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Characteristics of SnO_2-based $^{68}Ge/^{68}Ga$ generator and aspects of radiolabelling DOTA-peptides ☆

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ABSTRACT

Objectives: PET scintigraphy with ^{68}Ga-labelled analogs is of increasing interest in Nuclear Medicine and performed all over the world. Here we report the characteristics of the eluate of SnO_2-based $^{68}Ge/^{68}Ga$ generators prepared by iThemba LABS (Somerset West, South Africa). Three purification and concentration techniques of the eluate for labelling DOTA-TATE and concordant SPE purifications were investigated.
Methods: Characteristics of 4 SnO_2-based generators (range 0.4–1 GBq ^{68}Ga in the eluate) and several concentration techniques of the eluate (HCl) were evaluated. The elution profiles of SnO_2-based $^{68}Ge/^{68}Ga$ generators were monitored, while [HCl] of the eluens was varied from 0.3–1.0 M. Metal ions and sterility of the eluate were determined by ICP. Fractionated elution and concentration of the ^{68}Ga eluate were performed using anion and cation exchange. Concentrated ^{68}Ga eluate, using all three concentration techniques, was used for labelling of DOTA-TATE. ^{68}Ga-DOTA-TATE-containing solution was purified and RNP increased by SPE, therefore also 11 commercially available SPE columns were investigated.
Results: The amount of elutable ^{68}Ga activity varies when the concentration of the eluens, HCl, was varied, while ^{68}Ge activity remains virtually constant. SnO_2-based $^{68}Ge/^{68}Ga$ generator elutes at 0.6 M HCl $>100\%$ of the ^{68}Ga activity at calibration time and $\pm 75\%$ after 300 days. Eluate at discharge was sterile and Endotoxins were <0.5 EU/mL, RNP was always $<0.01\%$. Metal ions in the eluate were <10 ppm (in total). Highest desorption for anion purification was obtained with the 30 mg Oasis WAX column ($>80\%$). Highest desorption for cation purification was obtained using a solution containing 90% acetone at increasing molarity of HCl, resulted in a ^{68}Ga desorption of $68 \pm 8\%$. With all $^{68}Ge/^{68}Ga$ generators and for all 3 purification methods a SA up to 50 MBq/nmol with $>95\%$ incorporation (ITLC) and RCP (radiochemical purity) by HPLC $\pm 90\%$ could be achieved. Purification and concentration of the eluate with anion exchange has the benefit of more elutable ^{68}Ga with 1 M HCl as eluens. The additional washing step of the anion column with NaCl and ethanol, resulted in a lower and less variable $[H^+]$ in the eluate, and, as a result the pH in the reaction vial is better controlled, more constant, and less addition of buffer is required and concordant smaller reaction volumes. Desorption of ^{68}Ga-DOTA-TATE of SPE columns varied, highest desorption was obtained with Baker C_{18} 100 mg (84%). Purification of ^{68}Ga-DOTA-TATE by SPE resulted in an RNP of $<10^{-4}\%$.
Conclusions: Eluate of SnO_2-based $^{68}Ge/^{68}Ga$ generator, either by fractionated elution as by ion exchange can be used for labelling DOTA-peptides with ^{68}Ga at a SA of 50 MBq/nmol at $>95\%$ incorporation and a RCP of $\pm 90\%$. SPE columns are very effective to increase RNP.

1. Introduction

Since 1970s, several $^{68}Ge/^{68}Ga$ generator systems have been developed to provide a reliable source of the positron-emitter ^{68}Ga (half-life 68 min) that can readily be converted into radiopharmaceuticals for Positron Emission Tomography (PET) studies. There has been renewed interest in ^{68}Ga for several reasons. Firstly, PET has developed from a research tool to routine clinical application over the last decade. Secondly, the production of $^{68}Ge/^{68}Ga$ generators from which the eluate is suitable for labelling in

☆ Contributions by (iThemba Labs, South Africa): Clive Naidoo, Grant Sedres, Claudia Davids and Deidre Prince investigated ^{68}Ge and ^{68}Ga elution profile of the generators for 9 months, and performed tests in the eluates on Endotoxin, sterility and metal content by ICP.

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a clinical environment. Thirdly, the availability of compounds, such as small peptides that can be labelled with ^{68}Ga (Breeman et al., 2005; Decristoforo et al., 2007; Meyer et al., 2004; Velikyan et al., 2004; Zhernosekov et al., 2007).

The theoretical ingrowth of ^{68}Ga from the parent nuclide ^{68}Ge in a ^{68}Ge/^{68}Ga generator is shown in Fig. 1. Eight hours after the former elution there is an equilibrium ($>99\%$) of formation of ^{68}Ga and decay to ^{68}Zn (see Table 1). Already 68 min after the former elution 50% of the equilibrium value is reached, while after 4 h this is $>90\%$ (Fig. 1).

Recently Ocak et al. (2009) summarized the 3 different methods of purification and/or concentration of the ^{68}Ga from eluate of TiO_2-based generators by fractionated elution, and by anion- and cation-chromatography. In short, fractionated elution (a) results in a ready to use eluate containing approximately 80% of the elutable ^{68}Ga activity (Breeman et al., 2005; Decristoforo et al., 2007; Velikyan et al., 2004). Whereas anion (b) and cation (c) exchange resins are used to reduce ionic impurities and, to concentrate the generator eluate and to reduce acidity (Meyer et al., 2004; Velikyan et al., 2004; Zhernosekov et al., 2007). The major limitations for direct use of ^{68}Ga for radiolabelling of peptides for clinical PET applications are the large volume of generator eluate, high [H^+], ^{68}Ge breakthrough and potential metal ion impurities. More detailed data regarding competitions impurities and kinetics was described previously (Breeman et al., 2005; Velikyan et al., 2004; Breeman et al., 2003).

Loc'h et al. (1980) reported on the elution characteristics of a SnO_2-based ^{68}Ge/^{68}Ga generator, while [HCl] of the eluens was varied from 0.5–10 M and showed decreased activities of ^{68}Ga and ^{68}Ge in the eluate at lower [HCl]. McElvany et al. (1984) also confirmed the elution profile, low breakthrough and chemical impurities of the SnO_2-based generator.

Recently iThemba LABS (Somerset West, South Africa) launched a under Good Manufacturing Practice (GMP) conditions produced SnO_2-based ^{68}Ge/^{68}Ga generator Aardaneh and van der Walt (2006).

Here we report the characteristics and of the eluate of this SnO_2-based generator, the results of the 3 purification and concentration techniques of the eluate, and the results of labelling a DOTA-peptide.

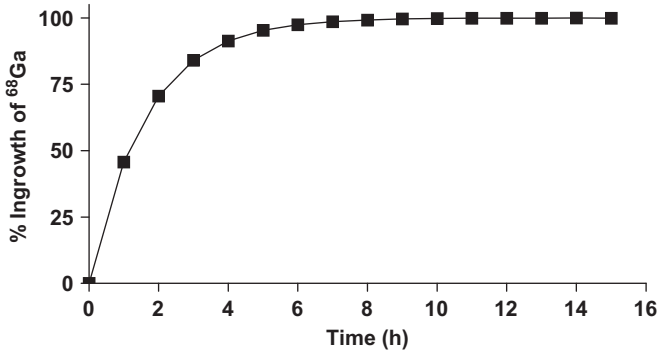

Fig. 1. Theoretical ingrowth of ^{68}Ga from a ^{68}Ge/^{68}Ga generator. 8 h after the former elution there is an equilibrium ($>99\%$) of formation of ^{68}Ga and decay to ^{68}Zn (see Table 1). Already 68 min after the former elution 50% of the equilibrium value is reached, while after 4 h this is $>90\%$.

Table 1
^{68}Ga $t_{\frac{1}{2}}$=68 min, specific activity $\equiv 3.63\times10^{-13}$ mol per 37 MBq ^{68}Ga. In theory 1 nmol DOTA-peptides can complex 10^{-9} gram-atoms Ga, corresponding to 102 GBq ^{68}Ga. After $\sim$2.5 h equal amounts of ^{68}Zn and ^{68}Ga are present in the generator, see Fig. 8.

^{68}Ge is a cyclotron-produced radiometal,formed from ^{69}Ga by a (p,2n) reaction:
$^{69}Ga \xrightarrow{p,\,2n} {}^{68}Ge \xrightarrow{EC} {}^{68}Ga \xrightarrow{\beta^+} {}^{68}Zn$

2. Methods and materials

2.1. ^{68}Ge/^{68}Ga generator eluate purification procedures

^{68}Ge/^{68}Ga generators were prepared by iThemba LABS (Somerset West, South Africa) by loading the parent ^{68}Ge (half-life 271 days) onto a modified SnO_2 column. ^{68}Ge/^{68}Ga generators of 370, 555, 740 and 1110 MBq $^{68}GaCl_3$ (10, 15, 20 and 30 mCi, respectively) were investigated. The generators were eluted 3–24 h after prior elution at a flow of 3–6 mL min^{-1}, total elution volume 6 mL, with Suprapur HCl (Merck, Modderfontein, South Africa) or Ultrapure HCl (J.T. Baker, Deventer, The Netherlands) in fractions of 0.5 mL at different [HCl], range 0.3–1.0 M, increments of 0.1 M. Eluted ^{68}Ga and ^{68}Ge activities were monitored and quantified per fraction as described earlier (Breeman et al., 2005). All ^{68}Ga activity data are expressed decay corrected. Used chemicals were reagent grade (Sigma-Aldrich, Zwijndrecht, The Netherlands), unless specified otherwise.

Elutions of the generators were optimized so that the eluate could be used for labelling DOTA-peptides, as described earlier (Breeman et al., 2005). The following parameters were investigated: elution profiles, volume peak activity of ^{68}Ga (a), and ^{68}Ge (b), the concentration in the eluate of Ga, Ge, Zn, Ti, Sn, Fe, Al and Cu by ICP (c) and sterility tests of the eluate (d).

2.2. Breakthrough and RadioNuclidic Purity (RNP)

In the literature breakthrough is used for different expressions, first to express the ratio of the activity of a parent radionuclide (e.g. ^{68}Ge or ^{99}Mo) in the eluate vs. activity of the parent on the column. However, the actual activity of the parent ^{68}Ge on the column is frequently not known or uncertain. Secondly, breakthrough is nowadays also in use to express the ratio of activities in the eluate of the parent radionuclide (here ^{68}Ge) vs. daughter radionuclide (here ^{68}Ga). Another expression is RadioNuclidic Purity (RNP), which is defined as the ratio of activity of a radionuclide vs. the activity of another radionuclide. We define RNP here as the ratio of activities of ^{68}Ge vs. ^{68}Ga in the eluate, expressed in %.

2.3. Analysis metal ions in eluate by ICP

Metal ions in the eluate were determined using a Jobin-Yvon Ultima ICP Spectrometer (Wirsam Scientific & Precision Equipment, Auckland Park, South Africa). Concentrations of the following metals were determined: Ga, Ge, Zn, Ti, Sn, Fe, Al and Cu 1000 ppm. Ga, Ge, Zn, Ti, Sn, Fe, Al and Cu standard solutions were obtained from Industrial Analytical (Halfway House, South Africa) and Alfa Aesar (Kyalami, South Africa). The standard solutions were used to prepare one standard solution containing 100 ppm of each of these metals. The 100 ppm standard solution was further diluted to prepare a 1, 5 and 10 ppm standard solution. The standard solutions, made up in 0.6 M HCl, were run to obtain a calibration curve. The undiluted eluate was analyzed.

2.4. Sterility tests of eluate

Dehydrated Tryptic soy broth and Thioglycolate broth were used to determine the sterility of the generator eluate. Tryptic soy broth was added aseptically (through a filter) to the sample provided and incubated at 25 ± 2.5 °C for 14 days. Thioglycolate broth was added aseptically (through a filter) to another sample and was incubated at 32 ± 2.5 °C for 14 days. Samples were checked daily for microbiological growth. The media remains clear if no microbiological growth occurs.

Endotoxins by the chromogenic method were measured by LAL test (Lonza, Walkersville, MD, USA).