was taken and measured by way of γ-ray spectrometry. The total activity in the aqueous phase was calculated, and the activity sorbed onto the resin was determined by the

subtraction of the total aqueous activity at equilibrium from the initial activity added. Distribution coefficients were then calculated using Equation (1), where $A_{100mg(res.)}$ is the activity in 100 mg of resin and $A_{100\mu L(sol.)}$ is the activity in 100 µL of solution. Meassured equlibrium distribution coefficients thus carry the unit mg/µL.

$$K_D = \frac{C^{eq.}_{phase1}}{C^{eq.}_{phase2}} = \frac{A_{1g(res.)}}{A_{1mL(sol.)}} = \frac{A_{100mg(res.)}}{A_{100\mu L(sol.)}} \qquad (1)$$

2.6.2. Preparation and conditioning of resins and stock solutions

For the determination of distributions coefficients, dry BDGA and ZR resins were weighed and used without preconditioning. Radiotracers ^{44}Ti ($t_{1/2}$ 60.0 a) and ^{46}Sc ($t_{1/2}$ 83.8 d; co-produced via secondary neutron induced reaction ^{45}Sc(n,γ)) were used for measurement of distribution coefficients. These tracers were obtained from the irradiated scandium target dissolution solution. Consequently, stable ^{45}Sc was also present in the samples (at 1 mg/ 64.3 kBq). 100 mg of resin was chosen so as to remain below the resin sorption capacity for scandium.

2.6.2.1. N,N,N',N'-tetrakis-2-éthylhexyldiglycolamide resin

Branched DGA (BDGA) is an extraction chromatographic material in which the extractant system is N,N,N',N'-tetrakis-2-éthylhexyldiglycolamide. Distribution coefficients for BDGA resin were determined as described in 2.6.1. Hydrochloric acid was used as the liquid phase in concentrations ranging from 0.1 – 10 M: 0.1, 1, 4, 6, 8, 10 M. The activities per sample were 0.302 ± 0.056 kBq for ^{44}Ti and 2.455 ± 0.489 kBq for ^{46}Sc, which corresponded to a ^{45}Sc mass of 38.2 µg.

2.6.2.2. ZR hydroxamate resin

ZR hydroxamate resin (ZR resin) was developed for Zr/Y separations, but according to the manufacturer's data, it seemed to show high selectivity for titanium (particularly in comparison with scandium). Distribution coefficients were determined as described in 2.6.1 for hydrochloric acid concentrations ranging from 0.1 - 10 M: 0.1, 1, 4, 6, 8, 10 M. Added activities were 0.148 ± 0.015 kBq for ^{44}Ti and 2.548 ± 0.408 kBq for ^{46}Sc -corresponding to a ^{45}Sc mass of 39.6 μg.

2.7. Dynamic column separation experiments

2.7.1. BDGA

2.7.1.1. BDGA separation experiment

Based on the results of the K_d study (Section 2.6.3), one BDGA resin dynamic column separation experiment was performed. BDGA resin (5mL bed volume) and water (5 mL) were placed in a plastic Bio-Rad column (20 mL), and the resin was preconditioned with 4 M HCl (15 mL). A 5 mL portion of the original target dissolution solution in 4 M HCl, containing 40 kBq of ^{44}Ti and 2.8 mg of ^{45}Sc (180 kBq of ^{46}Sc), was loaded on the column (f1). The gravity flow rate of the column was measured to be ~0.125 mL/min. After elution, the column was washed with 10(f2), 15(f3) and 10(f4) mL of 4 M HCl, respectively. Finally, the column was contacted with 0.1 M HCl (10 mL) (f5).

2.7.1.2. BDGA capacity experiments for scandium

To design a system for the recovery of ^{44}Ti from a bulk mass of scandium, it is mandatory to determine the sorption capacity of BDGA for scandium. The measurement of the sorption capacity was conducted as follows: BDGA (5mL) and water (5 mL) were placed in a plastic

Bio-Rad column (20 mL) and preconditioned with 4 M HCl (15 mL). A solution of 4 M HCl (15 mL), spiked with 40 kBq of ^{44}Ti and 50 mg of ^{45}Sc (1.93 MBq of ^{46}Sc), was loaded on the column (f1). The column was washed with 4 M HCl (10(f2), 11(f3), 6(f4) and 15(f5) mL).

2.7.2. ZR resin

2.7.2.1. ZR resin separation experiment

To make practical use of the obtained distribution coefficients, one ZR resin dynamic separation experiment was performed. ZR resin (1 mL) was mixed with water and loaded on a small plastic Bio-Rad column (5 mL). The resin was then preconditioned with 6 M HCl (10 mL). The loading solution of 6 M HCl (15 mL), spiked with 40 kBq of ^{44}Ti and 6 mg ^{45}Sc (167 kBq of ^{46}Sc), was then loaded on the column, and two fractions of 4(f1) and 11(f2) mL were collected. The gravity flow rate of the resin was measured to be ~0.11 mL/min. Elution of titanium was tested with several different eluents, including oxalic acid and citric acid and a mixture of 6 M HCl/0.65 M H_2O_2.

After scandium was eluted with 6 M HCl (4 mL (fraction f1) and 11 mL (f2), see Fig. 10 of the supplementary material), the column was washed with 0.1 M oxalic acid (5 mL, f3) and 1 M oxalic acid (5 mL (f4) and 1 mL (f5)). The column was subsequently plugged to allow for equilibration at ambient temperature for 24 hours prior to elution. This was followed by 0.1 M citric acid (1mL, f6), after which the column was again allowed to equilibrate at room temperature for 48 hours in contact with the resin. Next, the column was opened and the solution was eluted with citric acid (4 mL, f7) and measured for the presence of ^{44}Ti. The column was

subsequently contacted with a mixture of 6 M HCl/0.65 M H_2O_2 (6 mL (f8)) with no extended contact time.

The suitability of the 6 M HCl/0.65 M H_2O_2 mixture as an eluent for ^{44}Ti was evaluated by contacting the recovered titanium with anion exchanger resin (AG 1-X8, 200-400 mesh size). The recovered ^{44}Ti was evaporated to near dryness and re-suspended in 5 mL of 0.1 M oxalic acid/0.3 M hydrochloric acid (retention factor 370 and 0.3 for titanium and scandium, respectively [19]). This oxalic/hydrochloric acid mixture is expected to elute ^{44g}Sc while 4 M HCl was used to elute ^{44}Ti. Verification of this behavior was used to confirm the speciation behavior of the titanium eluted from hydroxamate based ZR resin.

2.7.2.2. ZR resin capacity experiments for scandium

In a bulk production process separation, microgram amounts of ^{44}Ti must be separated from 10's of grams of scandium. Therefore, the capacity of ZR resin for scandium was determined. 1 mL of the resin and water (5 mL) were loaded on a small Bio-Rad column (5 mL) and preconditioned with 6 M HCl (10 mL). Scandium metal (1 g) was dissolved in 6 M HCl (15 mL) and spiked with ^{44}Ti (40 kBq) and $^{46/45}$Sc (167 kBq). This "mock up" target solution was loaded on the preconditioned column. After loading (f1, f2, f3 5 mL each), the column was washed successively with 6 M HCl (5 mL volume, fraction f4; and 10 mL volume, fraction f5).

2.7.2.3. Scaled-up separation experiment

A scaled-up separation experiment was conducted remotely inside the LANL hot cell facility with an aliquot containing 4 g of the irradiated scandium metal target that contained 65.2 MBq of

^{44}Ti and (257 MBq of ^{46}Sc). ZR resin (5 mL) was placed into plastic Bio-Rad column (1.5x 12 cm, 20 mL) and preconditioned with 6 M HCl (10 mL). The column was then transferred into the hot cell. The scandium target portion was dissolved in 6 M HCl (150 mL) and loaded on the column. After loading, the column was washed with additional 150 mL and 15 mL of 6 M HCl (f2, f3) to remove residual scandium. The column was eluted with 6 M HCl/0.65 M H_2O_2 (5(f4), 10(f5), and 20(f6) mL). The final eluent was analyzed by γ-spectrometry for ^{44}Ti yield and for Ti/Sc separation factor determination. Additionally, the titanium fraction was analyzed via ICP-AES for the presence of other chemical impurities.

2.7.2.4. *Generator concept based on ZR resin*

Due to the high retention of ^{44}Ti on ZR resin, several experiments related to a possible ^{44}Ti/^{44g}Sc generator system design via direct elution and reverse flow elution modes were conducted.

Direct elution

ZR resin (1 mL) was placed in a Bio-Rad plastic column (0.8 x 4 cm, 5 mL) and preconditioned with 6 M HCl (5 mL). A portion of ^{44}Ti (9.8 kBq) in 6M HCl was loaded on to the column. The column was then eluted with 12 mL of 0.05 M HCl in 1 mL fractions, followed by 85 mL of 0.05 M HCl in 5 mL fractions. The activity of ^{44g}Sc and ^{44}Ti was measured immediately after elution using γ ray spectrometry.

Reverse elution

The schematic in Fig. 1 shows a prototypical generator design with “forward” and “reverse” flow elution mode capabilities. Alternating the direction of flow has been shown to minimize

breakthrough [19]. To evaluate the performance of a combined forward/reverse flow generator, 2 mL of ZR resin (conditioned in 0.05 M HCl) was loaded on a PEEK column from VICI Valco Instruments (Houston, TX, USA) (4x150 mm) such as to allow the placement of the ^{44}Ti fraction in the center of the column before loading an additional 1 mL resin. The loading fraction contained 3.7 MBq of ^{44}Ti. Elutions with 5 mL 0.05 M HCl were carried out not more frequently than once daily. This allowed for the ingrowth of ^{44g}Sc between elutions. Fractions were measured via γ-ray spectrometry immediately to determine ^{44g}Sc elution yields and again after 40 hours to assess ^{44}Ti breakthrough.

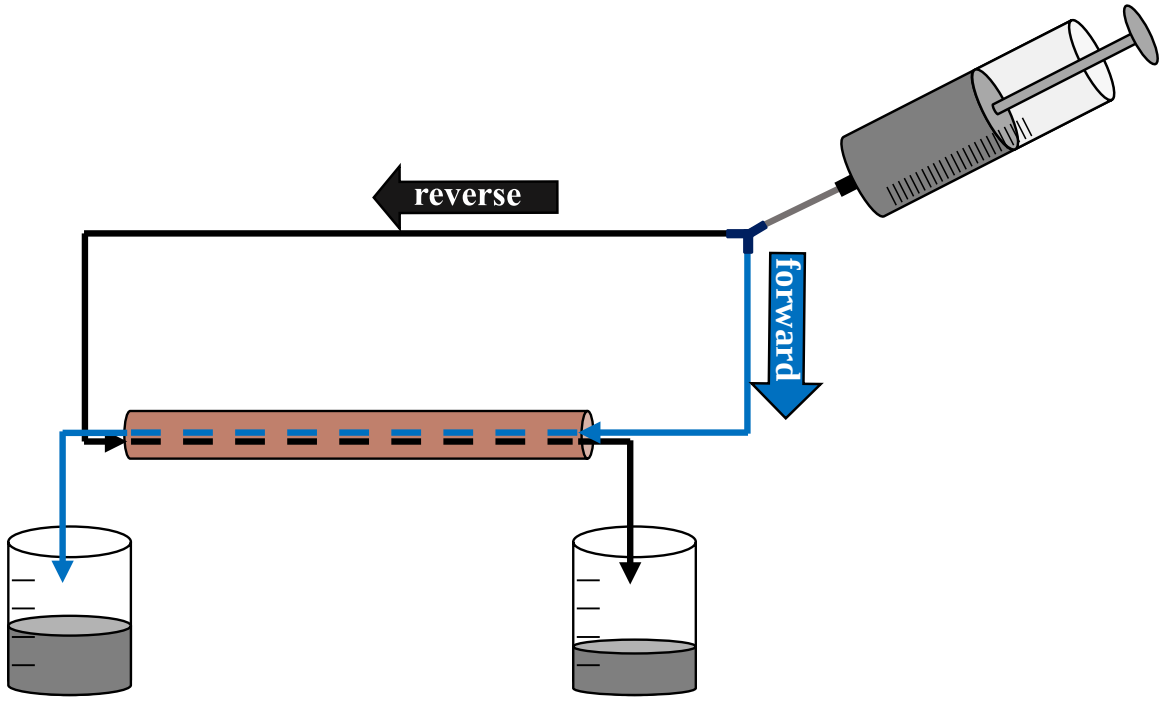

Fig. 1. Schematic concept of a forward/reverse flow radionuclide generator.

2.8. Labeling

The clinical viability of the ZR hydroxamate based resin as the potential solid phase in a ^{44}Ti/^{44g}Sc generator system and the purity of the subsequently obtained ^{44g}Sc was evaluated by way of a labeling verification study. DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic

acid) was used as the chelating agent in recognition of its wide use in radiopharmaceutical labeling. In a preliminary labeling experiment, ^{44g}Sc was eluted from a ZR hydroxamate column using 0.05M HCl. This solution (110 μL) contained ~300 kBq ^{44g}Sc and was added to 0.25 M ammonium acetate (1 mL), resulting in a pH of ~4. This was followed by the addition of 5 μL of DOTA in 0.25 M ammonium acetate solution to result in a final DOTA concentration of 30 nM. The sample was heated in a water bath at 80℃ for 15 minutes. The aliquot (5 μL) was analyzed by thin layer chromatography (TLC) using silica coated aluminum plates as solid support and 0.1 M sodium citrate (pH 4) as the mobile phase according to previously reported protocol [9]. The promising preliminary results then prompted an experiment varying the concentration of DOTA. Each sample contained ~120 kBq ^{44g}Sc in 1 mL $C_2H_3O_2NH_4$ and 5 μL DOTA of concentration varying from 0.01 – 10 nM. After 20 and 40 minute intervals, TLC plates were spotted with the solution and analyzed by γ-ray spectrometry to determine the yield.

3. Results

3.1. Production of ^{44}Ti and target dissolution

The physical production yield was reported previously [24] and amounted to 200 ± 15 MBq of ^{44}Ti via ^{45}Sc(p,2n) ^{44}Ti reaction and ~ 40 ± 3 GBq of ^{46}Sc via ^{45}Sc(n,γ)^{46}Sc at EOB. After dissolution, aliquots of the scandium target dissolution solution were used for K_d studies and dynamic column separation experiments.

3.2. K_d studies

3.2.1. Branched DGA resin

The procedure for determining the K_d function was performed as outlined in Section 2.6.1 and Section 2.6.2. Fig. 2 depicts the distribution coefficients of ^{46}Sc (as a scandium analogue radio-tracer) and ^{44}Ti on branched DGA resin as a function of HCl concentration. The ideal overall separation scheme is one in which ^{44}Ti from the loading fraction is selectively retained on the resin column while the bulk mass of scandium is eluted. On BDGA resin, however, a reverse behavior is exhibited: at concentrations below 8 M HCl, the resin preferentially sorbed the scandium over titanium. This separation behavior is most pronounced at a concentration of 4 M HCl, where scandium has a retention factor of ~730 while titanium is freely eluted ($K_d \leq 1$).

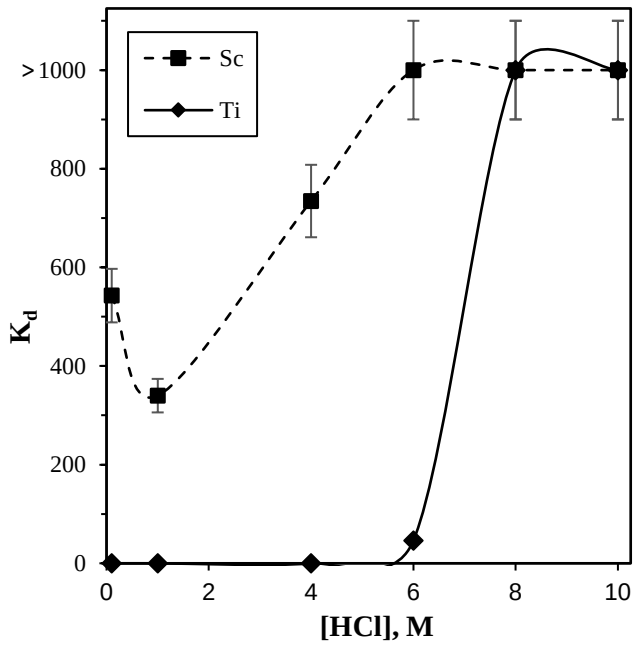

Fig. 2. Acid dependency of K_d for $^{44}Ti/^{46}Sc$ on branched DGA resin in HCl concentrations ranging from 0.1-10M.

This behavior resembles that of titanium and scandium on a strongly acidic cation exchanger resin [19, 22]. As discussed in introductory remarks, the current state of the art involves using a cation exchanger resin (AG 50W-X8, 100-200 mesh size, 10 mL volume) for fine purification of titanium from trace amounts of scandium. Given the similarities in behavior, it is conceivable

that DGA resin extraction chromatography may serve as an alternative to the cation exchange resin step for the fine purification stage of the incumbent chemical ^{44}Ti recovery procedure [24].

3.2.2. ZR hydroxamate resin K_d

The procedure for determining the K_d profile was performed as outlined in Section 2.3.1 and Section 2.3.3. Fig. 3 depicts the distribution coefficients of ^{46}Sc and ^{44}Ti on ZR hydroxamate resin as a function of HCl concentration. Unlike the DGA resin, titanium strongly sorbs on the ZR hydroxamate resin; the K_d is greater than 1000 across all tested concentrations (0.1 – 10 M HCl). Furthermore, the resin exhibits only weak affinity for Sc with K_d values less than 3 in all cases. The separation factor is highest at a concentration of 6 M HCl, though it is evidently high across the entire evaluated concentration range. The observed selectivity of ZR hydroxamate resin to titanium renders the resin suitable as a ^{44}Ti retention stationary phase in a ^{44}Ti/^{44g}Sc radiochemical generator.

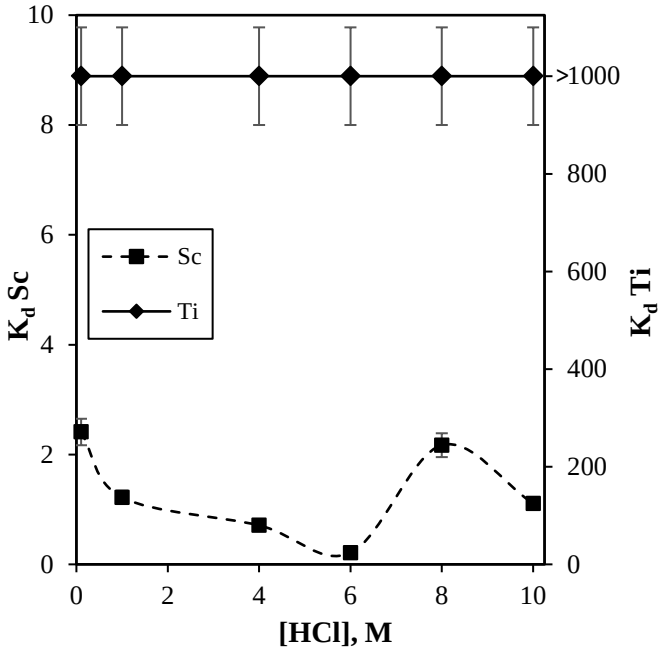

Fig. 3. HCl concentration dependency of K_d for ^{44}Ti/^{46}Sc on ZR hydroxamate resin.

3.3. Dynamic column separation studies

3.3.1. Branched DGA

3.3.1.1. Ti/Sc separation experiment

The elution profile for Ti/Sc on a DGA column (20 mL Bio-Rad 1.5x12cm, 5 mL resin) is shown in Fig. 4. Based on the results of the K_d study for Ti/Sc on DGA resin (see Fig. 2), 4 M HCl was used to load the initial ^{46}Sc and ^{44}Ti activities on the column. Titanium was eluted from the column with additional 4 M HCl washes (35 mL total wash volume) and contained no detectable scandium. The second fraction contained the largest amount of ^{44}Ti activity (~57%), followed by the loading fraction with ~27%. The final 4 M HCl wash (f4) contained less than 1% of the total activity. Overall, more than 98% of the ^{44}Ti was recovered. Elution of scandium was achieved using 0.1 M HCl. The Ti/Sc separation factor was determined by gamma spectrometry to be 10^5. For comparison, the experimental Ti/Sc separation factor for the cation exchanger resin step as reported earlier was approximately 10^4 [24].

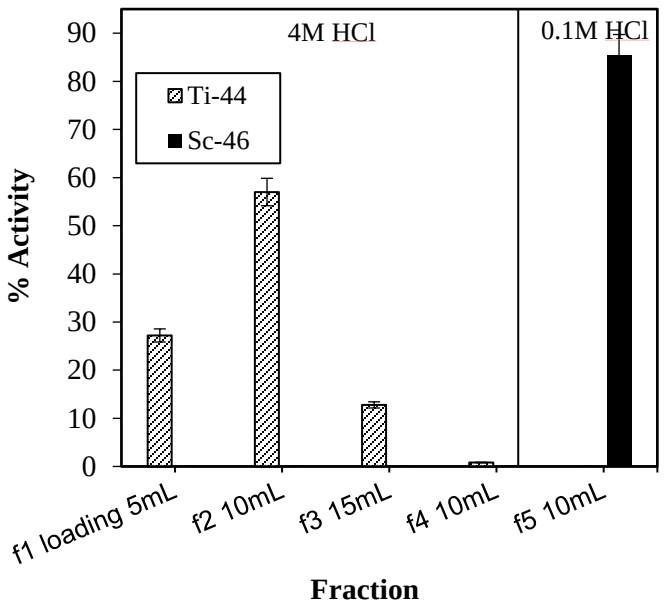

Fig. 4. ^{44}Ti/^{46}Sc elution profile using branched DGA resin with 2.8 mg of scandium.

3.3.1.2. Scandium capacity experiment

To determine the Ti/Sc sorption capacity for BDGA resin, an additional experiment was conducted using an increased mass of natural scandium. The elution profile for Ti/Sc on a BDGA column (20 mL Bio-Rad 1.5x12 cm, 5 mL resin) is available as supplementary information to this paper. The loading fraction (f1 15 mL) contained 30 mg of scandium (greater than ten times the scandium mass as compared to the preliminary separation experiment), which represents a more accurate reflection of the bulk production conditions. The loading matrix was 4 M HCl. The first fraction contained ~76% of the total titanium activity but also showed scandium breakthrough (~7% of total scandium activity). The most significant breakthrough is seen in the second fraction with ~21% of the total scandium activity eluted. This considerable

breakthrough of scandium at 4 M HCl demonstrates that this resin is not suitable to selectively elute titanium from matrices with larger scandium masses.

3.3.2. ZR hydroxamate resin

3.3.2.1. Ti/Sc separation experiment

The elution profile for Ti/Sc on a ZR hydroxamate column (5 mL Bio-Rad 0.8x4 cm, 1 mL resin) is available as supplementary information. The loading fraction contained ~6 mg of scandium. As informed by the K_d study for Ti/Sc (see Fig. 3), 6 M HCl was used for the loading fraction which was spiked with ~40 kBq of ^{44}Ti. The first 4 mL fraction contained the majority of the scandium activity (~62%), while neither f1 nor f2 contained detectable titanium. Given that the ZR hydroxamate resin strongly retains titanium in HCl media, eluents other than HCl were tested for the elution of titanium.

Low concentration oxalic acid (5 mL; 0.1 M) did not elute titanium, and with an increased concentration (1 M) only ~8% of the total titanium activity eluted after a contact time of 24 hours. Citric acid (1 mL, 0.1 M) further eluted 41% of the titanium after a contact time of 48 hours. Fraction 7 (4 mL, 0.1 M citric acid) was eluted without any extended contact time and yielded only ~1% of the total ^{44}Ti activity. Finally, a mixture of hydrogen peroxide and hydrochloric acid media (0.65 M H_2O_2/6 M HCl) eluted the remainder of the titanium (~43%) without any additional contact time. The total recovery of ^{44}Ti exceeded 95% of the original tracer activity, and a Ti/Sc separation factor of 10^5 was achieved.

To validate the suitability of hydrogen peroxide/hydrochloric acid mixtures as an eluent for titanium, the resultant titanium speciation was tested on an anion exchanger resin. Both the loading fraction eluate (5 mL 0.1 M $C_2H_2O_4$/0.3 M HCl) and an additional 5 mL wash showed elution of ^{44g}Sc without any titanium breakthrough. A subsequent 15 mL of 4 M HCl eluted more than 99% of the titanium from the column. This validates the suitability of the titanium speciation formed in the HCl/H_2O_2 matrix form of the titanium for further use in an anion exchanger based ^{44}Ti/^{44g}Sc generator.

3.3.2.2. Capacity experiment

The capacity of ZR hydroxamate resin was tested as described in Section 2.7.2.2. The 15 mL loading solution (f1, f2, f3) eluted 76% of the total ^{44g}Sc without any ^{44}Ti breakthrough. Subsequent washes with 6M HCl (f4, f5) did not containing detectable ^{44}Ti, and an overall separation factor of 10^5 was achieved (Fig. 10). Therefore, despite the higher scandium mass and the corresponding increased ionic strength, the resin remained selective for the titanium: a favorable quality for the isolation of ^{44}Ti from bulk scandium target masses.

3.3.2.3. Scaled-up experiment

To further confirm the suitability of ZR hydroxamate resin as an alternative stationary chromatographic phase for the bulk separation of Ti/Sc, a scale-up experiment was conducted (Section 2.7.2.3). The elution profile for this separation is shown in Fig. 5. The loading fraction (150 mL of 6 M HCl) contained more than 93% of the initial scandium activity. A further volume of 150 mL of 6 M HCl wash eluted the remaining ^{44g}Sc activity. After 300 mL of ^{44g}Sc elution without titanium breakthrough, a final 15 mL fraction of 6 M HCl (f3) stripped ~1.1% of

the total titanium activity. More than 95% of the sorbed titanium was then recovered using a total of 35 mL 0.65 M H_2O_2/6M HCl in which >82% of the titanium activity was eluted in the first 5 mL.

An aliquot of the titanium fraction (f4, f5, f6) was then evaluated by γ-ray spectrometry to reveal that a Ti/Sc separation factor of $\geq 10^3$ was achieved. In addition, this fraction was analyzed via ICP-AES to determine chemical purity and specific activity of the sample. The specific activity of titanium was found to be 0.342 MBq/μg Ti. The specific activity of scandium, an important indicator of medical application viability, was determined to be 3.527 MBq/mg Sc, corresponding to 19.4 mg of ^{45}Sc in the purified Ti fraction. Ultimately, results from ICP confirm the Ti/Sc separation factor determined by γ ray spectrometry as $\geq 10^3$.

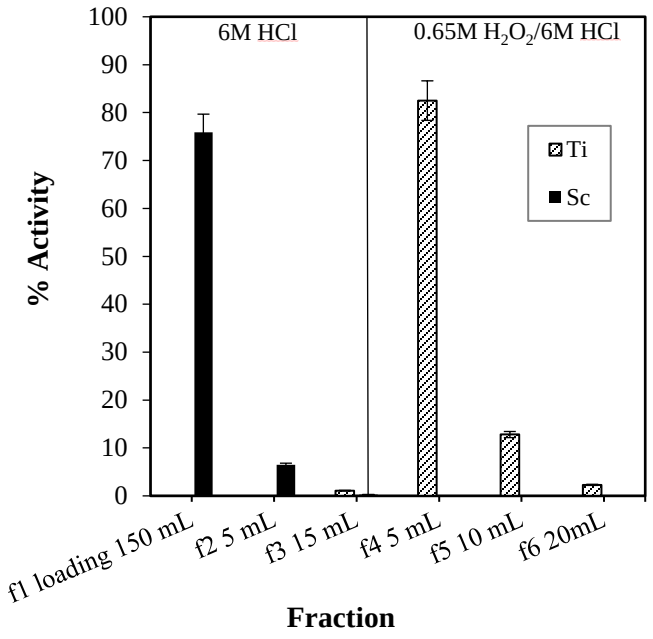

Fig. 5. ^{44}Ti/^{46}Sc elution profile using ZR hydroxamate resin with a load of 4g of scandium.

3.3.2.4. Generator study

Direct elution

Given that the ZR hydroxamate resin allows for efficient elution of scandium with high retention of titanium, this resin could potentially serve as stationary phase in a ^{44r}Ti/^{44g}Sc generator. The direct elution was tested using 0.05 M HCl as the eluent collecting 1 mL fractions (1 bed volume) for the first 12 mL then collecting 5 mL fractions for 17 more elution steps. This preliminary study revealed titanium breakthrough after ~40 resin bed volumes, representing about 0.23% of the total titanium activity on the column.

Reverse elution

As seen in Fig. 1, the reverse elution experiment involves loading the ^{44}Ti parent on the center section of a column and alternating the direction of flow after each elution. A photograph is available as supplementary information. A much smaller amount of titanium breakthrough was observed using the reverse-elution mode; at the time of publishing, 65 column volumes were tested. Please refer to the supplementary information for more details. Titanium-44 breakthrough was only observed in the reverse direction and amounted to ~15 Bq (corresponding to 4.1E-4 % of the initial activity load). No breakthrough was detectable in the forward direction as shown in Fig. 3. This was likely due to an off-center ^{44}Ti loading activity placement. Further work is required to evaluate the long-term performance of this generator configuration.

3.4. Labeling studies

Labeling studies focused on ^{44g}Sc labeling of the common trivalent cation chelator DOTA. In the first experiment, 30 nmols of DOTA were labeled with ^{44g}Sc. It was found that 6.0 % of the

initial ^{44g}Sc activity rose to the top of the plate (indicating the fraction of unchelated scandium) while 94% of the ^{44g}Sc were determined to bind with DOTA (please refer to supplementary data for a representative radiograph of the radiolabeling study). The Sc:DOTA ratio tested in this preliminary experiment was 1:15.

The high labeling yield obtained in this first experiment then prompted a follow-up study with increasing Sc:DOTA labeling ratios. The labeling yield plot is shown in Fig. 6 with DOTA concentrations varying from 0.01 – 10 nM, that is, Sc:DOTA ratios ranging from 1:0.017 – 1:16.7. With DOTA concentrations ≤ 0.1 nM, additional heating time did not increase the labeling yields and they remained consistently below 15%. After 40 minutes of heating, analyses showed that 1 nM DOTA (Sc:DOTA 1:1.67) can be used to achieve labeling yields greater than 60%.

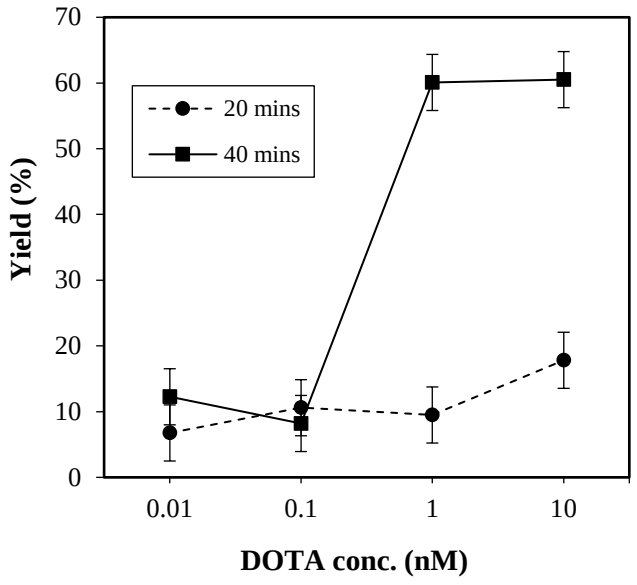

Fig. 6. Labeling yield of ^{44}Sc and DOTA in DOTA concentrations varying from 0.01 – 10 M.

4. Discussion

4.1. Ti and Sc sorption on BDGA resin

In the case of BDGA resin, scandium exhibits increasingly strong retention as the HCl concentration increases. Conversely, titanium shows relatively low sorption ($K_d \leq 1$) on BDGA resin at HCl concentrations below 6 $molL^{-1}$. Dynamic column separation experiments confirmed Ti/Sc behavior as deduced from determined equilibrium distribution coefficients. High retention of scandium in 6 M HCl was observed while titanium eluted. A Ti/Sc separation factor of 10^5 was achieved. A capacity test resulted in a rather low BDGA sorption capacity for scandium; immediate scandium breakthrough was observed for a ratio of 6 mg of Sc per 1 mL of resin. Considering this, BDGA may be used for Ti/Sc trace separations in the context of ^{44}Ti fine purification from residual scandium target material.

4.2. Ti and Sc sorption on ZR (hydroxamate) resin

ZR hydroxamate resin exhibited high sorption affinity for titanium, especially as compared to scandium, which was shown to elute at all tested HCl concentrations. K_d values revealed minimal retention of scandium ($K_d \leq 1$) and very high retention of titanium ($K_d > 1000$) in 6 M HCl matrices. Dynamic column separations confirmed K_d results, and a very high Ti/Sc separation factor ($\geq 10^5$) can be achieved in 6 M HCl. The strong sorption of ^{44}Ti was associated with some desorption challenges. Several eluents were tested, including citric acid, oxalic acid and a mixture of 6 M HCl/0.65 M H_2O_2. In the case of carboxylic acid eluents, significant contact time was required to re-chelate titanium and remove it from the stationary phase. A mixture of 6 M HCl/0.65 M H_2O_2 eluted the ^{44}Ti activity in 6 column bed volumes with no additional contact time. A scaled up experiment demonstrated the suitability of ZR resin for separating ^{44}Ti from

bulk scandium target mass. A Ti/Sc separation factor ≥10^3 and high recovery yield (≥94%) were achieved and support the claim that ZR resin can in principal be employed for the recovery of ^{44}Ti from a proton-irradiated scandium target. Due to very high titanium retention, the separation factor can be further increased by more washes with 6 M HCl, if needed.

4.3. ^{44}Ti/^{44g}Sc generator based on ZR resin

The strong retention of ^{44}Ti on ZR resin was exploited for a basic ^{44}Ti/^{44g}Sc generator design. Initially, a generator concept with direct elution was tested and showed increasing levels of ^{44}Ti breakthrough, from ~20 Bq to ~80 Bq (a four-fold increase), after 40 column bed volume elutions. This behavior would be not conducive to long-term ^{44}Ti/^{44g}Sc generator operations. An advanced generator concept involving alternating directions of elution flow, first proposed by Filosofov et al. [19], was then tested. The motivation for this "alternating flow" type generator was to prevent ^{44}Ti breakthrough and improve long-term generator stability. Reverse elution operation showed significant advantage over the direct elution by decreasing ^{44}Ti breakthrough. In our results, ^{44}Ti breakthrough was only detectable in the reverse elution direction. This can be explained by an off-center placement of initial ^{44}Ti activity on the column. Results still show promise for the practical application of a Ti/Sc generator based on ZR resin with a constant, very low ^{44}Ti breakthrough of ~15 Bq with roughly every other elution. The estimated yearly loss under these conditions would be 2.7 kBq, which corresponds to 0.07 % of the initial activity, which allows for years of operation. Optimal ^{44}Ti load activity placement could likely result in even lower breakthrough levels. Long-term performance of this prototypical system remains to be addressed.

As with other extraction chromatography resins, ZR resin operates on a liquid-liquid extraction mechanism and may as such be prone to leaching and sorption performance deterioration over time. For the purpose of separation of ^{44}Ti from bulk scandium mass, where a rather small amount of resin is needed (1 mL of resin/g of scandium), we designed the procedure under the assumption that fresh resin be utilized every time. However, the potential of capacity loss and leaching becomes a concern with respect to a possible ^{44}Ti/^{44g}Sc generator design based on ZR resin. For the number of elutions tested in our study, no elution yield or labeling efficiency losses owing to resin deterioration were observed. For a more robust ^{44}Ti/^{44g}Sc generator concept, authors are considering covalently bound hydroxamate moieties as stationary sorption bases. Covalently bound hydroxamate resins are currently in use for the separation of ^{89}Zr from bulk mass yttrium targets. [25]

4.4. Labeling experiments

To examine the suitability and purity of the ^{44g}Sc obtained, labeling tests were performed with DOTA, the most commonly used chelator for Sc^{3+}. DOTA labeling with a ratio of ^{44g}Sc:DOTA 1:15 exhibited >94% yield after 15 minutes of reaction time. Furthermore, radiolabeling testing with an increased 44Sc:DOTA ratio of 1:1.67 showed promising results, thereby suggesting the utility of ^{44g}Sc -as derived from recovered ^{44}Ti in this study- for radiopharmaceutical applications.

5. Conclusion

Both branched DGA and ZR (hydroxamate) resins hold promise for efficient and fast Ti/Sc separations. Since BDGA strongly sorbs scandium, it should preferably be used for ^{44}Ti fine

purification in the absence of larger scnadium amounts. ZR hydroxamate, on the other hand, proved to be highly suitable for the recovery of no carrier added ^{44}Ti from the bulk scandium matrices. This resin is also a candidate for long-term ^{44}Ti sorption in a possible ^{44}Ti/^{44g}Sc generator set-up. Results showed that ^{44g}Sc derived from ^{44}Ti as sorbed on ZR hydroxamate resin was associated with high radiochemical and radionuclidic purity and could be utilized for DOTA chelation with high specific activity. Depending on yet to be established pharmaceutical requirements, an additional sorption/elution step may have to be implemented for further purification of the ^{44g}Sc product. Further work towards commercialization of the generator for diagnostic applications will entail (1) a long-term generator performance study with a higher ^{44}Ti activity load addressing ^{44g}Sc elution yields, ^{44}Ti breakthrough levels as well as resin stability, and (2) systematic labeling and in vitro studies utilizing ^{44g}Sc derived from the generator.

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Appendix A. Supplementary data

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