

Radiochemical separation of ^{82}Sr and the preparation of a sterile $^{82}\text{Sr}/^{82}\text{Rb}$ generator column

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Ion-exchange chromatography using the chelating resins Purolite S950 and Chelex 100 was investigated for the radiochemical separation of ^{82}Sr from a RbCl target. 0.25M NH_4Cl solution was employed for the retention of Sr and elution of Rb, and 2M HCl for the elution of Sr. Although both resins showed very similar results, the conditions for adsorption of Sr were different. The ammonium chloride solution was directly used with Purolite S950 while it was necessary to adjust the pH between 9 and 10 with Chelex 100. Purolite S950 was, therefore, selected for routine production of ^{82}Sr . A procedure has been introduced for the preparation of a hydrous tin dioxide as supporting material for the $^{82}\text{Sr}/^{82}\text{Rb}$ generator column. All components of the generator column were made up of stainless steel. The column was 4 cm long, 9.5 mm O.D. and 7.1 mm I.D. Using isotonic saline (0.9% NaCl) for elution of ^{82}Rb , elution curves with different flow rates ranging from 5 to 20 ml/min were obtained. Maximum available ^{82}Rb was eluted in the first 20 ml. The column generator provided a sterile ^{82}Rb in isotonic saline. The breakthrough of ^{82}Sr over 4 weeks of elution using 7 liter of saline was on average $4.5 \cdot 10^{-5}\%$ (based on the first 20 ml eluate).

Introduction

Rubidium-82 ($T_{1/2}=75$ s) is one of the positron-emitting radionuclides that is used in the positron emission tomography (PET) as a myocardial imaging agent and in terms of sensitivity and accuracy it has been proved to be superior to ^{201}Tl imaging using Single photon emission computer tomography (SPECT).¹ Rubidium-82 is conveniently obtained by the decay of its parent ^{82}Sr ($T_{1/2}=25.55$ d), a cyclotron radionuclide produced by the proton-induced nuclear reaction $^{85}\text{Rb}(p,xn)^{82}\text{Sr}$, using a generator system.

Recently, a great effort has been made to establish PET centres in South Africa and the Radionuclide Production Group of iThemba LABS plays an important role in production of PET radionuclides. This role becomes more significant in regard to the production of ^{82}Sr , since it needs a high-energy proton beam that is available from the 66-MeV separate sector cyclotron (SSC) at the site.

In the past nearly three decades several $^{82}\text{Sr}/^{82}\text{Rb}$ generator systems have been developed that employed both organic and inorganic ion exchangers as supporting material with different eluents.^{1–6} Among these generators, hydrous tin dioxide eluted with isotonic saline (0.9% NaCl) is the most widely used system and was, therefore, considered as the system of our choice in the present work.

Target materials for proton irradiation can be either RbCl or metallic rubidium. iThemba LABS uses RbCl. The target is prepared by pressing 7 g RbCl into a disc of 19 mm diameter. The radiochemistry, therefore, involves the separation of Sr radionuclides from Rb. Distribution coefficients data for Rb and Sr on the Chelex 100 and Dowex A-1 (which is identical to

Chelex 100)^{5–7} chelating resin suggest that a good separation can be achieved at a rather high pH. Using Chelex 100 and a pH 9–10 buffer solution of $\text{NH}_4^+/\text{NH}_3$ has been employed for the separation by Fassbender et al.⁸ In this work, chelating resins Chelex 100 and Purolite S950 were investigated for the separation of Sr from Rb.

Experimental

Reagents and equipment

Hydrous tin dioxide, $\text{SnO}_2 \cdot x\text{H}_2\text{O}$ (100–500 μm particle size), was supplied by STMI–DID (Société des Techniques en Milieu Ionisant – Direction Industrialisation et Développement, France). Chelex 100 and Purolite S950 cation exchange resins marketed by Bio-Rad Laboratories (USA) and Purolite International Ltd. (UK), respectively. All other chemicals were of analytical grade and were used without further purification. The solutions were prepared using deionized water ($18 \text{ M}\Omega \cdot \text{cm}^{-1}$) from a Milli-Q water purification system supplied by Microsep (Pty) Ltd. (South Africa). An HPGe detector coupled to a multi-channel analyzer was used for the measurement of the radionuclides involved in the radiochemical separation and ^{82}Sr breakthrough of the generator. For loading ^{82}Sr on to the column generator with very low flow rate a syringe pump, Model 402 Gilson (USA), and for the column generator elution yield measurement a radionuclide assay calibrator, Isocal II (Vinten Instrument Ltd., England), were used. A Varian GTA 100 atomic absorption spectrophotometer was used for measurement of natural Rb in the ^{82}Sr product as well as Sn in the eluates of the $^{82}\text{Sr}/^{82}\text{Rb}$ generator.

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Ion-exchange chromatography for the separation of ^{82}Sr

The irradiated RbCl target was dissolved in 100 ml 0.25M NH_4Cl solution. The column (10 cm \times 1 cm) was packed with the Purolite resin (in NH_4^+ form) up to 5 cm height and conditioned with 50 ml 0.25M NH_4Cl at a flow rate of 2 ml/min using a peristaltic pump. The solution obtained from target dissolution containing Sr radionuclides was loaded onto the column, followed by washing the column with 200 ml of the ammonium chloride solution for the elution of Rb. The column was then washed with 50 ml distilled water to remove ammonium chloride from the column. For the elution of Sr radionuclides, 25 ml 2M HCl was used. The eluate was evaporated to dryness and the residue was reconstituted in 10 ml 0.9% NaCl for the preparation of the generator column.

Manufacturing the $^{82}\text{Sr}/^{82}\text{Rb}$ generator column

All the components of the generator column, made from stainless steel type 316, were washed thoroughly with soap and water, then rinsed with water and dried. The dried components and spanners were then soaked in acetone to remove oils and lubricants, dried and finally washed with ethanol. The solutions for conditioning the column were autoclaved before use.

Preparation of the hydrous tin dioxide: An excess hydrous tin dioxide (with 100–500 μm particle size) was sifted with a 150 μm laboratory stainless steel sieve and 100–150 μm particles were selected for the column. Approximately 4 g of the sifted oxide was placed in a 100 ml Erlenmeyer flask and washed with 2M NaCl solution to remove fine particles. 20 ml 2M NaCl solution was added to the flask and the suspended hydrous tin dioxide titrated with a 0.1M NaOH – 2M NaCl solution until the pH was 9.5–9.8 and remained in this range at least for 20 minutes.

Assembling and conditioning the generator column: The generator column consisted of two 3/8"–1/8" Swagelok reducing adaptors with nuts and ferrules, one column was made up of a 4 cm stainless steel tube with 9.5 mm O.D. and 7.1 mm I.D., and two 20 μm frits (Fig. 1). The column together with one of the 20 μm frits was attached to one of the reducing adaptors, to which the outlet line (a 1/8" stainless steel tubing) was connected. The hydrous tin dioxide suspended in 2M NaCl solution, obtained from the titration procedure (former section), was transferred into the column under a very mild vacuum provided by a peristaltic pump attached to the other end of the outlet line. The tin dioxide was added until it levelled to the top of the column. The second 20 μm frit was then placed on top of the column and connected to the other reducing adaptor, to which was

attached another 1/8" stainless steel tubing as the inlet. Using a peristaltic pump attached to the outlet line, the lines were filled with 2M NaCl solution. The peristaltic pump was then connected to the inlet line. 200 ml 2M NaCl solution was pumped through the column at a flow rate of 0.5 ml/min followed by 300 ml 0.9% NaCl solution at 10 ml/min. If no leakage was observed during the last pumping steps, the column was ready for loading the ^{82}Sr activity.

Loading ^{82}Sr onto the generator column: A programmable syringe pump was connected to the inlet line of the column generator and the line was filled with 0.9% NaCl solution. The solution containing ^{82}Sr , obtained from the radiochemical separation, was adjusted to pH 7.5–8.5 with addition of a 0.1M NaOH solution and then pumped onto the column at a flow rate of 6 ml/h followed by a further 30 ml 0.9% NaCl solution (pH 7.5–8.5) at the same flow rate. Using either a peristaltic pump or the syringe pump, the saline solution was pumped through the column at a flow rate of 10 ml/min until the ^{82}Sr breakthrough was reached to a value of approximately 5 \cdot 10 $^{-5}$ %.

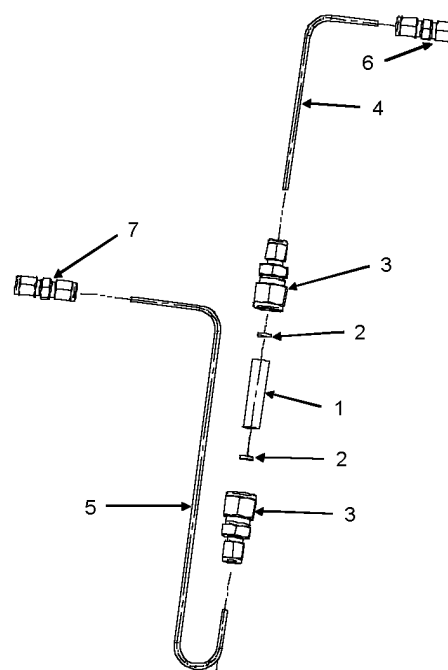


Fig. 1. Components of the $^{82}\text{Sr}/^{82}\text{Rb}$ generator column made of stainless steel: (1) column; (2) 20 μm frit; (3) 3/8"–1/8" Swagelok reducing adaptor with ferrules inside; (4) 1/8" generator inlet; (5) 1/8" generator outlet; (6) 1/8" end fitting to be connected to infusion pump and 0.9% NaCl reservoir; (7) 1/8" end fitting to be connected to the infusion tube of the patient

Sterility and endotoxin tests of the saline eluates

The test for sterility was carried out under aseptic conditions, e.g., inside a laminar airflow cabinet, to avoid accidental contamination of the eluates. These precautions were taken so as not to affect any micro-organisms that might be revealed by the test. Soybean casein digest medium (tryptic soy broth), which had previously been tested for growth promotion, was used for the test. Media was added to the sample aseptically and incubated for 14 days.

The chromogenic Limulus Amebocyte Lysate (LAL) test was used for the quantitative determination of endotoxin. The sample (eluate) was mixed with the LAL supplied in the test kit and incubated at 37 °C for 10 minutes. A substrate solution was then mixed with the LAL sample and incubated at 37 °C for an additional 6 minutes. The reaction was stopped with stop reagent. The developing of a yellow color indicated the presence of endotoxin in the sample. The absorbance of the sample, which was proportional to the amount of endotoxin, was determined spectrophotometrically at 405–410 nm. The concentration of the endotoxin was then calculated from a standard curve.

Results

Radiochemical separation of ^{82}Sr

The radiochemical separation experiments were first achieved on 7 g natural RbCl ("cold test") using ^{82}Sr and ^{83}Rb as tracers. The experiments were performed on Chelex 100 and Purolite S950 resins under the same conditions except that the ammonium chloride solution was directly used on Purolite S950 while its pH was adjusted to 9–10 when using Chelex 100. Elution curves on these resins showed a very similar pattern. Figure 2

illustrates the elution curve on Chelex 100. When the final product containing ^{82}Sr was analyzed for stable Rb, however, it was found that Rb content in the solution was different; the total Rb obtained from Chelex 100 was 180 μg and from Purolite S950 was almost half, 92 μg . The Purolite resin was, therefore, selected for routine production and in order to obtain a more purified product, the volume of the ammonium chloride solution was increased from 100 to 200 ml. An elution curve was also drawn under the new conditions. For better observation of ^{82}Sr elution by 2M HCl, 2-ml fractions were collected (Fig. 3). This elution curve showed that less than 25 ml 2M HCl would be sufficient to elute Sr completely. The total Rb in the final product was found to be about 6 μg , which is far less than that obtained from previous experiments.

The $^{82}\text{Sr}/^{82}\text{Rb}$ generator and ^{82}Sr breakthrough

At the early stages of development the generator column was 3.3 cm long, the particle size of the hydrous tin oxide 100–300 μm and the preparation of the tin oxide as described in ALVAREZ-DIEZ's work.¹ No attempt was made to measure the backpressure by flushing 0.1M NH_3 solution through the column, however. Although the ^{82}Sr was quantitatively retained by the column, the breakthrough was quite high (up to 0.17%). The experiment was then repeated with a smaller range of particle size of the hydrous tin dioxide, 100–150 μm , using a longer column, 4 cm long. The ^{82}Sr breakthrough dramatically dropped to a value of about $5 \cdot 10^{-5}\%$. However, after 6 weeks elution of the column, using 3 liter 0.9% NaCl, the breakthrough had increased by a factor of more than two orders of magnitudes. Using 0.1M NaOH – 2M NaCl solution, a pH titration was conducted on the hydrous tin oxide to facilitate the saturation of the oxide with Na^+ .

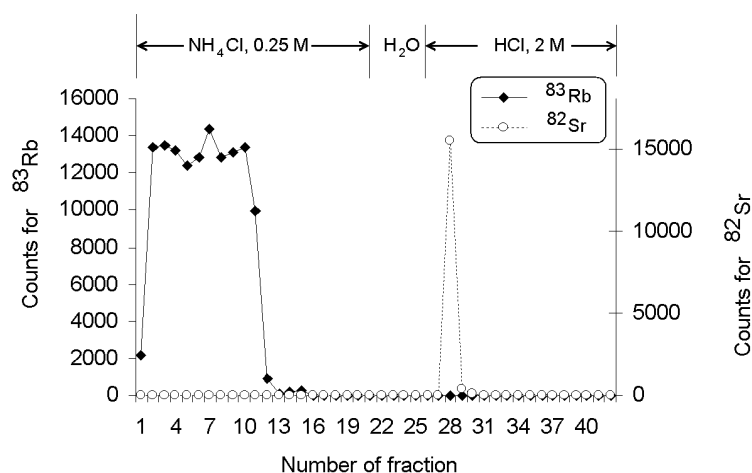


Fig. 2. Elution curves for Sr and Rb on Chelex 100; flow rate: 2 ml/min; volume of each fraction: 10 ml

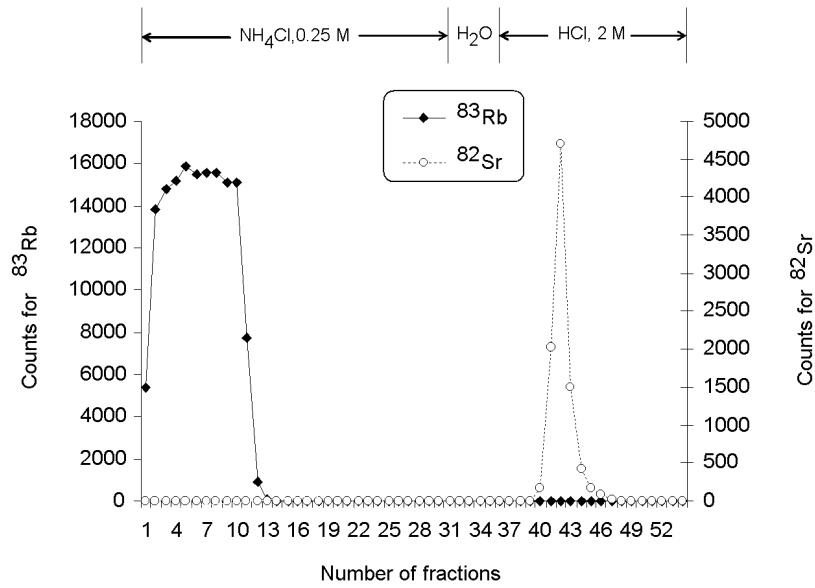


Fig. 3. Elution curves for Sr and Rb on Purolite S950; flow rate: 2 ml/min; volume of each fraction: 10 ml for water and NH_4Cl , 2 ml for 2M HCl

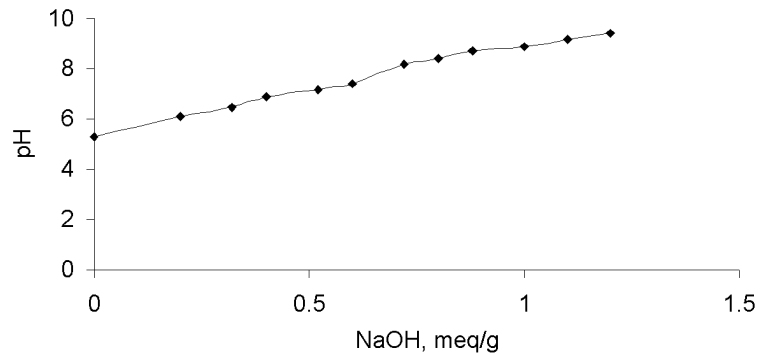


Fig. 4. The pH titration curve for hydrous tin dioxide; exchanger: 0.25 g; total volume: 8.1 ml; titrant: 0.1M NaOH – 2M NaCl solution

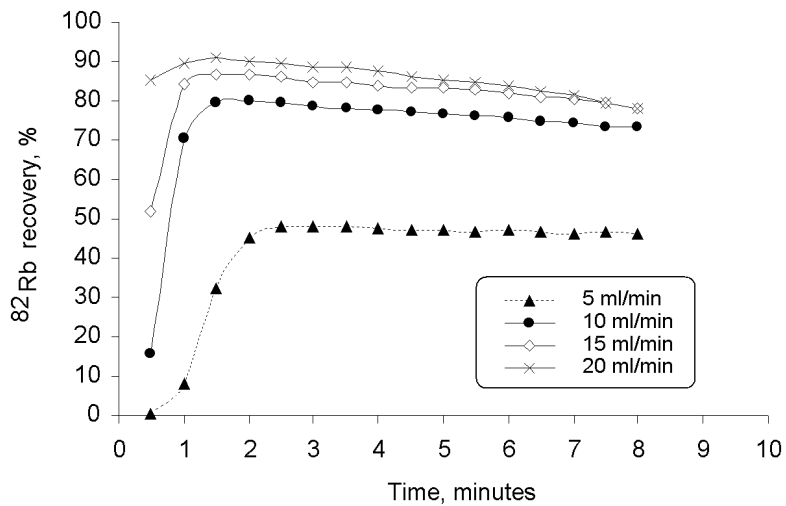


Fig. 5. Elution yields of ^{82}Rb on the $^{82}\text{Sr}/^{82}\text{Rb}$ generator column at various flow rates; exchanger: hydrous tin dioxide; size of the column: 0.7 cm I.D. and 4 cm length; eluent: 0.9% NaCl

The titration curve is shown in Fig. 4. A generator column was then prepared with the titrated hydrous tin oxide and tested for ^{82}Sr breakthrough. The breakthrough remained as low as $4.5 \cdot 10^{-5}\%$ after 4 weeks elution with over 7 liter of 0.9% NaCl solution.

The elution yield of ^{82}Rb was studied at four flow rates; 5, 10, 15 and 20 ml/min. The corresponding elution curves are shown in Fig. 5 indicating that the level of radioactivity of ^{82}Rb reached a maximum and remained almost at that level with continuous elution. The maximums correspond to 90, 86, 79 and 48.5% elution yields for 5, 10, 15, and 20 ml/min flow rates, respectively.

The sterility test of ^{82}Rb showed no visible growth and the endotoxin concentration was less than 10 eu/ml.

Discussion

Radiochemical separation of ^{82}Sr

According to the elution curves on the chelating resins investigated for the separation of Sr from Rb (Figs 2 and 3), both resins showed very similar behavior to the adsorption/desorption of Rb and Sr. As mentioned earlier, Purolite S950 resin showed two advantages over Chelex 100 and was, therefore, chosen for the routine production of ^{82}Sr .

For destruction of NH_4^+ ions, which come from either Chelex 100 or Purolite S950, FASSBENDER et al.⁹ used a few steps including evaporation of product and reconstituting the residue in concentrated nitric acid. In this work no attempt was made to destroy NH_4^+ ions in the final product, since we found that the ammonium ions do not interfere with the preparation of $^{82}\text{Sr}/^{82}\text{Rb}$ generator.

Preparation of the generator column

The distribution coefficient (K_D) for Sr and Rb on hydrous tin dioxide are very dependent on the pH. As the values are very low for Rb in the pH range of 4–9, they become very high only at pH range of 5.8–9.^{5,6} These K_D values suggest that for a good adsorption and low breakthrough of ^{82}Sr , the eluent must have a $\text{pH} \geq 5.8$. The physiological saline solutions, 0.9% NaCl solution, which have a pH between 4.5 and 5.5 (the saline used in this work had a pH 5.2), can be used as the eluent for elution of ^{82}Rb provided that the hydrous tin dioxide has been previously converted to Na^+ form. It was also concluded that the hydrous tin dioxide in Na^+ form can keep its properties unchanged even if up to 30 liter saline solution is passed through. And this conversion could be achieved by percolating a 2M NaCl solution through the generator column.⁶ In preparation of the generator column, ALVAREZ-DIEZ et al.¹ incubated the hydrous tin dioxide in a $\text{NH}_3/\text{NH}_4\text{Cl}$

buffer (pH 10) to “activate” the cation-exchange properties of the oxide first and then pumped 120 ml 2M NaCl solution through the column to saturate the exchanger with Na^+ ions. This procedure was also followed in our work on the first generator column using hydrous tin dioxide of particle size ranges of 100–300 and 100–150 μm . The results obtained from these experiments (as mentioned earlier) showed that the procedure for the saturation of the tin oxide with Na^+ ions, which is vital for a low Sr breakthrough when a slightly acidic solution like 0.9% NaCl (pH 4.5–5.2) is used,⁵ was inefficient.

Ion-exchange behavior of metal oxides, including hydrous tin dioxide, to particular ions strongly depend on pH as well as the ionic strength of the solution, the higher the pH the higher the capacity towards cations.⁸ Using a 0.1M NaOH – 2M NaCl solution, a pH titration on the hydrous tin dioxide was, therefore, conducted to observe the relation between pH and the exchange of Na^+ . Since the hydrous tin oxide is not soluble in the pH range of the titration ($\text{pH} < 10$), the amounts of OH^- added is practically proportional to the uptake of Na^+ by the oxide.⁸ The titration curve in Fig. 4 reflects that the uptake of Na^+ is insignificant at the pH 5.4, which was the pH of 2M NaCl solution. In other words, the hydrous tin oxide could not be necessarily saturated with Na^+ simply by passing through 2M NaCl solution. This incomplete saturation of column with Na^+ resulted in high ^{82}Sr breakthrough in the previous experiments.

For complete conversion of the exchanger into Na^+ form, 2M NaCl solution was pumped through the generator column packed with the titrated hydrous tin dioxide at a flow rate of 0.5 ml/min. It was observed that at the beginning of this process the pH of the 2M NaCl solution, which originally was 5.4, was increased to 9.5–10 when leaving the column. Pumping the sodium chloride solution was, therefore, continued until the effluent reached a pH of ~ 8.5 and a volume of ~ 250 ml 2M NaCl was consumed. This buffering action of the hydrous tin dioxide, which can be attributed to the OH^- ions adsorbed on the dioxide during the titration process,⁸ in turn, further facilitated the uptake of Na^+ and also the adsorption of Sr^{2+} at the next stage. After passing 300 ml 0.9% NaCl solution through the column at a flow rate of 10 ml/min, the pH of the eluate was still greater than 8. The pH of the eluate was measured during the elution of ^{82}Rb with saline solution. After using 7 liter solution, the eluate had a pH 5.9, in which the distribution coefficient Sr^{2+} is still at the highest level. This explains why the breakthrough decreased when using the titrated hydrous tin dioxide.

Both ^{82}Rb elution yield and ^{82}Sr breakthrough are functions of the flow rate of saline, the higher the flow rates the higher ^{82}Rb yield and ^{82}Sr breakthrough.^{4,5} The patterns of the elution curves in Fig. 5 are very similar to the one reported by YANO et al.⁴ Should the

generator be used for bolus elution, Fig. 5 also shows that the available ^{82}Rb can be obtained in the first 20 ml eluate. The ^{82}Sr breakthrough measurements were, therefore, achieved on the first 20 ml applying 20 ml/min flow rate elution.

In the protocol introduced by ALVAREZ-DIEZ et al.¹ for manufacturing the generator, the back pressure of the generator had been measured by passing a 0.1M ammonia solution through the column at high flow rates (no data for the backpressure has been mentioned, though). This test was, however, avoided in our generator preparation, as 0.1M ammonia solution provides a pH of 11.3¹⁰ and hydrous tin dioxide is soluble in solutions with pH>10.⁸ Furthermore, since a peristaltic pump was used throughout the preparation and elution of the generator pumping 0.9% NaCl at flow rates of up to 20 ml/min and no leakage was observed, it was concluded that the backpressure built in the column was insignificant.

Conclusions

From the investigation of the radiochemical separation of Sr radionuclides from Rb target, it is concluded that the chelating resin Purolite S950 (with amino phosphonate functional group in NH_4^+ form) can be used efficiently in a routine production of ^{82}Sr . The presence of ammonium chloride in the product, which resulted from the elution of Sr, do not interfere with the preparation of $^{82}\text{Sr}/^{82}\text{Rb}$ generator.

As to Sr adsorption and the low breakthrough of ^{82}Sr , the results of this paper showed that the ion-exchange properties of hydrous tin dioxide can be activated by a titration procedure using 0.1M NaOH – 2M NaCl solution. The titration facilitates the conversion of the exchanger into Na^+ form, which in turn, improves the retention of ^{82}Sr resulting in a low ^{82}Sr breakthrough.

The elution curves using commercial physiological NaCl solution indicate that the column generator can be operated in a continuous elution mode for myocardial and blood flow studies. The required dose can be delivered by changing the flow rate of the saline solution.

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