A ^{44}Ti/^{44}Sc radionuclide generator for potential application of ^{44}Sc-based PET-radiopharmaceuticals

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Summary. ^{44}Ti/^{44}Sc radionuclide generators are of interest for molecular imaging. The 3.97 h half-life of ^{44}Sc and its high positron branching of 94% may stimulate the application of ^{44}Sc labelled PET radiopharmaceuticals. However, both ^{44}Ti production and ^{44}Ti/^{44}Sc generator design represent challenges for basic radiochemistry.

About 5 mCi (185 MBq) of ^{44}Ti were obtained *via* the Sc($p, 2n$) nuclear reaction. ^{44}Ti was separated from 1.5 g of massive scandium targets in multi-step procedures, including exchange chromatography on cation exchange resin (AG 50W-X8, 200–400 mesh, H$^+$-form). In order to design a robust ^{44}Ti/^{44}Sc generator concept, distribution coefficients of Ti(IV) and Sc(III) on both AG 1-X8 (200–400 mesh, Cl$^-$-form) and AG 50W-X8 (200–400 mesh, H$^+$-form) resins eluted with HCl and HCl/H$_2$C$_2$O$_4$ solution of various concentrations were investigated systematically. Optimal conditions for efficient separations of both radionuclides have been determined for AG 1-X8 resin and mixtures of 0.07 M HCl and 0.005 M H$_2$C$_2$O$_4$.

A 5 mCi generator was prepared on an AG 1-X8 column ($H = 150$ mm, $D = 3$ mm, $V_0 = 0.55$ mL). The system achieved elution of 180 MBq ^{44}Sc in 20 mL of eluate solution. The breakthrough of ^{44}Ti is 90 Bq. This corresponds to an excellent separation factor of 2×10^6.

In the context of long-term stability of ^{44}Ti/^{44}Sc generators, a "reverse" type of washing steps after each elution using 0.07 M HCl/0.005 M H$_2$C$_2$O$_4$ mixtures appeared to be essential.

1. Introduction

Radionuclide generator systems as means of separating a desired radioisotope from its parent and other contaminants have proved to be extremely useful. The radiochemical separation concept, however, should meet certain requirements in order to be of real value: (1) it must rapidly yield the generator daughter in high purity in a chemical form suitable for further application; (2) the parent material must remain, or be reconverted readily to a form with which the process can be repeated and (3) it should be simple to handle, oper-

ate and shield. Potential radionuclide generators have been considered since decades, *e.g.* [1].

For PET imaging, the ^{82}Sr/^{82}Rb generator with its relatively long-lived mother radionuclide has already shown significant clinical value. Recently, the ^{68}Ge/^{68}Ga radionuclide generator has shown significant potential for molecular imaging [2–4]. The high positron branching of 89% and the kit-type of radiopharmaceutical syntheses offer excellent parameters for the routine use of ^{68}Ga labelled tracers in nuclear medicine using state-of-the-art positron emission tomography (PET) and PET/CT. A clinical breakthrough was achieved demonstrating the superior possibilities of ^{68}Ga-DOTA-octreotide derivatives for localising neuroendocrine tumours, in particular if PET/CT is used. However, the physical half-life of ^{68}Ga ($T_{1/2} = 67.71$ min) might limit the spectrum of clinical applications of ^{68}Ga labelled radiodiagnostics. Furthermore, ^{68}Ga labelled analogues of endoradiotherapeuticals of longer biological half-live such as ^{90}Y- or ^{177}Lu-labelled peptides and proteins cannot be used to determine individual radiation dosimetry.

Thus, radionuclide generator systems providing positron emitting daughters of extended physical half-life are of renewed interest [4].

An important characteristic of positron-emitting ^{44}Sc is its cyclotron-independent availability *via* the ^{44}Ti/^{44}Sc radionuclide generator system. The long-lived ^{44}Ti produces a short-lived ^{44}Sc, which subsequently decays to stable ^{44}Ca. The physical half-life of ^{44}Sc is $T_{1/2} = 3.97$ h, its positron branching is 94.27% [5]. The ^{44}Ti half-life varies in different studies from 39 to 66.6 years [8–12]. Most recent studies revealed a half-life of 60.6 ± 1.3 years [10–12].

A crucial issue in the development of ^{44}Ti/^{44}Sc radionuclide generator systems consists of the production of ^{44}Ti. The ^{45}Sc($p, 2n$)^{44}Ti process seems to be an effective nuclear reaction, however, due to the long physical half-life, cyclotrons of high proton flux are mandatory.

For preparation of ^{44}Ti/^{44}Sc radionuclide generators, several radiochemical criteria are relevant, such as effective separation strategies providing high ^{44}Sc elution yields and low ^{44}Ti breakthrough, high long-term stability, and type of Sc eluates useful for subsequent labelling reactions (*i.e.* low volume, low pH, high purity *etc.*), *cf*. [4].

Sc(III) is strongly adsorbed from oxalic acid solution, and its oxalate complex is selectively destroyed by the

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addition of hydrochloric acid. These data can be used as the basis of a method for the anion exchange separation of Sc(III) and Ti(IV) in oxalic acid/hydrochloric mixtures [13]. In fact, there are only a few reports on studies to develop ^{44}Ti/^{44}Sc radionuclide generators. Using 0.2 M HCl/0.1 M H$_2$C$_2$O$_4$ mixture on Dowex-1 resin, 60–70% elution yield of ^{44}Sc in 30–50 mL was reported [14]. A solvent extraction technique with an organic phase of 1% 1-phenyl-3-methyl-4-capryl-pyrazolone-5 in methyl isobutyl led to > 90% recovery of Sc in less than 10 mL with a Ti contamination of < 10^{-6} [15]. Elution yields of 42–46% and decontamination factor of 5×10^4 were reported in studies with 0.01 M HCl as an eluent and ^{44}Ti being adsorbed on inorganic ZrO$_2$ as an analogue of Ti(IV) [16].

2. Materials and methods

2.1 Chemicals and reagents

Oxalic acid of analytical reagent grade was obtained from Merck (Darmstadt, Germany). All other chemicals were pure reagent grade and used as received unless otherwise specified. The ion exchange resins AG 1-X8 (200–400 mesh, Cl$^-$-form) and AG 50W-X8 (200–400 mesh, H$^+$-form) were purchased at Bio-Rad Laboratories (Richmond, CA, USA). Deionized Milli-Q water (18.2 MΩ cm; Millipore) was used in all reactions.

2.2 Production of ^{44}Ti

Five mCi of ^{44}Ti were produced utilizing the ^{45}Sc$(p, 2n)^{44}$Ti process at an internal proton beam of $E_p \approx 25$ MeV. The 1.5 g Sc targets were covered by intermediate layers of Ag in order to reduce ^{65}Zn contaminations. The target system was capable to withstand long-term irradiations at up to 200 μA as described in [17].

^{46}Sc ($T_{1/2} = 83.79$ d) was produced *via* neutron capture on ^{45}Sc at the TRIGA research reactor Mainz as a tracer both for the determination of Sc(III) distribution coefficients and to follow the separation of ^{44}Ti from the irradiated scandium target.

2.3 Measurement of radioactivities

The absolute radioactivity of ^{44}Sc, ^{46}Sc and ^{44}Ti was measured by γ-spectrometry using a high-purity germanium (HPGe) detector. For detection of ^{44}Ti(^{44}Sc), the 1157 and 1499 keV γ-emissions were used for decay equilibrium. The ^{44}Ti radioactivity was measured also at 67.9 (88%) and 78.4 (94.5%) keV. Measurements of ^{44}Ti radioactivity were performed 120 h after ^{44}Sc measurements (more than 30 half-lives of ^{44}Sc).

All radioactive materials were handled according to approved protocols at the Institute of Nuclear Chemistry at Johannes Gutenberg University.

Measurement of ^{44}Sc high radioactivities was accomplished in a dose calibrator M2316 (Messelektronik, Dresden GmbH). The Curie-meter ^{18}F setting was used with a multiplication factor of 0.7, to account for absolute ^{44}Sc activity.

A small animal PET scanner (Siemens MicroPET Focus 120) was used to analyse the local distribution of ^{44}Ti(^{44}Sc) on the generator columns.

2.4 Separation of ^{44}Ti and ^{44}Sc

The 1.5 g irradiated scandium target was dissolved in 18 mL of 2 M HCl and basic separations of silver as well as co-produced ^{65}Zn and ^{109}Cd have been performed as described earlier [17].

For further separations of macroscopic scandium two aliquots A) ^{44}Ti in 9 mL 2 M HCl (96.6 MBq) and B) ^{44}Ti in 9 mL 2 M HCl (103.5 MBq) were spiked with ^{46}Sc. For cation exchange chromatography, a large column ($H = 350$ mm, $S = 2$ cm^2, $V_0 = 35$ mL) of AG 50W-X8, 200–400 mesh (H$^+$-form) was washed with 1500 mL of 4 M HCl and 50 mL H$_2$O. Aliquot A was brought in the column followed by 7.5 mL H$_2$O and 8 mL 1 M HCl consecutively. After that, aliquot B was brought in the column, then 9 mL H$_2$O and 17.5 mL 1 M HCl. The column was washed with 45 mL 1 M HCl, 30 mL 2 M HCl, 160 mL 3 M HCl, 200 mL 0.5 M H$_2$C$_2$O$_4$ consecutively, *cf*. Table 1.

The second separation was performed using a similar column ($H = 360$ mm, $S = 2$ cm^2, $V_0 = 36$ mL) with AG 50W-X8, 200–400 mesh (H$^+$-form). The column was washed with 1000 mL of 4 M HCl and 50 mL H$_2$O. The fraction N 5 from the first separation was brought in the column, then 170 mL H$_2$O, 45 mL 1 M HCl, 180 mL 2 M HCl and 190 mL 4 M HCl, consecutively. Measurements have been performed similar to the protocol used for the first separation, *cf*. Table 2.

2.5 Final purification of ^{44}Ti

The first purification was performed on a medium-scale column ($H = 170$ mm, $D = 3$ mm, $V_0 = 0.6$ mL) with AG 1-X8 resin, 200–400 mesh (Cl$^-$-form). The column was washed with 10 mL 12 M HCl, 10 mL 1 M HCl, 10 mL H$_2$O and 10 mL 12 M HCl, consecutively. The fractions N 23–24 (from second separation) were brought in the column, then 8.6 mL 12 M HCl, 4.5 mL 8 M HCl, 8 mL 1 M HCl, consecutively, *cf*. Table 2, Fig. 1.

The second purification was made on a smaller column ($H = 150$ mm, $D = 3$ mm, $V_0 = 0.55$ mL) using cation exchange chromatography with the resin AG 50-X8, 200–400 mesh (H$^+$-form). The column was washed with 40 mL 4 M HCl and 5 mL H$_2$O. The ^{44}Ti fraction N 4 (from first separation) as isolated in the anion exchange chromatography in 2 M HCl, as well as the fractions N 22, 25 (from second separation), and N 13–18 (from first purification) as obtained in the initial separations in 2 M HCl, were brought in the column, then 10 mL 1 M HCl and 20 mL 0.3 M HCl were applied. The ^{44}Ti was eluted with 1 M HCl and ^{56}Co with 4 M HCl beginning with fraction N 122, *cf*. Fig. 2.

2.6 Determination of K_d values of Ti and Sc in HCl/ H$_2$C$_2$O$_4$ solution of various concentrations

K_d values for both Sc(III) and Ti(IV) were determined in batch experiments using different concentrations of HCl/H$_2$C$_2$O$_4$ mixtures. ^{44}Ti(^{44}Sc) and ^{46}Sc were used as iso-

topic tracers for Ti(IV) and Sc(III) distributions. A stock solution of ^{44}Ti (30 kBq) and ^{46}Sc (1 mg, 20 mCi) was dried and dissolved in 100 µL 0.1 M $C_2H_2O_4$ (solution X). Aliquots were prepared in Eppendorf 1.5 mL vials with 100 mg of AG 1-X8 (200–400 mesh, Cl$^-$-form) or AG 50-X8 (200–400 mesh, H$^+$-form). To all solutions 1 mL of a HCl/$H_2C_2O_4$ mixture was added, than 5 µL of solution X was added to the solutions 001–009.

A solution Y – an aliquot with ^{44}Ti (117 kBq) with the remaining solution of X and with ^{46}Sc – was dried and dissolved in 0.025 M $H_2C_2O_4$. 5 µL of solution Y was added to vials 010–016.

Another solution Z was prepared from a fraction of ^{44}Ti (27 kBq) with the remaining solution Y and with ^{46}Sc dried and dissolved in 0.005 M $H_2C_2O_4$. 5 µL of solution Z was added to vials 017–026. Each of the vials was shaken for 2 d. Then 400 µL of the liquid phase was taken from every vial, and radioactivity A was measured on the Curie-meter.

K_d was calculated by the following equation:

$$K_d = (4A - 10A')/A'$$

A – activity of a whole vial with resin, A' – activity of a 400 µL sample of the solution after ion-exchange reaction.

2.7 Preparation and evaluation of pilot ^{44}Ti/^{44}Sc radionuclide generators

Two columns made of PEEK (diameter 3 mm, length 40 mm) were prepared in the institute's workshop. Both columns were filled with AG 1-X8, 200–400 mesh, in Br$^-$-form. The columns were washed with 5 mL 12 M HCl and 5 mL H_2O two times. Finally, they were washed with 5 mL 0.1 M $H_2C_2O_4$.

A sample of ^{44}Ti was evaporated to dryness and taken up with 420 µL of 0.1 M $H_2C_2O_4$. The solution obtained was divided into two parts. To each sample 2 mL 0.1 M $H_2C_2O_4$ were added. The two ^{44}Ti fractions of 300 kBq activity were transferred to the generators.

Both generators were eluted using 10 mL of 0.2 M HCl/0.1 M $H_2C_2O_4$ solutions. While generator G1 was eluted in a standard procedure, *i.e.* in a single direction ("direct"), the generator G2 was additionally regenerated after each elution using 0.2 M HCl/0.1 M $H_2C_2O_4$ in alternating direction ("reverse"). Elution of both generators was carried out 3 times a week.

2.8 Improvement of ^{44}Ti distribution profiles of a pilot ^{44}Ti/^{44}Sc radionuclide generator

Elution of the generator G2 was carried out 3 times a week as described earlier. After 50 elutions, the ^{44}Ti distribution profile was analysed using µPET registration. Subsequently, the generator was washed using 4 mL of 0.1 M $H_2C_2O_4$ and 2 mL of 1 M HCl/0.1 M $H_2C_2O_4$ mixture and again 4 mL of 0.1 M $H_2C_2O_4$ consecutively in "reverse" direction. This operation was repeated 5 times. After the "regeneration" protocol, the generator G2 was scanned again.

^{44}Ti distribution profiles on the column of the pilot generator G2 after 50 elutions (a) and after regeneration with 0.1 M $H_2C_2O_4$ and 1 M HCl/0.1 M $H_2C_2O_4$ solutions (b) are compared graphically in Fig. 5.

2.9 Preparation of a 5 mCi ^{44}Ti/^{44}Sc radionuclide generator

For the final generator, a larger column ($H = 150$ mm, $D = 3$ mm, $V_0 = 0.55$ mL) was made of PEEK and filled with resin AG 1-X8 (200–400 mesh, Br$^-$-form). The column was washed with 20 mL 12 M HCl and 10 mL H_2O. Finally, it was washed with 10 mL 0.1 M $H_2C_2O_4$. The fractions with purified ^{44}Ti (185 MBq) were dried and dissolved in 20 mL 0.1 M $H_2C_2O_4$. This solution was brought into the generator which was then washed with 0.07 M HCl/0.005 M $H_2C_2O_4$ mixture in "reverse" direction. Two days later, the generator was eluted for the first time using 20 mL of 0.07 M HCl/0.005 M $H_2C_2O_4$. Eluate aliquots were collected for each 2 mL. One week later, the activity of ^{44}Ti in these samples was analysed by means of γ-spectrometry.

Within several months, the generator was eluted each week according to the same protocol.

3. Results and discussion

3.1 Separation of ^{44}Ti and macroscopic Sc

About 99.9% of the ^{44}Ti produced in the Sc($p, 2n$) nuclear reaction have been isolated in a 48 mL fraction of 2 M HCl. As expected, the scandium separation was not complete with about 500 µg scandium still present in that ^{44}Ti fraction. A second chromatographic separation provided a more complete separation with a separation factor of about 10^5, *i.e.* less than 10^{-3}% (about 15 µg) of the initial scandium still remaining in the ^{44}Ti fraction. In total, about 99.6% of the ^{44}Ti activities have been recovered following cation exchange purification of the no-carrier-added radionuclide from about 1.5 g of a macroscopic scandium target.

Tables 1 and 2 show the results for the ion exchange chromatography for two basic separations, the activities of ^{44}Ti and ^{44}Sc measured in the different fractions using different detectors, *i.e.* Curie-meter and γ-ray spectroscopy. Further purification appeared to be useful. The fractions N 23 and N 24 (Table 2) thus were further purified using anion exchange chromatography, as described later.

3.2 Purification of ^{44}Ti

After the second purification ^{44}Ti still contained about 15 µg ^{nat}Sc and ^{56}Co (7 MBq), therefore further purification was performed.

Results for the ion exchange chromatographic purifications are shown in Figs. 1–2 for the two purification procedures. Activities have been analysed using a Curie-meter at two different time points. The ^{44}Ti fraction N 1–12, *cf.* Fig. 1, contains > 99% of the overall ^{44}Ti activity. The amount of ^{nat}Sc and the contamination of ^{56}Co are below the detection limit. This highly-pure ^{44}Ti was finally used to prepare the first ^{44}Ti/^{44}Sc generator. The fractions 13–18 (Fig. 1), as well as sample N 4 from the 1st (Table 1) separation and 22, 25 from 2nd (Table 2) were transferred to another purification process, *cf.* Fig. 2.

Fractions N 108–119 were very pure, with ^{nat}Sc and ^{56}Co separated quantitatively. The highly-pure fractions N 110–111 were used for the preparation of two low-activity pilot-generators G1 and G2, in order to investigate the radiochem-

Table 1. Initial ^{44}Ti/Sc separation. AG 50W-X8, 200–400 mesh (H$^+$-form), $H = 350$ mm, $S = 2$ cm^2, $V_0 = 35$ mL. Sample N 5 is emphasized as this aliquot was used for subsequent separation.

N	Solution	V (mL)	Activity (MBq)			
			Curie-meter		γ-spectroscopy	
			1st day	2nd day	^{44}Ti	^{46}Sc
1	1 M HCl	40	0.197	0.153	0	0
2	1 M HCl	45	0.888	0.827	0	0
3	1 M HCl	45	0.386	0.314	0	0
4	2 M HCl	47	1.089	1.271	0.18	0
5	*3 M HCl*	*48*	*924.7*	*1040.0*	*204.35*	*2.56*
6	3 M HCl	49	56.96	14.91	0.100	2.36
7	3 M HCl	41	23.84	7.231	0.410	1.14
8	3 M HCl	18	8.260	2.752	0.007	0.280
9	0.5 M H$_2$C$_2$O$_4$	32	13.33	6.570	∼0.020	∼0.150
10	0.5 M H$_2$C$_2$O$_4$	47	2.367	0.667	0.025	0.109
11	0.5 M H$_2$C$_2$O$_4$	37	1.661	0.398	0.008	0.077
12	0.5 M H$_2$C$_2$O$_4$	39	–	0.420	0.008	0.850

Table 2. Second ^{44}Ti/^{nat}Sc separation. AG 50W-X8, 200–400 mesh (H$^+$-form), $H = 360$ mm, $S = 2$ cm^2, $V_0 = 36$ mL. Samples N 23 and 24 are emphasized as these aliquots were used for subsequent purification.

N	Solution	V (mL)	Activity (MBq)			
			Curie-meter		γ-spectroscopy	
			1st day	2nd day	^{44}Ti	^{46}Sc
13	1 M HCl	30	0.036	0.002		
14	1 M HCl	30	0.055	0.008		
15	1 M HCl	30	0.062	0.014		
16	1 M HCl	30	0.082	0.018		
17	1 M HCl	30	0.082	0.020		
18	1 M HCl	30	0.088	0.020		
19	1 M HCl	30	0.088	0.019		
20	1 M HCl	30	0.082	0.014		
21	2 M HCl	30	0.077	0.011		
22	2 M HCl	30	1.692	2.206	0.54	
23	*2 M HCl*	*30*	*561.4*	*771.4*	*188.42*	
24	*2 M HCl*	*30*	*154.8*	*209.0*	*51.04*	
25	2 M HCl	30	13.80	12.99	0.06	
26	2 M HCl	30	0.844	0.295	0.004	
27	4 M HCl	30	1.546	0.064	0.003	0.0004
28	4 M HCl	30	95.90	5.139		1.017
29	4 M HCl	30	46.52	3.105		0.614
30	4 M HCl	30	24.16	1.631		0.323
31	4 M HCl	30	12.85	0.704		0.140
32	4 M HCl	30	8.974	0.358		0.071
33	4 M HCl	30	5.440	0.181		0.036

ical design of ^{44}Ti/^{44}Sc generators in terms of high ^{44}Sc elution yields and low ^{44}Ti breakthrough, but also long-term stability.

3.3 Determination of distribution coefficients of Ti(IV) and Sc(III) on ion exchange resins

Results of the K_d values obtained for the two different ion exchange resins and the various mixtures are shown in Table 3.

Accordingly, the optimum conditions for efficient separations and for the design of generators could be to elute AG 1-X8 resins with 0.2 M HCl/0.1 M H$_2$C$_2$O$_4$, 0.125 M HCl/0.025 M H$_2$C$_2$O$_4$ or 0.06–0.08 M HCl/0.005 M H$_2$C$_2$O$_4$ mixtures.

In the context of further use of ^{44}Sc, *e.g.* for radiopharmaceutical syntheses, it appeares favourable to use eluate mixtures of lower salt concentration, such as 0.06–0.08 M HCl/0.005 M H$_2$C$_2$O$_4$. For other studies, such as, *e.g.*, to investigate the breakthrough of ^{44}Ti even at increasing numbers of elutions, 0.2 M HCl/0.1 M H$_2$C$_2$O$_4$ mixtures can be used as well (*cf*. "Evaluation of ^{44}Ti/^{44}Sc radionuclide generators"). In this case, the somewhat lower K_d values for Ti(IV) compared to the more diluted eluate mixtures, may allow following the eventual breakthrough of ^{44}Ti at a limited number of elutions already.

Table 3. Distribution coefficients of Ti(IV) and Sc(III) in various HCl/H$_2$C$_2$O$_4$ mixtures for cation and anion exchange resins. HCl/H$_2$C$_2$O$_4$ mixtures providing the most useful K_d values are indicated in italic numbers.

| | N | Concentration of solution, mol/L | | K_d | | | |
| | | | | AG 50-X8 | | AG 1-X8 | |
		H$_2$C$_2$O$_4$	HCl	Ti(IV)	Sc(III)	Ti(IV)	Sc(III)
X	001	0.1	0	–	–	> 1000	184
	002	0.1	0.05	–	–	> 1000	41
	003	0.1	0.1	–	–	> 1000	14
	004	0.1	0.15	≪ 1	12.0	> 1000	5.1
	005	*0.1*	*0.20*	≪ 1	10.7	*> 1000*	*1.7*
	006	0.1	0.30	≪ 1	7.0	370	0.2
	007	0.1	0.50	≪ 1	11.2	105	≪ 1
	008	0.1	0.75	~ 0.5	14.0	–	–
	009	0.1	1.0	≪ 1	8.1	17	≪ 1
Y	010	0.025	0	1.0	201	> 1000	954
	011	0.025	0.025	1.0	148	> 1000	168
	012	0.025	0.050	0.6	129	> 1000	40.9
	013	0.025	0.075	1.8	128	> 1000	14.2
	014	*0.025*	*0.125*	3.3	124	*1050*	*2.68*
	015	0.025	0.175	3.1	120	410	0.3
	016	0.025	0.250	2.9	119	290	≪ 1
Z	017	0.005	0	32	7619	> 1000	2340
	018	0.005	0.025	30.4	2378	> 1000	67.2
	019	0.005	0.0375	34.2	2242	> 1000	24.0
	020	0.005	0.05	33.6	2665	> 1000	10.9
	021	*0.005*	*0.065*	*28.2*	*1872*	*> 1000*	*4.0*
	022	0.005	0.08	33	1715	844	1.27
	023	0.005	0.10	33	1646	688	0.71
	024	0.005	0.125	25.6	1398	457	≪ 1
	025	0.005	0.25	–	–	46	≪ 1
	026	0.005	0.5	–	–	3.8	≪ 1
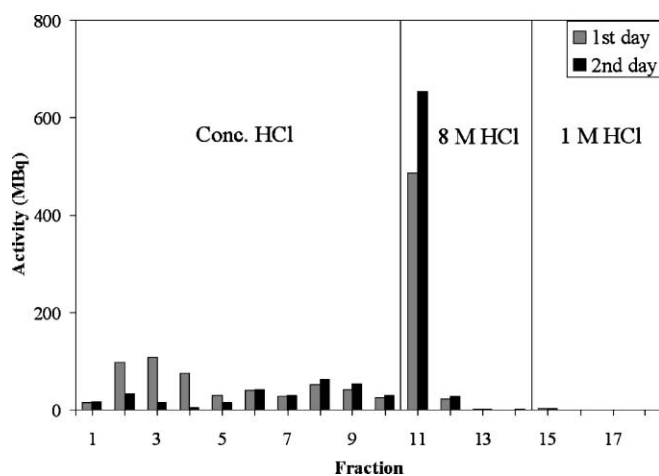
Fig. 1. The distribution of ^{44}Ti after the 1st purification. AG 1-X8 (200–400 mesh Cl$^-$-form). Each fraction volume is 1.2 mL. Activity measured on Curie-meter.
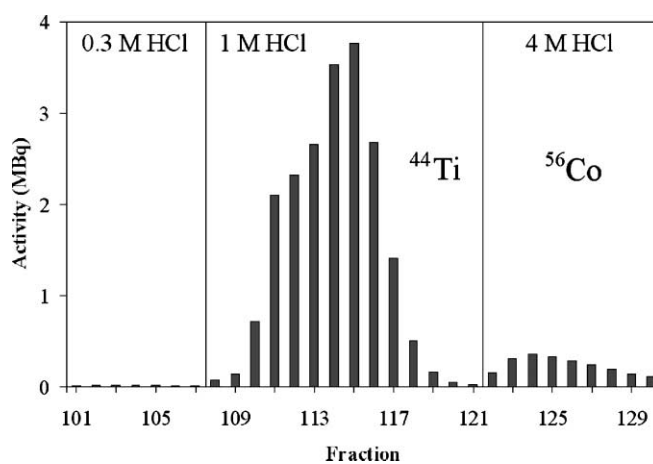
Fig. 2. Distribution of ^{44}Sc and ^{44}Ti after the 2nd purification. AG 50-X8 (200–400 mesh, H$^+$-form). Each fraction volume is 2 mL. Activity measured on Curie-meter.

### 3.4 Evaluation of pilot ^{44}Ti/^{44}Sc radionuclide generators

Fig. 3 illustrates the yield of ^{44}Sc obtained for the increasing number of elutions for both generator types G1 and G2. After about 15 elutions for G1, the eluted activity of ^{44}Sc started to drop, which is due to the increasing breakthrough of ^{44}Ti.

In contrast to G1, the "reverse" elution protocol applied to generator G2, showed a constant yield of ^{44}Sc elutions for the complete 50 elution runs applied. This ^{44}Sc elution profile corresponds to the breakthrough of ^{44}Ti as shown in Fig. 4.

The "direct" elution strategy of pilot generator G1 results in an increasing breakthrough of ^{44}Ti, which results in a 50% desorption of ^{44}Ti after about 30 elutions, and an almost complete release of ^{44}Ti after 50 elutions. In contrast, the breakthrough of ^{44}Ti in the case of the "reverse" type elution scheme is negligible for the first 10 elutions, and increases only slightly in the following 40 elutions. The maximum breakthrough of ^{44}Ti is about 0.2%.
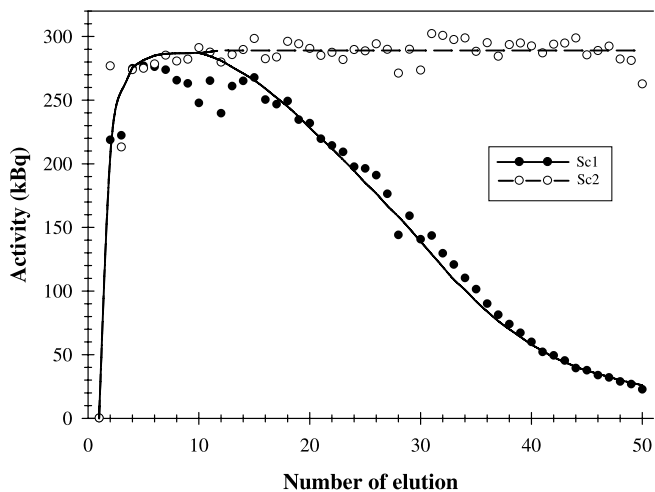
Fig. 3. Yield of ⁴⁴Sc for increasing number of elutions for "direct" (Sc1) and "reverse" (Sc2) elution modes after 50 elutions.
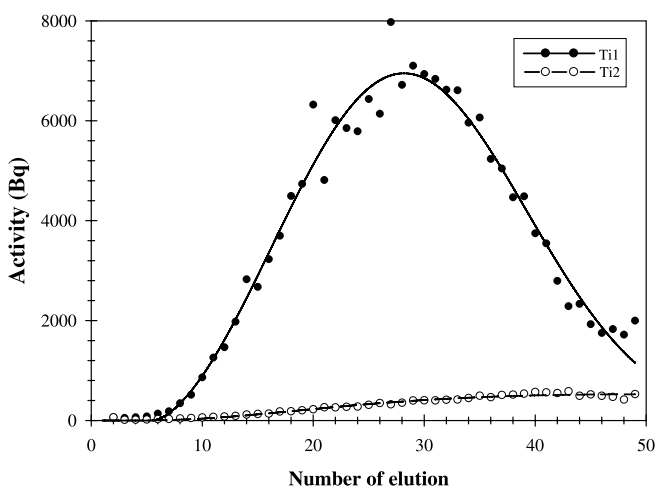
Fig. 4. Breakthrough of ⁴⁴Ti for increasing number of elutions for "direct" (Ti1) and "reverse" (Ti2) elution modes after 50 elutions.
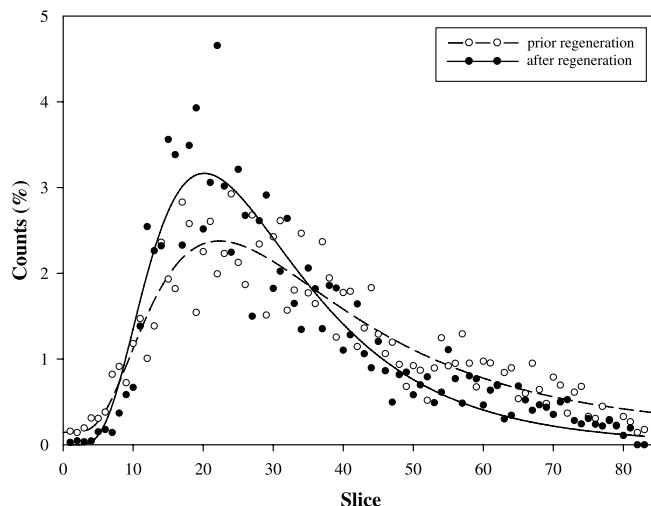
3.5 Regeneration of ⁴⁴Ti distribution profiles on the column

"Reverse" elution modes obviously provide high retention of ⁴⁴Ti on the column. However, the distribution of ⁴⁴Ti is still changing with increasing elutions. We tested the possibility to improve the distribution profile by "reverse" elution with different composition of $H_2C_2O_4$/HCl solutions.

The coronary distribution of ⁴⁴Ti(⁴⁴Sc) on the pilot generator G2 after 50 elutions was analysed by μPET imaging. Quantitative data on the distribution of ⁴⁴Ti/⁴⁴Sc of the generator column are illustrated in Fig. 5. Each slice unit is about 0.5 mm. Obviously, the zone of ⁴⁴Ti became narrower after a "regeneration" procedure.

### 3.6 Preparation of 5 mCi ⁴⁴Ti/⁴⁴Sc radionuclide generator

Typical ⁴⁴Ti/⁴⁴Sc radionuclide generator elution profiles of ⁴⁴Sc are shown in Fig. 6 for the initial experiments (mean data for the elutions 4–7) as well as a typical result for an elution done after one year (elution number 54). The corresponding activities of ⁴⁴Ti in the individual fractions are given in Fig. 7.

**Fig. 5.** Distribution profile in percent for ⁴⁴Ti prior and after "regeneration" as measured by PET.
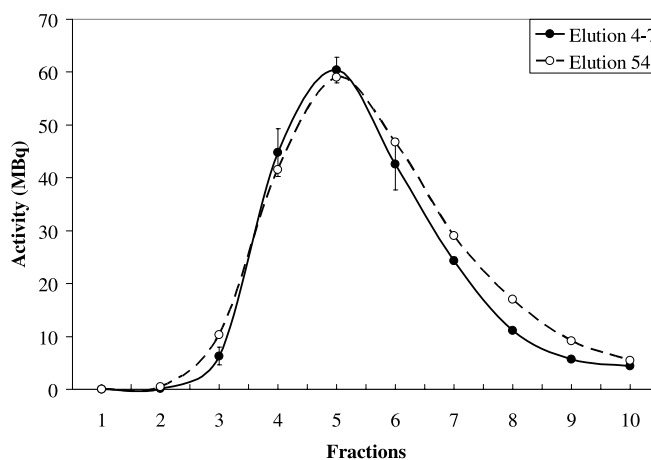
Fig. 6. Elution profile of ⁴⁴Sc (mean of elutions 4–7) and 54 (after 1 year). Curie-meter measurements. Each fraction contains 2 mL.
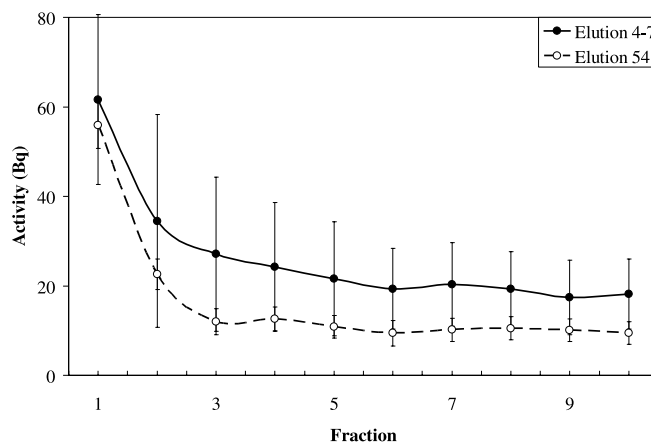
Fig. 7. Breakthrough of ⁴⁴Ti (mean for the elutions 4–7) and 54 (after 1 year). γ-spectroscopy. Each fraction contains 2 mL.

After the first seven elutions of the ⁴⁴Ti/⁴⁴Sc radionuclide generator, the profile of ⁴⁴Sc elutions indicates that the aliquots 4–7 contain $85 \pm 2\%$ of the total ⁴⁴Sc activity. The breakthrough of ⁴⁴Ti (γ-spectroscopy) in all 10 aliquots (20 mL) is less than $10^{-4}\%$ (150 Bq).
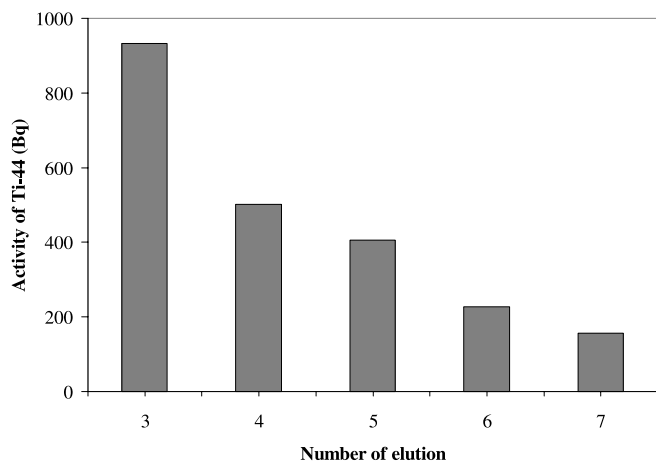
Fig. 8. Total breakthrough of ^{44}Ti for the elutions 3–7. Each fraction contains 20 mL.

Fig. 8 illustrates the overall breakthrough of ^{44}Ti in the total 20 mL eluate volume for the elutions 3 to 7. It indicates an improvement of the breakthrough with increasing number of elutions. While about 1 kBq of ^{44}Ti was found for the third elution, this value is 0.1 kBq only for the 7th elution.

3.7 Final apparative scheme of the "reverse" ^{44}Ti/^{44}Sc-generator

A modular system of the 5 mCi generator was constructed. It presents the central generator column in a horizontal position (II). Two reservoirs for the eluate solutions are connected to the inlet (I) and the outlet (III) position of the generator column. The reservoirs I and III are connected to an air pressure *via* filter F to avoid contaminations of eluate composition with metals from the air. Transfer of eluates from the reservoirs through the generator is achieved by air pressure (elution of ^{44}Sc) or vacuum ("reverse" elution) using empty syringe S.

All parts of the 5 mCi ^{44}Ti/^{44}Sc-generator are connected *via* tubing and 3-way valves. The generator works in a "reverse" scheme of elutions. The initial elution is organized by transferring 20 mL of the eluate solution of reservoir (I) through the generator into the ^{44}Sc vial (IV). After each elution the generator is eluted with the same eluate composition in a reverse way using reservoir (III). While the eluate in
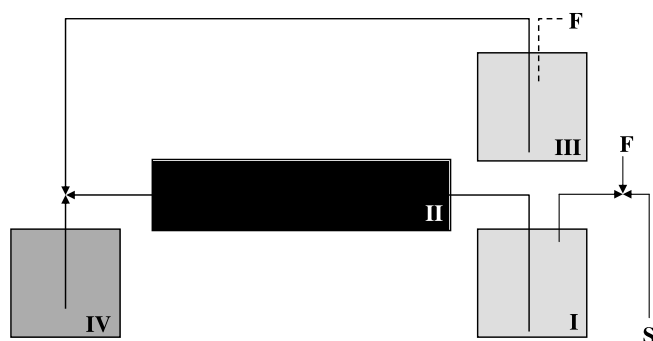
Fig. 9. The final scheme of the "reverse" ^{44}Ti/^{44}Sc-generator. I – 20 mL reservoir, II – generator, III – 500 mL reservoir with 0.005 M $H_2C_2O_4$/0.07 M HCl mixture, IV – collecting vial, F – filter, S – syringe.

Table 4. Comparison of different ^{44}Ti/Sc radionuclide generators.

Year of study	Activity of ^{44}Ti (MBq)	Yield of ^{44}Sc (%)	Eluate volume (mL)	Separation factor
1967 [14]	not given	60–70	30–50	2×10^4
	not given	60–70	50	10^3 (after 40 elution)
1973 [16]	0.037	42–46	30	5×10^4
This work	185	97	20	2×10^6

reservoir (III) is refreshed routinely, the eluate in bottle (I) can be used for next elution of the generator. Eluted ^{44}Sc in collecting vial (IV) can be used for further experiments.

The scheme guarantees for safe handing, as it represents an inherently closed system with respect to ^{44}Ti.

4. Conclusions

Using optimum K_d values of Ti(IV) and Sc(III) for HCl/$H_2C_2O_4$ mixtures, *i.e.* 0.2 M HCl/0.1 M $H_2C_2O_4$ and 0.07 M HCl/0.005 M $H_2C_2O_4$, two low-activity pilot generator and a 5 mCi ^{44}Ti/^{44}Sc generator, respectively, were constructed and evaluated.

After one year of regular elution of the 5 mCi ^{44}Ti/^{44}Sc radionuclide generator, the yield of ^{44}Sc and ^{44}Ti is stable and the breakthrough of ^{44}Ti is very low. The system achieves elution of 97% (180 MBq) ^{44}Sc in 20 mL of eluate solution. The breakthrough of ^{44}Ti is 5×10^{-5}% (90 Bq). This corresponds to an excellent separation factor of 2×10^6. Compared to other ^{44}Ti/^{44}Sc radionuclide generators, higher elution yields of ^{44}Sc in less volume and a higher separation factor were achieved, at higher ^{44}Ti activity compared to only kBq in other studies (Table 4).

Regarding long-term stability of ^{44}Ti/^{44}Sc generators, "direct" generator elutions may not be adequate. In comparison, a "reverse" elution strategy definitely guarantees a very low breakthrough of ^{44}Ti. This does not affect the elution yield of ^{44}Sc.

There is a possibility to even improve the distribution profile, to further reduce the ^{44}Ti breakthrough and to extend the generators shelf-life by "reverse" elution with different composition of HCl/$H_2C_2O_4$ solutions.

Finally, the new radionuclide generator design provides stable high-purity elution of significant activities of ^{44}Sc of 180 MBq per elution. In the context of medical applications of ^{44}Sc eluted from the present generator system, its absolute activities are sufficient for initial studies. However, the ^{44}Sc solution that is obtained from generator appears to be too diluted and too acidic for use in direct labelling procedures. Nevertheless, concentrating the ^{44}Sc solution and reducing the acidity in that ^{44}Sc solution may be added on line to the generator performance described.

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