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Manufacture of strontium-82/rubidium-82 generators and quality control of rubidium-82 chloride for myocardial perfusion imaging in patients using positron emission tomography

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Abstract

We describe a protocol to manufacture ^{82}Sr/^{82}Rb generators and ^{82}RbCl for myocardial imaging with PET. The generators are manufactured in 3 stages: (1) preparation of a tin oxide column, (2) leak test of the generator column and (3) loading of the generator with ^{82}Sr. The generators produced sterile and non-pyrogenic ^{82}RbCl for i.v. injection. No significant ^{82}Sr/^{85}Sr breakthroughs were observed after elution with 20 l of saline. The automated system delivered human doses of ^{82}RbCl accurately. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Conventional diagnostic techniques used to assess coronary artery disease and its severity are of limited sensitivity. Stress SPECT (single photon emission computed tomography) using thallium-201 (^{201}Tl) or technetium-99m-sestamibi (^{99m}Tc-sestamibi) has been used extensively in clinical practice to determine myocardial perfusion. However, this technique is limited by attenuation artifacts and does not permit in some cases the accurate distinction between hypoperfused but viable myocardium and infarcted tissues, thus underestimating the areas of viable tissue (Wackers et al., 1976; Cloninger et al., 1988; Galli et al., 1988).

Cardiac positron emission tomography (PET) using several short half-life ($t_{1/2}$) radionuclides has been used to characterize myocardial perfusion and metabolism non-invasively (Bergmann et al., 1985; Goldstein et al., 1986; Brunken et al., 1987; Camici et al., 1989; Demer et al., 1989; Saha et al., 1992). It has been demonstrated that rubidium-82 (^{82}Rb), a positron emitter radionuclide with ultra-short half-life ($t_{1/2} = 75$ s) (Woods et al., 1987), permits the assessment of myocardial perfusion with high sensitivity and specificity (Goldstein et al., 1983; Gould et al., 1986, 1988; Go et al., 1990; Stewart et al., 1991; Grover-McKay et al., 1992). The accuracy of ^{82}Rb PET has been shown to be superior to ^{201}Tl SPECT imaging (Go et al., 1990; Stewart et al., 1991). This is especially important for the detection of early coronary artery disease and for the evaluation of therapy designed to protect or salvage myocardium. Some clinical studies have also demonstrated the utility of ^{82}Rb as a quantitative marker of myocardial necrosis/viability (Gould et al., 1991; Dahl et al., 1996).

There are two other advantages of ^{82}Rb. It has a ultra-short $t_{1/2}$ which allows the sequential perform-

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ance of scans every 10 min and reduces the exposure of the patients to radiation. Secondly, ^{82}Rb is a PET radiopharmaceutical which is generator-produced from its parent radionuclide strontium-82 (^{82}Sr). This makes it feasible for institutions to participate in investigational and clinical PET studies without the need to have expensive on-site cyclotrons.

Several studies have been reported in the literature about the manufacture of strontium-82/rubidium-82 (^{82}Sr/^{82}Rb) generators. Neirinckx et al., 1982 demonstrated the efficiency of hydrous tin oxide, a cationic exchanger material, for the separation of ^{82}Rb from ^{82}Sr in a column. A special issue of this journal (Waters and Coursey, 1987) contained 11 papers dealing with ^{82}Sr production and the physics and chemistry of the ^{82}Sr/^{82}Rb generators and 2 papers dealing with the clinical applications of ^{82}Rb. However, much of these works were taken from on-going projects and there are in several cases discrepancies reported. Also, our group had previously described the development of a ^{82}Rb generator system while focusing on the safe production of ^{82}Sr from metallic rubidium targets (Cackette et al., 1993). However, the ^{82}Rb eluted from these generators is not suitable for clinical studies but only for research applications. In addition, a detailed protocol for the manufacture of ^{82}Sr/^{82}Rb generators from simple components that include quality control procedures and specifications of ^{82}Rb for clinical imaging has not yet been reported in the literature.

Thus, we describe in this manuscript a novel and simple manufacturing protocol which include the quality control procedures for the production of ^{82}Sr/^{82}Rb generators and ^{82}Rb chloride doses for i.v. administration in patients. Other nuclear pharmacies could produce generators in a similar fashion and participate in clinical PET studies. We discuss some of the factors that determine the performance of ^{82}Sr/^{82}Rb generators for clinical imaging: (1) the type and properties of the cationic exchanger material used in the manufacture of the generator column; (2) the tightness of the cationic exchanger packing and (3) the type of eluent. We also report the quality control tests and specifications of ^{82}Rb chloride suitable for clinical myocardial imaging.

2. Materials and methods

All components of the generator column and the wrenches used during manufacturing were thoroughly washed with laboratory soap and water, rinsed with sterile water and then autoclaved. Soaking of the generator components in soap and water for at least 20 min removes the traces of lubricant used during its manufacturing. Rinsing the stainless steel components with a diluted solution of hydrochloric acid before autoclaving is not recommended because we observed that HCl with repeated autoclaving accelerates the oxidation of the components. The buffers and solutions used during the manufacturing of the generator column are sterile and pyrogen free. ^{82}Sr/^{82}Rb generators are manufactured under aseptic conditions in 3 stages.

2.1. Manufacturing of the generator column

2.1.1. Preparation of hydrous tin oxide

The generator column is prepared in a clean manufacturing room dedicated to the compounding of radiopharmaceuticals. An excess of hydrous tin oxide (International Tin Research Institute, Middlesex, England) was sieved with a 150 μm stainless steel sieve for 10 min. The sieved tin oxide (with particle size < 150 μm) was then sieved thoroughly with a 75 μm sieve in order to separate the fines (tin particles of < 75 μm) from the tin oxide particles with sizes in the interval of interest, 150 μm $> x >$ 75 μm. The fines were discarded. The tin oxide, approximately 3.5 g, was washed with 30 ml of 0.1 N NH_4OH/NH_4Cl, pH 10 and incubated overnight with 10 ml of this buffer in order to activate its cationic exchanger properties. The tin oxide was kept in the buffer of incubation until loaded in the column subassembly.

2.1.2. Preparation of the generator column assembly

The generator column assembly consists of two 9.5–1.5 mm Swagelok reducing adaptors with nuts and ferrules, one column and two 25 μm filters (frits) (Fig. 1). The dimensions of the generator column are: 2.6 cm length, 6 mm internal diameter and 0.5 mm wall thickness. All components are made of stainless steel type 316. A generator column subassembly is first prepared by attaching the column to a reducing adaptor containing a 25 μm filter. This column subassembly is then loaded with about 3.5 g of the wetted α-hydrous tin oxide ($Sn_2O{\cdot}xH_2O$ where $x = 1–2$) in 10 ml of 0.1 N NH_4OH/NH_4Cl buffer. A 3-cm Teflon reservoir containing 0.1 N NH_4OH/NH_4Cl is connected to the upper end of the generator column and a vacuum aspirator to the lower end to facilitate the packing of the tin oxide into the column. It is important not to draw air through the column. An alternative method that facilitates the packing of the tin oxide column is to gently shake the reducing adaptor of the loaded column with a small electrical vibrator (i.e. an engraver). When the packing is finished (the resin is leveled with the top of the column), a 7.9 mm Swagelok capping nut (plug) is screwed onto the column outlet to prevent fluid drainage. The column assembly is then completed by attaching a second Swagelok reducing adapter containing a 25 μm filter to the upper end of the column. The Swagelok nut is tightened one full turn past finger tight.

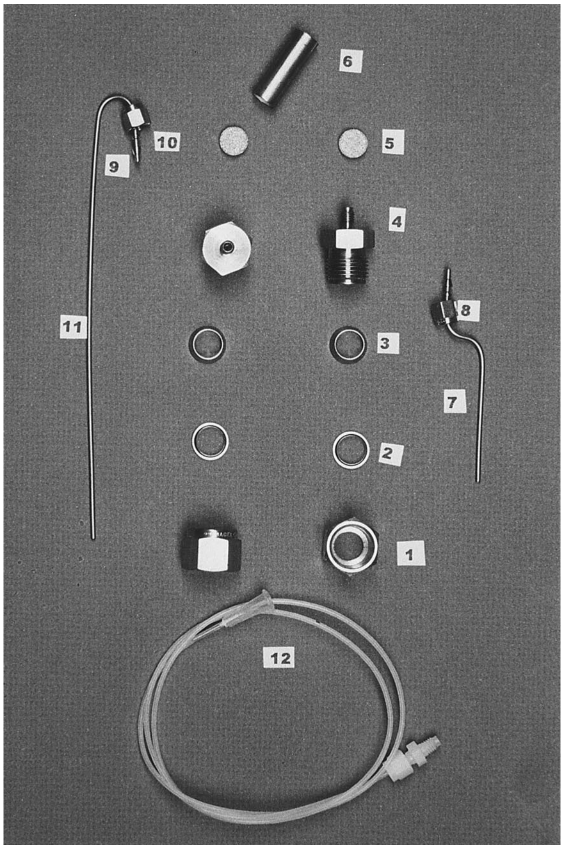

Fig. 1. Physical components of the strontium-82/rubidium-82 generator column and infusion line used during generator's manufacture. The components (1–11) are made of stainless steel type 316. The generator column assembly consistent of two 9.5–1.5 mm Swagelok reducing adaptors (4) with nuts and ferrules (1–3), one column (6), two 25 µm filters (frits) (5) and the generator inlet (7) and outlet (11) lines which are attached to the column using 7.9 mm Swagelok nuts (8) and ferrules (9–10). The Teflon infusion line (12) is attached to the syringe pump (luer end) and to the column inlet in order to infuse the buffers through the hydrous tin oxide column.

The column backpressure is measured by passing approximately 120 ml of 0.1 N NH_4OH through the column at flow rates of 10 and 20 ml/m using a syringe connected to a syringe pump and an in-line pressure gauge.

The tin oxide exchanger is then saturated with sodium cations by passing 120 ml of 2 M NaCl through the column at a flow rate of 0.5 ml/m followed by 500 ml of 0.9% saline at a flow rate of 10 ml/m.

The cationic exchanger properties of hydrous tin oxide were tested by passing through the column about 10 ml of sterile water for irrigation USP that was acidified with 0.1 N HCl to pH 4. A rapid shift on the pH of the eluate from pH 6 to pH 9 to 10, after passing a few ml of the eluent, will indicate that the tin oxide of the generator column is in the Na^+ form and that it is successfully working as a cationic exchanger material, exchanging some of its Na^+ for the H^+ of the eluent. If the pH of the eluate is outside this pH interval, the generator column is discarded.

After this test has been performed the hydrous tin oxide is replenished with sodium cations by repeating the 2 M NaCl flush.

2.2. Generator assembly and leak test

The generator inlet and outlet lines used are preformed 1.5 mm stainless steel tubes of 7 and 15.5 cm, respectively (Triumf, Vancouver, Canada) (Fig. 1). The outlet line is filled with 0.9% NaCl and attached to the column outlet using a 7.9 mm Swagelok nut and ferrules. The nut is tightened 3/4 of a turn past finger tight. The inlet line is attached to the generator column inlet in the same way. This assembly is then attached to the lid of the shielding body (Fig. 2). The generator

Fig. 2. This figure shows the generator column assembled, packed with hydrous tin oxide and attached to the lid of the shielding body. Both shielding lid and body (not shown) contain depleted uranium and weigh approximately 17 kg.

lines are passed through the hexagonal holes in the shielding lid until the lines protrude at least 1 cm above the surface of the lid. Female threaded luer fittings are attached to the inlet and outlet lines with 7.9 mm Swagelok nuts and ferrules. The nut is tightened 1/4 of a turn. The outlet line is then capped with a male luer plug. The inlet line of the generator is attached to a research purity argon gas cylinder using a 7.9 mm Swagelok nut. The generator column is then leak tested by passing a static pressure of 50 psi of argon. Leaks are detected by immersing the generator column into a 1 l beaker containing a sufficient volume of sterile water to cover the column assembly (Fig. 3). If no leaks are observed, this assembly is detached from the argon gas cylinder and the generator column inserted into the shielding body. The shielding body is a cylinder of 2.8 cm internal diameter, 3 cm wall thickness and 15.5 cm length (not shown). Both shielding lid and body contain depleted uranium and weigh approximately 17 kg in total. The shielding lid is secured to the body using lever clamps.

Fig. 3. Leak test of the generator column. The generator column is leak tested by capping the outlet line of the column and passing a static pressure of 50 psi of argon through the column. The generator column is immersed in a beaker containing 1 liter of sterile water. If a leak occurs, argon bubbles will be observed in the water.

Spent generators are disassembled and the columns stored for decay. Generator shielding assemblies are reused to manufacture subsequent generators.

2.3. Loading ^{82}Sr into the generator column

Loading of the generator with ^{82}Sr is performed inside a lead castle in the Radiopharmacy laboratory. This castle has a front and back wall thickness of 7.5 cm. Mirrors placed on the inside front and back walls of the castle provide an inside view. Lead bricks at the top of the side walls can be removed to enable manual access during the manufacturing procedure.

The arrangement for loading the generator with ^{82}Sr is shown in Fig. 4. It consists of the assembled generator column, a 30 ml vial containing the ^{82}Sr stock solution (MDS Nordion, Vancouver, Canada), a syringe isolator and a waste container. The stock vial, syringe isolator and the waste container are shielded with lead. The syringe isolator (Triumf, Vancouver, Canada) consists of a 20 ml syringe attached by its plunger to the plunger of a 60 ml syringe. The plunger of the 60 ml syringe was modified to permit attachment of a 20 ml syringe. The 60 ml syringe of the isolator is connected to a remote 60 ml syringe which can be operated using a syringe pump.

50 mCi of ^{82}Sr in 0.1 N HCL with a concentration of <50 mCi/ml (MDS Nordion, Vancouver, Canada) was mixed with 15 ml of 0.5 M Tris buffer pH 7.5. Using the remote syringe the ^{82}Sr was withdrawn into the shielded syringe of the isolator with a 19G sterile spinal needle. The ^{82}Sr solution is then pumped from the isolator through the generator column at a flow rate of 2 ml/h. After the generator column has been loaded with ^{82}Sr, the column is purged with 500 ml of 0.9% NaCl at a moderate flow rate of 0.5 ml/m. Washing the column with a large volume of saline is intended to remove any possible impurities contained in the ^{82}Sr stock solution.

2.4. Quality control of the ^{82}Sr/^{82}Rb generators

The ^{82}Sr/^{82}Rb generators were eluted with sterile and pyrogen free 0.9% NaCl. The initial quality control tests performed on the ^{82}Rb chloride eluate included: visual inspection, pH measurement, radionuclide purity (^{82}Sr and ^{85}Sr breakthrough), chemical purity, sterility and pyrogen tests.

The radionuclide purity of a sample of ^{82}Rb was measured by quantitative gamma spectrometry using a calibrated and certified multi-channel analyzer (Tracor Northen, Middleton, Wisconsin, USA) with an intrinsic germanium lithium detector (Ge(Li)) and computer analysis (AccuSpec). (The multi-channel analyzer was

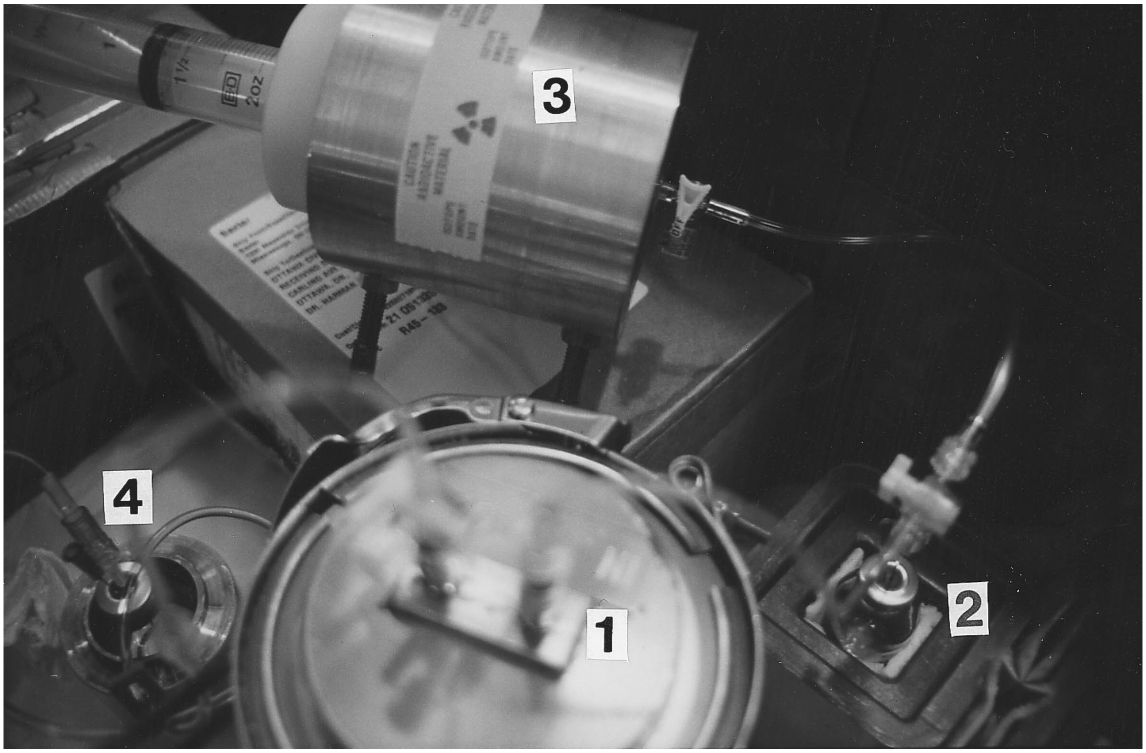

Fig. 4. Arrangement for loading strontium-82 into the generator column. It consists of the assembled generator column (1), a vial containing the strontium-82 chloride solution (2), a syringe isolator (3) and a waste container (4).

certified by the Institute for National Measurements Standards; National Research Council, Ottawa, Canada). The amounts of ^{82}Sr and ^{85}Sr in the eluate were measured relative to eluted yield of ^{82}Rb. The ^{82}Sr/^{82}Rb ratio limit established was 0.02 μCi/mCi. The limit of the ^{85}Sr/^{82}Rb ratio was 0.2 μCi/mCi. The elution yield of ^{82}Rb was also measured with a dose calibrator and corrected for decay of the measured ^{82}Rb activity to the time of loading.

A sample of ^{82}Rb was sent to an independent laboratory for trace metal analysis by inductively coupled plasma/atomic emission spectroscopy (ICP/AES).

A sterility test of ^{82}Rb was performed by inoculation in trypticase soy broth and thioglycolate media for the detection of aerobic and anaerobic microorganisms.

The absence in the eluate of pyrogenic substances was established using the limulus amebocyte lysate (LAL) test.

^{82}Sr and ^{85}Sr breakthrough measurements using a dose calibrator and pyrogen tests of ^{82}Rb were also performed daily before administration to the patients. The ^{82}Sr and ^{85}Sr breakthroughs relative to the peak of ^{82}Rb radioactivity eluted were measured in 20-ml samples of ^{82}Rb eluted at a rate of 20 ml/m and which were allowed to decay for 1 h after elution. 30 ml of the first elution of ^{82}Rb is discarded every day. ^{82}Sr breakthrough was calculated using the following equations:

$$^{82}Sr\ breakthrough = \frac{^{82}Sr}{^{82}Rb} \tag{1}$$

where ^{82}Sr is the amount of ^{82}Sr in the sample in μCi calculated from Eq. (2); ^{82}Rb is the peak of ^{82}Rb radioactivity in mCi measured at the end of the elution.

$$^{82}Sr = \frac{\text{Radioactivity at one hour}}{1 + 0.48 \times R'}. \tag{2}$$

The numerator of Eq. (2) is the radioactivity of the sample in μCi measured in a dose calibrator (on the ^{82}Rb and/or ^{82}Sr setting) at least 1 h after elution (at complete decay of ^{82}Rb) and R' is the ^{85}Sr/^{82}Sr ratio on the date of the measurement, calculated from the manufacturer specifications. The correction factor 0.48 is used to compensate for the contribution of the ^{85}Sr to the reading. The ^{85}Sr breakthrough was calculated by multiplying the ^{82}Sr (μCi) by R'.

$$^{85}Sr\ breakthrough =^{82} Sr\ Breakthrough \times R'. \tag{3}$$

A sterility test of a sample of ^{82}Rb was also performed every day retrospectively.

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