Applied Radiation and Isotopes 107 (2016) 113–120

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Preparation of ^{68}Ga-labelled DOTA-peptides using a manual labelling approach for small-animal PET imaging

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HIGHLIGHTS

- Concentration and purification method using anion exchange resin for ^{68}Ga synthesis.
- Manual radiolabelling DOTA-TATE.
- Small-animal pheochromocytoma tumour PET imaging.

ARTICLE INFO

Article history:
Received 2 June 2015
Received in revised form
21 September 2015
Accepted 4 October 2015
Available online 13 October 2015

Keywords:
Gallium-68
Concentration
Purification
NaOH
Radiolabelling
DOTA-peptides
Pheochromocytoma

ABSTRACT

^{68}Ga-DOTA-peptides are a promising PET radiotracers used in the detection of different tumours types due to their ability for binding specifically receptors overexpressed in these. Furthermore, ^{68}Ga can be produced by a ^{68}Ge/^{68}Ga generator on site which is a very good alternative to cyclotron-based PET isotopes. Here, we describe a manual labelling approach for the synthesis of ^{68}Ga-labelled DOTA-peptides based on concentration and purification of the commercial ^{68}Ga/^{68}Ga generator eluate using an anion exchange-cartridge. ^{68}Ga-DOTA-TATE was used to image a pheochromocytoma xenograft mouse model by a microPET/CT scanner. The method described provides satisfactory results, allowing the subsequent ^{68}Ga use to label DOTA-peptides. The simplicity of the method along with its implementation reduced cost, makes it useful in preclinical PET studies.

1. Introduction

The most common positron emitters used for positron emission tomography (PET) in the field of nuclear medicine are ^{18}F, ^{11}C, ^{13}N and ^{16}O due to their ability to be used for labelling bio-molecules. Fluorine-18 is the most widely used and has a significant role in oncology, neurology and cardiology. These radionuclides have relatively short half-lives ($T_{1/2,}$ less than a few hours to at most a few days). There are definite advantages in using short lived radionuclides; for example, there is a low radiation dose associated with each study and radioactive waste disposal problems are minimised if not eliminated. The disadvantages are the need for an accelerator nearby or within easy shipping distance for the longer lived species (a few hours), and for rapid chemical procedures, especially for the formation of more complex compounds (IAEA, 2008). There is also the possibility of producing some positron emitters using a generator, such as ^{82}Rb, ^{62}Cu and ^{68}Ga, being produced using ^{82}Sr/^{82}Rb, ^{62}Zn/^{62}Cu and ^{68}Ge/^{68}Ga generators, respectively. Radionuclide generators represent a cost effective strategy for obtaining daughter radionuclides, provided the generator is used effectively. Other advantages are that the daughter product can be easily obtained on demand and with high specific activity, in a no-carrier-added form. Radionuclide generators can routinely provide radionuclides on demand without on-site availability of an accelerator or a research reactor; thus, in-house availability of daughter radionuclides is one of the key advantages of using radionuclide generator systems (IAEA, 2010).

^{68}Ga is a radionuclide of great interest as it decays to ^{68}Zn by emitting a positron (88.88%) with a maximum energy of 1899 keV and by electron capture (11.11%). Moreover, its short half-life, ($T_{1/2}$=67.845 (18) min) (García-Toraño et al., 2014), makes it compatible with the pharmacokinetics of numerous molecules of interest in PET-based diagnostics, radiotherapy planning and

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http://dx.doi.org/10.1016/j.apradiso.2015.10.005
0969-8043/

follow-up of the response to chemo/radiotherapy. Finally, the long half-life of the parent radionuclide, ^{68}Ge, ($T_{1/2}$=270.95 (26) d) (DDEP, 2015), allows ^{68}Ga can be obtained over a relatively long period of time.

In aqueous solution the only stable oxidation state of this metal is +3, where the free hydrated Ga^{3+} ion is stable only under acidic conditions. In the pH range of 3–7 it can hydrolyse to insoluble hydroxyl complexes, $Ga(OH)_3$, if its concentration exceeds nanomolar level, while at physiological pH its solubility is high due to the almost exclusive formation of $[Ga(OH)_4]^-$ ions (Moerlein and Welch, 1981; Green and Welch, 1989; Bernstein, 1998). To prevent $Ga(OH)_3$ formation, buffers with weak metal-complexation properties as acetate or HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid) have been used during ^{68}Ga labelling, adjusting the pH to 3.5-5 to optimise the synthesis (Buchmann et al., 2007; Maecke et al., 2005; Meyer et al., 2004; Velikyan et al., 2004; Heppeler et al., 1999; Koukouraki et al., 2006). Although HEPES has shown good radiochemical yields with ^{68}Ga (Bauwens et al., 2010; Velikyan et al., 2004), its use in humans has not been authorised (Velikyan et al., 2008, 2010).

The $^{68}Ga^{3+}$ cation can form stable complexes with many ligands containing oxygen, nitrogen and sulphur as donor atoms (Fani et al., 2008). This makes ^{68}Ga suitable for complexation with chelators and various macromolecules. Several bifunctional chelating agents (BFCA) have been developed for complexation of ^{68}Ga and they have been coupled to biologically active targeting agents. Open chain complexing agents have almost completely been displaced by macrocyclic DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and NOTA (1,4,7-triazacyclononane-1,4,7,10-tetraacetic acid) derived conjugates (Boschi et al., 2013). The most important driver for the development of ^{68}Ga radiopharmacy has come up with the development of small tumour-affine peptides, most notably those targeting somatostatin receptors which are overexpressed in neuroendocrine tumours (NETs). ^{68}Ga-based somatostatin analogues such as ^{68}Ga-DOTA-D-Phe^1-Tyr^3-octreotide (^{68}Ga-DOTATOC), ^{68}Ga-DOTA-D-Phe^1–Nal^3-octreotide (^{68}Ga-DOTANOC) and ^{68}Ga-DOTA-D-Phe^1-Tyr^3-Thr^8-octreotate (^{68}Ga-DOTATATE) are currently the most common (Antunes et al., 2007; Fani et al., 2008; Banerjee and Pomper, 2013). In general, DOTA derivatives require high temperatures (80–100 °C) since the synthesis reaction of ^{68}Ga with DOTA exhibits slow formation kinetics. It has been reported that the use of microwave activation substantially improves the efficiency and reproducibility of the ^{68}Ga-chelating agent complex formation; furthermore, microwave activation also leads to fewer side reactions and for increasing radiochemical yield (Elander et al., 2000; Velikyan et al., 2004).

Although ^{68}Ge/^{68}Ga generators have been known since the 1960s (Gleason, 1960; Greene and Tucker, 1961; Yano and Anger, 1964), it was not until the last decade when it has been seen a rise in the use of ^{68}Ga. Increasing applications of generator-based ^{68}Ga radiopharmaceuticals for diagnosis alone, but increasingly for treatment planning by an individual patient-based approach, applying pre-therapeutic evaluation, as well as dosimetric calculations, and for measuring treatment response after radionuclide therapy (known as Theranostics), (Roesch and Baum, 2011), ask for progressive developments towards the optimization of ^{68}Ge/^{68}Ga generators both from chemical and regulatory points of view (Roesch, 2013). Depending on the type of matrix on which they are based, these generators can be classified as either organic or inorganic. Examples of organic-type generators include those based on pyrogallol–formaldehyde (Schuhmacher and Maier-Borst, 1981; Neirinckx et al., 1982), on organic macroporous styrene–divinylbenzene co-polymer containing N-methylglucamine groups (Nakayama et al., 2003) and those silica derivate (Zhernosekov et al., 2010) (marketed by ITG Isotope Technologies Garching GmbH, Germany). Inorganic-type generators include those based on Al_2O_3 (Egamediev et al., 2000), $Al(OH)_3$ y $Fe(OH)_3$ (Kopecky et al., 1973; Kopecky and Mudrová, 1974), SnO_2 (Loc´h et al., 1980; Tang et al., 1997) (iThemba LABS, South Africa, commercially available from I.D.B. Holland), ZrO_2 (Malyshev and Smirnov, 1975), TiO_2 (Razbash, 2003; Neirinckx and Davis, 1979) (commercially available from Cyclotron Co Ltd., Russia and Eckert & Ziegler, USA), Fe_2O_3 (Ambe, 1987; Cao et al., 1998), CeO_2 (Bao and Song, 1996; Song and Bao, 1995) with more recent ones being based on nano-ZrO_2 and nano-CeO_2 derivate (Chakravarty et al., 2010, 2011). Commercial generators currently available are eluted with hydrochloric acid solutions (0.05–1 M) obtaining ^{68}Ga in cationic form suitable for the subsequent labelling, with ^{68}Ga yields range from about 65% to 80% and ^{68}Ge "breakthrough" is at levels of about 0.01–0.001% for fresh generators, with increasing percentages over longer periods of generator usage.

However, these generators have a series of drawbacks, including: (1) the presence of ^{68}Ge in the eluate, (2) the ^{68}Ga obtained is not chemically pure and may contain metals that can compete in the ^{68}Ga synthesis reactions, (3) the use of hydrochloric acid solutions for ^{68}Ga elution. The relatively high H^+ in such solutions may lead to protonation of functional groups on the bifunctional ligands or chelating agents needed for the labelling of ^{68}Ga, and an adequate control of the pH and volume of the eluate improves radiochemical yield (Roesch, 2013; Velikyan et al., 2004).

Different strategies have been employed to overcome these problems, and the most common ones are described in greater detail below. The fractionation method is based on performing a fractionated elution from the generator; approximately 2/3 of the activity is contained in 1–2 mL of eluate and this method minimises the ^{68}Ge content (Breeman et al., 2005). Moreover, the obtained ^{68}Ga is more concentrated and is ready for using in synthesis reactions. A subsequent solid phase extraction (SPE) phase can be included for purification of the labelled product, thereby minimising the ^{68}Ge content in the final product (Decristoforo et al., 2007). A further method is based on direct adsorption of $^{68}Ga^{3+}$ on a cation exchange resin which allows selective separation of Ga^{3+} from Ge^{4+} and other metals due to the different affinity for the resin at different acetone/HCl concentrations (Zhernosekov et al., 2007). Other variants based on this method have been devised to minimise and simplify the process (Mueller et al., 2012; Loktionova et al., 2011; Seemann et al., 2015). Another alternative is the anion exchange method, which is based on the fact that Ga^{3+} forms complex chlorides $[^{68}GaCl_4]^-$ at HCl concentrations higher than 4.0 M (Meyer et al., 2004; Velikyan et al., 2004). These chlorides are strongly adsorbed by an anion exchange resin whereas Ge^{4+} does not interact with this, thus allowing them to be readily separated. The ^{68}Ga adsorbed on the anion exchange resin can subsequently be recovered in a small volume of deionized water. This method was further modified in order to remove the excess of $[H^+]$, by the addition of a cartridge washing step using 5 M sodium chloride solution (Velikyan et al., 2012; Seemann et al., 2015). Several of the strategies described are based on the combination of various methods (Mueller et al., 2011), initially using a cation exchange resin and then an anion exchange resin due to the formation of cationic and anionic gallium species due to changes in pH and HCl concentration.

The following study describes our experience with a manual system to purify and concentrate the ^{68}Ga obtained from a generator based on use of an anion exchange resin. We also describe the subsequent use of the ^{68}Ga using this method to label DOTA-peptides to use in microPET-based imaging studies in a xenograft mouse model for NETs.

2. Materials and methods

2.1. Elution, purification and concentration of ^{68}Ga

Gallium was obtained in $^{68}Ga^{3+}$ form using a commercial TiO_2-based $^{68}Ge/^{68}Ga$ generator (Eckert & Ziegler) according to the manufacturer's instructions using 7 mL of 0.1 M HCl prepared from 30% HCl (TraceSELECT Ultra, Fluka) as eluent. Activity eluted was experimentally measured using a dose calibrator VDC-405 (Veenstra Instruments) and the elution yield was defined as the proportion of ^{68}Ga separated during the elution process, and expressed as percentage of the ^{68}Ge loaded activity at the moment of the measurement. ^{68}Ga was purified and concentrated using a manual method (Fig. 1) according to a slightly modified version of the method described by Meyer et al. (2004) and Velikyan et al. (2004). Elution was performed employing a syringe coupled to an infusion pump at a flow rate of 2 mL/min and the eluted volume was collected in a vial (number 1, located inside a lead-shielded container) containing 30% HCl for forming the corresponding chlorides $[^{68}GaCl_4]^-$. The final concentration of the mixture was 4.3 M HCl, in a final volume of 10.6 mL. This solution was then passed through a commercial cartridge containing the anion-exchange resin Chromafix 30-PS-HCO_3 (Macherey Nagel), previously equilibrated with 3 mL of 5 M HCl, 3 mL of deionized H_2O and 3 mL of 4.3 M HCl, using a multi-channel peristaltic pump at a flow rate of 3 mL/min. The gallium chlorides formed were adsorbed on the exchange resin whereas the ^{68}Ge and other metals did not interact and were collected in waste vial (number 2) by opening the three-way taps A and B in that order. The tubing line and resin used during the process were then washed with 200 μL of 4.3 M HCl for preventing any loss of activity by opening three-way tap C. Subsequently, two elution procedures for ^{68}Ga extraction from the resin were compared: Milli-Q water and NaOH. ^{68}Ga was eluted in Milli-Q water following the method described by Meyer et al. (2004) and Velikyan et al. (2004) and, alternatively, in 0.5 M NaOH. This last procedure minimises the relatively high concentrations of H^+.

Both options (water/NaOH) require opening the three-way taps A and B in that order and were collected in the reaction vessel (number 3), located inside a laboratory microwave, Discover Benchmate (CEM). ^{68}Ga was desorbed with 0.5 mL (11 fractions of 45 μl each) or 1 mL (non-fractionated) of water/NaOH using the peristaltic pump at a flow rate of 0.5 mL/min. The ^{68}Ga elution profiles from the ion-exchange cartridge (using water/NaOH, either continuously or in fractions) were determined using a Foxy R1 fraction collector (Teledyne Isco), collecting fractions of 45 μL and subsequently being measured each in a dose calibrator. The purification yield was expressed as % recovered ^{68}Ga in the the anion-exchange resin of total ^{68}Ga loaded activity at the moment of measurement.

The ^{68}Ge content in the eluent from the generator and the purified samples was calculated by gamma spectrometry using a coaxial germanium spectrometer (Ortec). All samples were allowed to decay for more than 48 h to ensure the complete radioactive decay of ^{68}Ga. At this moment, when both radionuclides are in secular radiochemical equilibrium, ^{68}Ge can be calculated by quantifying the 511 keV peak which corresponds to ^{68}Ga obtained from ^{68}Ge decay.

2.2. Radiolabelling

^{68}Ga obtained as described previously was eluted directly to the reaction vessel containing 25 nmol DOTATATE (ABX) dissolved in 12.5 μl of deionised H_2O and 1 mL of 0.31 M of non-ionic HEPES (Sigma). The pH of the reaction mixture was 3.5–4. Next, the reaction mixture was immediately heated to 90 ± 5 °C for 5 min using a microwave with monomodal radiation and then cooled to room temperature with nitrogen.

The crude product was purified by solid-phase extraction cartridge Sep Pak light (Waters) previously conditioned and equilibrated with 4 mL of pure ethanol and 4 mL of deionized water. The mixture was transferred onto Sep Pak where the ^{68}Ga-DOTATATE and colloidal ^{68}Ga were retained, whereas free ^{68}Ga passed through the cartridge (Bauwens et al., 2010). Next, the cartridge was rinsed with 4 mL of deionised water and the activity on the Sep Pak cartridge was reversely eluted with 0.5 mL ethanol 96% (v/v); during this procedure, all colloidal ^{68}Ga is retained on the Sep Pak cartridge. Finally, ethanol was evaporated to dryness using a Speed Vac (Savant) and ^{68}Ga-DOTATATE was dissolved with normal saline (0.9% NaCl solution). An aliquot was taken to analyze by HPLC the possible peptide radiolysis damage and radiochemical purity of the final product.

The free ^{68}Ga, colloidal ^{68}Ga and radiolabelling yield, were determined as % activity in the waste of the cartridge, activity retained on the cartridge and activity in ethanol respectively of total activity used, correcting measures for decay at the beginning of this step.

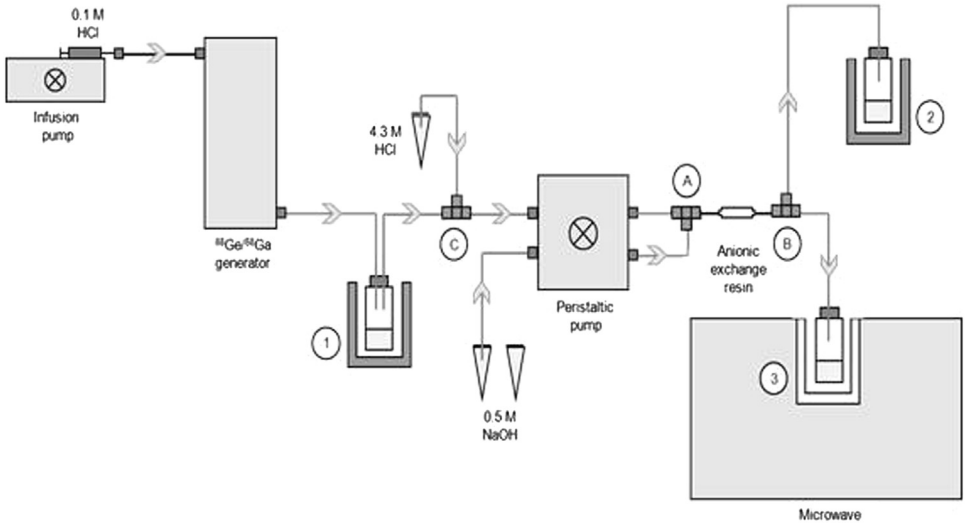

Fig. 1. Manual system for ^{68}Ga elution, purification, concentration and synthesis of ^{68}Ga-DOTA-peptides.

2.3. Quality Control

Radiochemical purity (RQP) is defined as the % of the activity of the radionuclide present in the desired radiopharmaceutical form of the total radioactivity. RQP of ^{68}Ga-DOTATATE was determined by radio-High Performance Liquid Chromatography (radio-HPLC) under the following conditions: an Alliance 2695 system (Waters) controlled by Millenium software, equipped with a diode array UV-detector (996 Waters) and a radioactivity detector LB 507A (Berthold); column: Jupiter Proteo 90 Å column (Phenomenex), 4 μm, 250 × 4.6 mm; UV chromatograms were recorded at 220 nm; eluents were: 0.1% trifluoroacetic acid (TFA) in 5% acetonitrile (solvent A) and 0.1% TFA in 95% acetonitrile (solvent B), gradient: 0–2 min, 5% B; 2–12 min, 5–100% B; 12–15 min, 100% B, 15–18 min, 100–5% B; 18–24 min, 5% B; flow rate: 1 mL/min.

2.4. Small-animal PET imaging

Animal experiments were approved by the Animal Ethical Committee and conducted in compliance with Centre for Energy, Environmental and Technological Research (CIEMAT) Guidelines. Two male Swiss nude mice (Charles River), housed in the CIEMAT laboratory's animal house were used. Both animals were inoculated subcutaneously, in both flanks, with one million cells from a rat pheochromocytoma cell line (PC 12), supplied by Dr. Rodríguez, Membrane Biology and Axonal Repair Laboratory (Hospital Nacional de Parapléjicos, Toledo, Spain).

PET studies were performed 25 d after tumour inoculation in an Argus PET/CT (SEDECAL). Images were acquired under anaesthesia (2–2.5% isoflurane in 5% oxygen using a Fluovac device) 1 h after the i.v. inyection into the caudal tail vein of around 2–2.5 MBq of ^{68}Ga-DOTA-TATE in 0.15–0.2 ml and pH 6 with the following parameters: energy window 250–700 keV and 45 min static acquisition. For the CT image: voltage 40 kV, current 150 μA, 2 exposures, 360 projections and standard resolution. PET images were corrected for random events and scatter with and without attenuation correction and reconstructed using the 2D–OSEM (Ordered Subset Expectation Maximisation) algorithm (16 subsets and two iterations). For image-reading purposes, ^{68}Ga-DOTA-TATE PET images were fused with the corresponding CT images, which were used as a reference for image coregistration.

Quantification of ^{68}Ga-DOTATATE uptake was performed calculating tumour to non-tumour ratios using a reference tissue with low SSTR expression such as muscle (Poeppel et al., 2011). The mean activity was then corrected by injected dose and weight of the animals to derive SUV values (SUV, standardized uptake value = mean activity (kBq/mL)*weight of the animals (g)/injected dose (kBq)). The peak SUV (SUV_{max}) was calculated by automatically drawing (around tumours seen on the coregistered axial CT images) a region of interest ROI having a threshold of 30% of the SUV_{max}. A value of 30% was arbitrarily used, since the volume of interest determined with this threshold appeared to fill the apparent tumour size on PET images. We also calculated the SUV of the tumours relative to the background by dividing the SUV_{max} of the tumours by the SUV_{mean} of the muscle ($SUV_{T/M}$)

2.5. Statistical analysis

All values are expressed as mean ± standard deviation. Statistical significance was tested using the *t*-test using GraphPad software (available at: http://www.graphpad.com/quickcalcs/). Difference was considered statistically significant if $p < 0.05$

3. Results and discussion

3.1. Elution, purification and concentration of ^{68}Ga

^{68}Ga yield obtained from the generator elution was around 70% during this study, results in accordance with the manufacturer's specifications. ^{68}Ga as $[GaCl_4]^-$ in 9.1 mL 4.3 M HCl was adsorbed (99.8 ± 0.1%, n = 32) on the Chromafix 30-PS-HCO_3 cartridge. Of total ^{68}Ga column activity, a 77 ± 3% was desorbed in the first 0.2 mL of Milli-Q water and 95 ± 1% with 0.5 mL (Fig. 2) when it was eluted with small fractions of deionised water (45 μl). If Milli-Q water was continuously passed through the cartridge, it could be obtained approximately 64 ± 1% of the available activity with 0.5 mL and 85 ± 3% with 1 mL (Fig. 3). Therefore, the percentage of desorbed ^{68}Ga from the resin was significantly higher when the eluent was passed in fractions through the anion exchange column. We have had higher values for ^{68}Ga recovery than those reported by de Blois et al. (2011), although slightly lower with respect to those reported by others (Velikyan et al., 2004; Vosjan et al., 2011).

The ^{68}Ge/^{68}Ga generators utilize hydrochloric acid solutions for ^{68}Ga elution. It has been described that minimising the pH and volume of ^{68}Ga eluted prior for labelling should facilitate syntheses yields (Roesch, 2013). In order to decrease acidity of the eluate of the exchange resin, a 5 M NaCl solution has been used by any authors (de Blois et al., 2011; Mueller et al., 2012; Schultz et al., 2013). In the present work, we have chosen sodium hydroxide as neutralising agent. As it can be seen in Fig. 2, the highest desorption was obtained with NaOH as eluent. Of total ^{68}Ga column activity, a 75 ± 2% was desorbed in the first 0.2 mL and 99 ± 1% in 0.5 mL when it was eluted with small fractions of NaOH (45 μl). When NaOH was continuously passed through the cartridge approximately 73 ± 2% of the available activity with 0.5 mL and 96 ± 1% with 1 mL could be obtained (Fig. 3). These data show that the percentage of recovered ^{68}Ga activity was significantly higher with NaOH than deionised water. Therefore it was decided that the best procedure for desorbing ^{68}Ga from anion exchange resin was to elute with only 400 μL of NaOH 0.5 M (2 × 200 μl); this method allowed a 95 ± 3% (n=20) recovery of the loaded ^{68}Ga activity on the anion exchange column.

It is interesting to note that the use of 400 μl of 0.5 M NaOH for recovering ^{68}Ga from the anion exchange cartridge has several advantages as it concentrates the generator eluate 17.5-times and

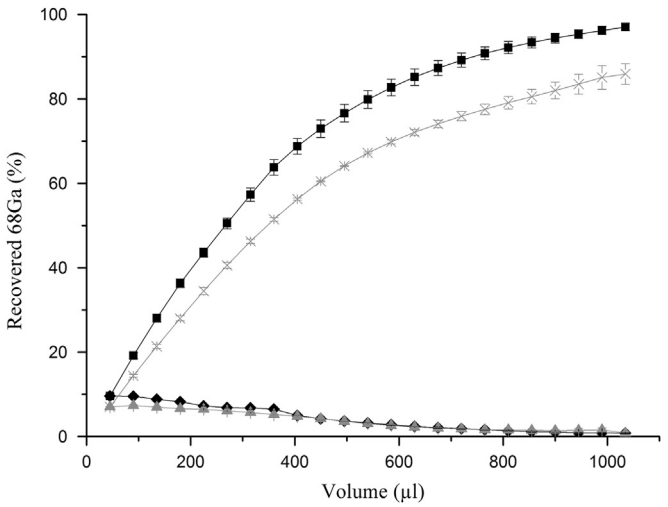

Fig. 2. Desorption of ^{68}Ga activity from total ^{68}Ga activity on the Chromafix cartridge. Elution profile non-fractionated using as eluent 1 mL of deionised water (-X-) or NaOH (-■-), and cumulative curve respectively (-▲-), (-◆-).

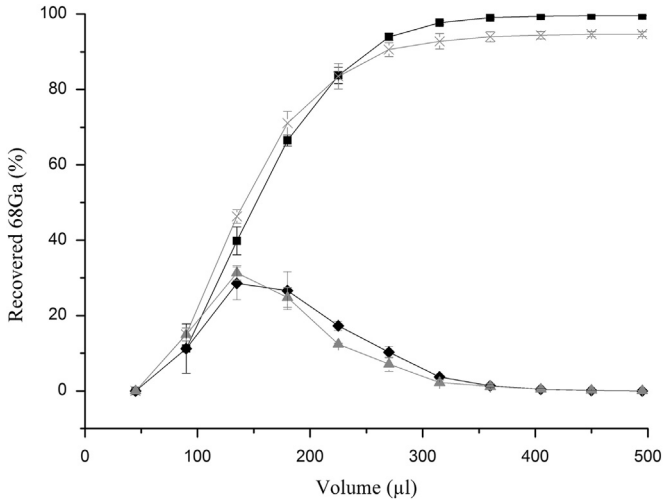

Fig. 3. Desorption of ^{68}Ga activity from total ^{68}Ga activity on the Chromafix cartridge. Elution profile fractions 45 µl of deionised water (-X-), or NaOH (-■-) and cumulative curve respectively (-▲-), (-◆-).

the highly concentrated ^{68}Ga in small volume improves radiochemical yield. Thus, some authors had used ^{68}Ga highly concentrated to obtain ^{68}Ga-carbon nanoparticle aerosol for examination acute pulmonary emboli (Borges et al., 2011; Kotzerke et al., 2010).

Moreover, this method minimises the relatively high concentrations of H^+, thus reducing the amount of buffering agent required for the ^{68}Ga synthesis. This could be important because recent works tend to prove that HEPES, such as sodium acetate buffer, may, to some extent, compete with BFCA for the ^{68}Ga complexation (Clausén et al., 2002; Martins et al., 2013). Another advantage of this method is that it can be translated to any generator type eluted with HCl, independently on its molarity. It can also be used for the preconcentration of several generator eluates independently on the total volume.

Regarding ^{68}Ge breakthrough, it was found that the ^{68}Ge content with respect to eluted ^{68}Ga activity from the generator was not more than 1×10^{-3}% ($n=5$) according to the manufacturer's specifications. Since ^{68}Ge is not retained on the anion resin, the concentrating step above described can also be seen as a purification of ^{68}Ga from the parent nuclide. Hence, ^{68}Ge breakthrough decreased to $4.1 \pm 0.2 \times 10^{-6}$% ($n=5$) in the samples obtained from the anion exchange resin. An almost complete removal of ^{68}Ge in addition to an effective eluate volume reduction and concentration of H^+ decrease provide concentrated ^{68}Ga an aqueous solution ready for using.

3.2. Radiolabelling and Quality Control

The use of 25 nmol de DOTATATE led to a high labelling yield (95.6 ± 2.3%, $n=20$), in agreement with the results published by Velikyan et al. (2004) and Bauwens et al. (2010). We decided to use HEPES buffer since it has been published that is the best buffer regarding for labelling yield. HEPES is currently not approved for human use; however, despite the restriction by legal issues, we believe it is the most appropriate buffer for the preparation of ^{68}Ga-DOTA-peptides for small animals. NaOH addition to the reaction mixture before addition of the buffer led to lower total volumes and slightly higher yields. Therefore, the elution of ^{68}Ga with NaOH from the anion exchange column could stabilize the pH of the reaction mixture and improve the reproducibility of the^{68}Ga-radiolabelling procedure. Our experiments are consistent with previous results (Bauwens et al., 2010; Velikyan et al., 2004).

As it has been reported, ^{68}Ge/^{68}Ga generator eluates are not chemically pure since no-radioactive metals such as Zn may represent elements competing with ^{68}Ga for coordinative labelling of radiopharmaceuticals precursors (Roesch, 2013). As mentioned by Velikyan et al. (2004), a preventive elution 4 h prior to the synthesis is recommended since the interfering metal ion concentration can be kept at its minimum value. Although it was not possible for determining the metal ion content of the generator eluate in the present work, we have not found differences between to use the first elution of the generator of each day or an elution 3–4 hours later with regard to labelling yield (data not shown), possibly due to excess amount of DOTA-ligand used.

In agreement with the results published by Bauwens et al. (2010), about the presence of ^{68}Ga-colloid in the synthesis of ^{68}Ga-DOTA-TATE, estimated from the activity retained during the solid-phase extraction step, we found that the percentage was extremely low (1.41 ± 1.13%; $n=20$). This low retention could also be due to nonspecific binding.

RQP of ^{68}Ga-DOTATATE was measured by radio-HPLC. Fig. 4 shows a typical radio-HPLC chromatogram of ^{68}Ga-DOTATATE which was prepared as described previously. The radio-HPLC chromatogram gives a peak with retention time of 10.02 min (Fig. 4); the RCP result from HPLC was ≥ 95%. In the chromatogram can be observed a relatively broad peak with an unresolved front shoulder before the mean peak; this poorly separated shoulder was estimated to be < 2% of the total amount of radioactivity regardless of the amount of starting activity. These radio impurities could partially attribute to the radiolytic oxidation of ^{68}Ga-DOTA-TATE, which is consistent with previous results (Breeman et al., 2009; Mu et al., 2013). Addition of radical scavengers such as ascorbic acid could significantly suppress the formation of by-products (Mu et al., 2013).

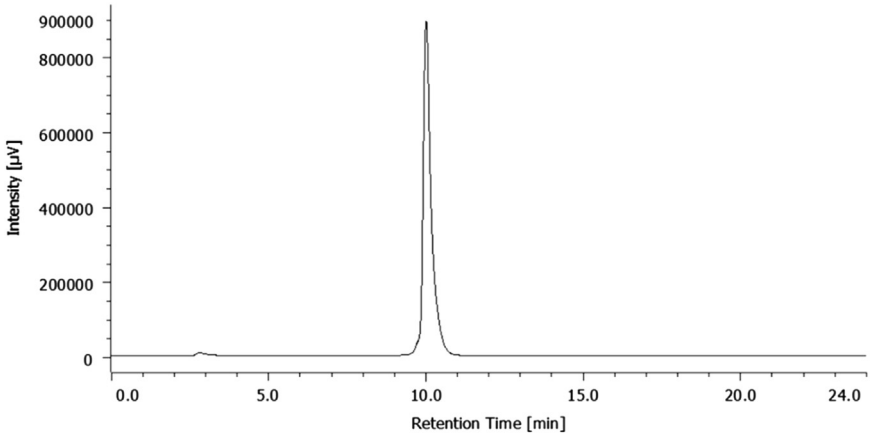

Fig. 4. Radio-HPLC chromatogram for ^{68}Ga-DOTA-TATE, tr = 10.02 min, RCP = 95%.

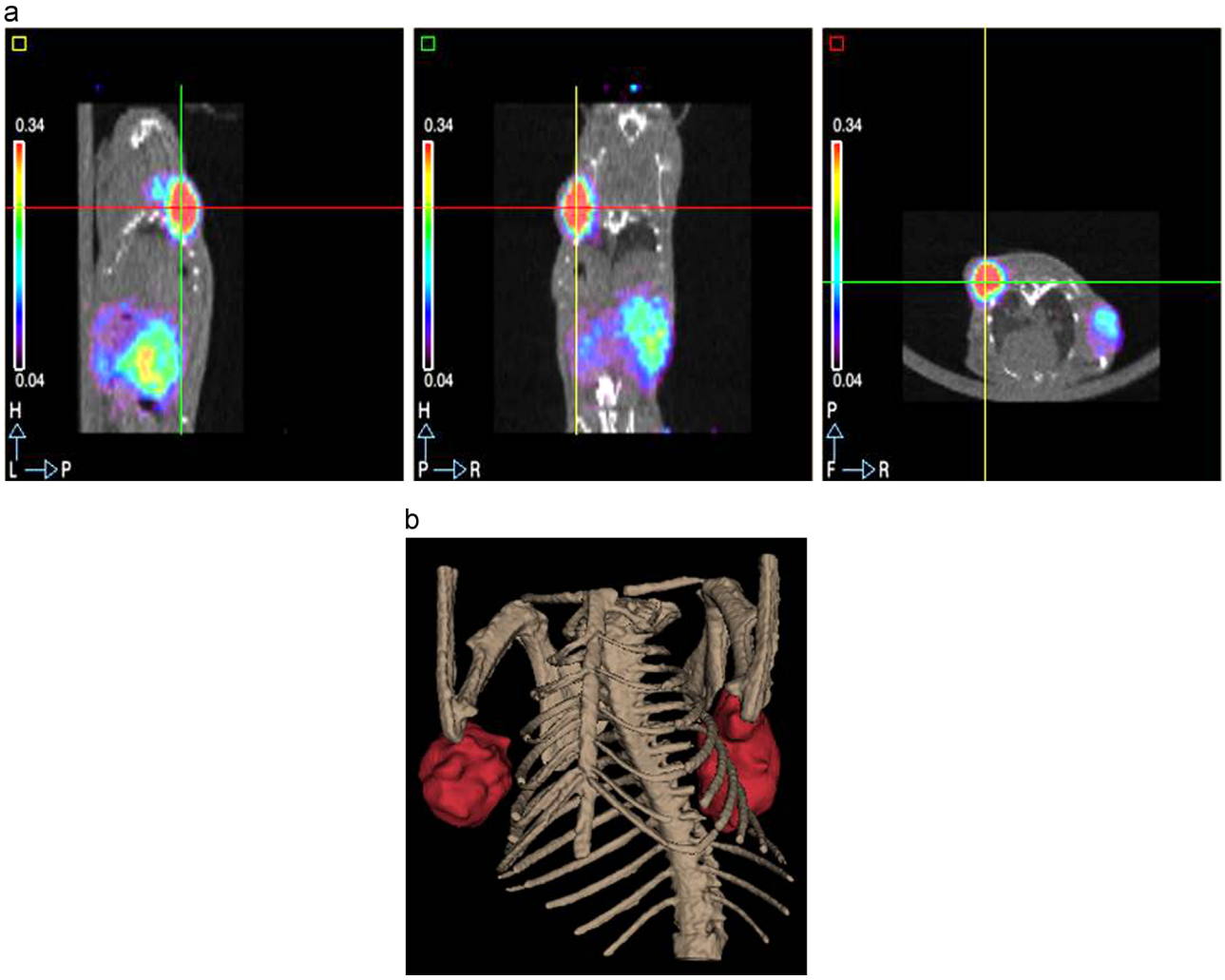

Fig. 5. (a) Fused PET/CT images of pheochromocytoma in a xenograft mouse model, (b) segmented image using ITK-SNAP (Yushkevich et al., 2006).

3.3. Small-animal PET/CT study

Pheochromocytomas (PCC) are rare catecholamine-secreting neuroendocrine tumours derived from chromaffin cells. Although ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) remains as the gold standard in PCC diagnosis (Naji and AL-Nahhas, 2012), ^{68}Ga-labelled peptides are promising agents for the diagnosis and management of pheochromocytoma since SSTR receptors are overexpressed in malignant PCC (Win et al., 2007; Maurice et al., 2012; Naji and AL-Nahhas, 2012). This last imaging approach has become well established and, in some clinical centres, it has become part of the routine practice (Naji and AL-Nahhas, 2012).

In order to confirm that the ^{68}Ga obtained from the purification and concentration process involving 0.5 M NaOH was suitable for labelling DOTA-ligands and the subsequent application therefore in PET, ^{68}Ga-DOTA-TATE (2–2.5 MBq) was injected in an in vivo pheochromocytoma xenograft mouse model. Tumours and kidneys showed intense ^{68}Ga-DOTA-TATE uptake (Fig. 5a and b). Tumour SUV_{max} ranged from 0.26 to 0.63 (mean, 0.37 ± 0.17) and mean $SUV_{T/M}$ ratio was 31.2 ± 17.7 (range 19–57) in the non-attenuation corrected images. In order to evaluate the effect of attenuation on quantification, the same analysis on the tumours and muscle was performed. Although some authors have suggested that CT-based attenuation correction should be applied for accurate quantitative small animal PET imaging (El Ali et al., 2012), we have not found differences. Thus, tumour SUV_{max} ranged from 0.24 to 0.63 (mean, 0.37 ± 0.18) and mean $SUV_{T/M}$ ratio was 26.4 ± 13.2 (range 17–46) in the attenuation corrected images. On the other hand, ^{68}Ga-DOTATATE, being hydrophilic, was primarily excreted through the kidneys; this contributed to the high uptake in the kidneys.

4. Conclusion

A simple ^{68}Ga purification and concentration method followed by ^{68}Ge/^{68}Ga generator elution has been described. This protocol can be carried out in a time-frame which allows the subsequent use of ^{68}Ga-DOTA-peptides. Furthermore, the minimal financial investment required and its simple and practical design mean that it can easily be implemented in research centres in which ^{68}Ga-based PET studies are performed in experimental small-animals. On the other hand, the analogue of somatostatin labelled with ^{68}Ga was useful for detecting neuroendocrine tumours such as pheochromocytomas in a xenograft mouse model.

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