J Radioanal Nucl Chem (2012) 292:683–687
DOI 10.1007/s10967-011-1482-3

Speciation of no-carrier-added ^{68}Ga prior to its labeling for PET imaging

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Received: 27 September 2011 / Published online: 20 October 2011

Abstract The present article describes the probable speciation of ^{68}Ga radionuclide just before labeling to DOTA peptides for PET imaging. The ^{68}Ga eluted from an anion exchange column after its purification was analyzed for its elemental composition and pH at several stages. Neutron activation analysis of the eluted fractions yields the concentrations of Na and Cl, pH measurements indicate the concentration of free H^+ ions in the medium and specific activity calculations indicate the concentration of ^{68}Ga in the solution. Using all these information we get the idea of speciation of no carrier added Ga in the eluted fractions from CHEAQS programme. The estimations indicate that Ga is mostly present as $GaCl^{2+}$ in the total MiliQ eluate. However, just before labeling of DOTA the pH of the Ga-containing eluate is adjusted to ~3.5 using HEPES buffer and at that condition Ga remains as Ga^{3+} species which is responsible for a successful and efficient labeling. The MilliQ eluate collected before actual labeling was estimated for trace elements using inductively coupled plasma atomic emission spectrometry was found to contain a few ppb of Al, Co, Pd and Pt that did not interfere in the actual labeling. A clear idea about the prerequisite of ^{68}Ga species before labeling to a peptide might be of special interest for its judicious application as a radiopharmaceutical.

Keywords Speciation · ^{68}Ge/^{68}Ga generator · Neutron activation · CHEAQS

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Introduction

In recent years ^{68}Ga has gained a renewed interest for use in positron emission tomography (PET) imaging. It readily forms stable complexes with DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), allowing peptides and other small molecules to be radiolabeled at high specific activities [1]. ^{68}Ga, with a positron yield of 89% and a half-life of 68 min is compatible with the pharmacokinetics of many peptides of interest in PET imaging. The 9 months half-life of the parent ^{68}Ge isotope allows PET application even without a cyclotron. Much work has been done with commercially available ^{68}Ge/^{68}Ga radionuclide generator based on a TiO_2 phase (Cyclotron Co., Obninsk, Russian Federation) [2, 3]. The eluted Ga^{3+} from the generator is further purified by converting it into $[GaCl_4]^-$ followed by adsorption on an anion exchange column. Prior to the labeling of DOTA-octreotides (DOTATOC, DOTANOC, etc.) and desferrioxamine-B-succinyl-octreotide (DFOOC) with ^{68}Ga, it is eluted with low volume water.

The method of utilization of anion exchange chromatography was introduced in order to increase the ^{68}Ga concentration and remove competing impurities to obtain a fast and quantitatively ^{68}Ga-labeled peptide conjugate, which could be used in humans without further purification [1, 4]. In combination with microwave heating, the

DOTA-peptide conjugates could be labeled within 15 min with 95% ^{68}Ga radioactivity incorporation. The optimal incorporation yield for Ga^{3+} in the DOTA cage was also achieved in the pH range of 3.5–4.0 by Meyer et al. [5]. At higher pH, Ga^{3+} tends to form hydroxy-aquo complexes, while the complex formation yield decreases at lower pH values. In order to control the pH they found the non-ionic buffer HEPES most suitable.

According to the earlier labeling experiment of Breeman et al. [2], the rate of incorporation of ^{68}Ga in DOTA-peptides was found to be pH dependent [2]. There was no radioactive incorporation at pH 1, a slow start at pH 2.5, and completion of incorporation at pH 4 was observed after 5 min at 80 °C. Maximal achievable specific activities were up to 1 GBq ^{68}Ga per nmol for DOTATOC and DOTA-tate. Rösch et al. [6] also preconcentrated and purified $^{68}Ga^{3+}$ eluted from the cation exchange resin for labeling reactions with a final pH of 2.3 [6].

It is clearly understood that the labeling of ^{68}Ga is very much pH sensitive, but the chemical species responsible for the actual labeling is still not well understood. To have an idea about the real chemical form of Ga actually undergoing reaction for incorporation in the DOTA, experiments as well as theoretical simulations are necessary. To the best of our knowledge, a detailed study of the chemical speciation of ^{68}Ga at this stage before labeling has never been reported. In the present work we report about the speciation possibility of ^{68}Ga after purification from the anion exchanger. A tin(II) oxide generator was used in our study.

Experimental

A tin(II) oxide generator for ^{68}Ge/^{68}Ga was obtained from iThemba Labs, Somerset West, South Africa. The generator was eluted with 1 M ultrapure HCl (Ultrapure HCl 30% was obtained from J.T. Baker, Deventer, The Netherlands). All chemicals were of the highest available grade. The eluate containing ^{68}Ga was mixed with 10 M HCl in order to achieve a 6 mL solution of an overall HCl concentration of 5 M. The ^{68}Ga solution having a total activity of 175 MBq was adsorbed on an anion exchange resin Oasis WAX (weak anion exchanger SPE cartridge 1 cc, 30 mg, Waters, Etten-Leur, The Netherlands) as $[GaCl_4]^-$. The micro column containing ^{68}Ga in the anionic form was washed with 2 mL of 5 M NaCl solution to reduce of the acidity while keeping the Cl^- concentration constant at 5 M. ^{68}Ga was then eluted with MilliQ for further labeling experiments. Fractions of $\approx$0.09 mL of this MilliQ eluate were collected in small plastic vials using a fraction collector in small plastic vials. The empty vials were previously weighed. The exact weights of the vials were again measured after collection of the ^{68}Ga containing MilliQ fractions. The vials were then closed and its activity for ^{68}Ga was measured in a dose callibrator (VDC-405, Veenstra, Joure, The Netherlands) while ^{68}Ge activity was measured after $\geq$24 h in a well-type gamma counter (COBRA, Packard Instruments Co, Groningen, The Netherlands) [2]. The ^{68}Ga in the vials was allowed to decay for at least 24 h and then again irradiated with thermal neutrons with a flux of 3.9×10^{12} cm^{-2} s^{-1} to measure the concentration of Na and Cl. The irradiation was done in the fast irradiation system, CAFIA (acronym for Carbon Fiber Autonomous Facility for Irradiation and Analysis) of the 2 MW research reactor at the Reactor Institute, Delft, The Netherlands [7]. In a separate but similar set of experiment, the pH of all the fractions were measured using a pH meter with a micro probe. With the idea of concentrations of Ga, Na, Cl and H (from pH measurements) theoretical calculations were done using CHEAQS programme for evaluation of chemical species in each of the fractions. The pH measurement and elemental measurements were done in two similar but separate measurements to avoid weight loss due to sticking of solution to the pH meter probe. The collection of the total (2 mL) eluate from the anion exchanger was also done and analysis for the above elements as well as pH measurement and multielement analysis was also done to have the overall idea of speciation and contamination from other elements. Multielemental measurement was also done with the HCl solution that was loaded initially to the anion exchange column to estimate the behavior of contaminants. Inductively coupled plasma atomic emission spectrometry (ICPOES, Perkin Elmer, OPTIMA 4300) was used to analyze the contaminants in the total MilliQ eluate. All the elements were measured both in axial and radial modes using a nebulizer. The nebuliser flow rate was maintained at 1.5 mL/min. The results reported here are the mean data of three successive measurements.

Results and discussion

Chelated gallium species are widely studied for their applications in medical therapy and diagnostic processes. For example, rituximab pharmaceutical solution labeled with ^{67}Ga was used to study an antigen–antibody reaction in mouse [8]. Bleomycin labeled with ^{66}Ga was studied for its possible diagnostic properties in PET [9]. The efficacy of the chelates depends on their chemical behavior, which in turn depends on the chemical speciation of the metal ion. Speciation study of medically active metals is an emerging field of research as it yields valuable information regarding its mode of action [10–15]. In diagnostic imaging, several radiopharmaceuticals and magnetic resonance contrast reagents are based on chelates of Gd, ^{99m}Tc, and ^{67}Ga. The

Table 1 Ga, ^{68}Ga, Ge, Na and Cl concentrations along with pH in MilliQ fractions

Weight of MilliQ fractions (g)	Ga (M/L)	^{68}Ga (MBq)	Ge (M/L)	pH	Na (M/L)	Cl (M/L)
1 (0.113)	1.5E−9	1.73	7.63E−010	0.59	3.4	3.4
2 (0.0916)	1.8E−9	1.68	6.66E−010	0.76	0.81	0.81
3 (0.077)	1.6E−9	1.26	3.70E−010	0.88	0.42	0.42
4 (0.079)	1.4E−9	1.13	7.90E−011	1.07	0.2	0.21
5 (0.071)	1.3E−9	0.94	5.64E−011	1.17	0.15	0.16
6 (0.078)	6.7E−10	0.53	4.37E−011	1.27	0.09	0.09
7 (0.077)	4.8E−10	0.38	6.83E−011	1.44	0.06	0.06
8 (0.079)	4.9E−10	0.39	6.07E−011	1.48	0.05	0.05
9 (0.078)	3.02E−10	0.24	5.95E−011	1.58	0.04	0.04
10 (0.076)	2.4E−10	0.19	6.15E−011	1.63	0.03	0.03
11 (0.076)	2.26E−10	0.18	3.85E−011	1.64	0.02	0.02
12 (0.078)	2.9E−10	0.23	3.06E−011	1.65	0.01	0.01
13 (0.063)	2.9E−10	0.19	2.14E−011	1.71	0.02	0.02
14 (0.076)	2.76E−10	0.21	2.14E−011	1.79	0.01	0.01
15 (0.074)	7.8E−11	0.06	2.02E−011	1.81	0.01	0.01

goal of diagnostic imaging is to direct the element to a particular site of interest, e.g., to achieve selective accumulation in a tumor. The distribution will clearly be affected by speciation, and the labile chelates are expected to exchange the metal with a variety of biological ligands. Concurrent with the recent interest in the design of metal compounds as drugs and diagnostic agents and in understanding the molecular mechanisms of action of metallopharmaceuticals, the present study gives a lot of relevant information. Central to progress in this area is investigation of the speciation of the metal actually used for labeling. A precise knowledge about the actual metal species taking part in any chemical reaction may lead to quantitative and reproducible results. In our present study we have tried to figure out all the associated elements present with our radionuclide of interest, ^{68}Ga, to find its actual chemical form that takes part in labeling. The results of neutron activation give an idea about the concentrations of Na and Cl, pH measurements indicate the concentration of free H^+ ions in the medium and specific activity calculations indicate about the concentration of ^{68}Ga in the solution (Table 1) [2]. When all this information is taken into in the CHEAQS programme [16] we get the idea of speciation of no carrier added Ga in the solution to be tagged to DOTA. An essential process in the development of any speciation model is to define a series of chemical equilibria which represent the system under investigation. In defining the equilibria all the chemical species involved and the equilibrium constants for the reactions have to be specified. In this case theoretical calculations are done using CHEAQS, a computer program for calculating chemical equilibria in aquatic systems. It gives information about the selection process for the equilibria and equilibrium constants from CHEAQS database. Selecting equilibrium constants is an important step in equilibrium modelling. To ensure that the database, CHEAQS contains a consistent set of correct constants, values are taken from the NIST database 46 (version 8) where available. The programme based on a well documented huge database taking into account adsorption equilibria, redox equilibria as well as models for organic complexation helps to identify the species under study.

An extra washing using the anion exchanger has been included to reduce the acidity of the Ga containing eluate. 5 M NaCl was used instead of HCl. This is because high concentration of Cl^- salt keeps the complex in the form of $GaCl_4^-$. The anion exchanger remains bonded with this anionic complex. On washing with water, Cl^- gets slowly removed and the speciation of Ga changes to free Ga^{3+} ions, which then gets eluted. With progress in elution with MilliQ the pH of the fractions slowly increase from 0.6 till 1.8 and the ^{68}Ga concentration increase slightly and then

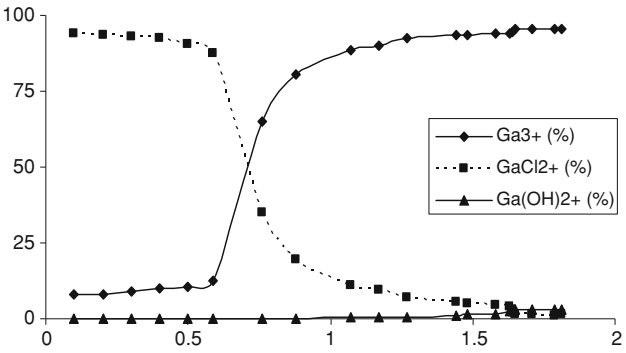

Fig. 1 Distribution of Ga species in the MilliQ fractions

Table 2 Estimation with the total MilliQ collection

Total MilliQ fraction	mol/L Ga	pH	mol/L Na	mol/L Cl	Species distribution in solution		
					Ga^{3+} (%)	$GaCl^{2+}$ (%)	$Ga(OH)^{2+}$ (%)
1.5 mL	1.24E−11	0.28	5	5	6.57	93.19	0.25

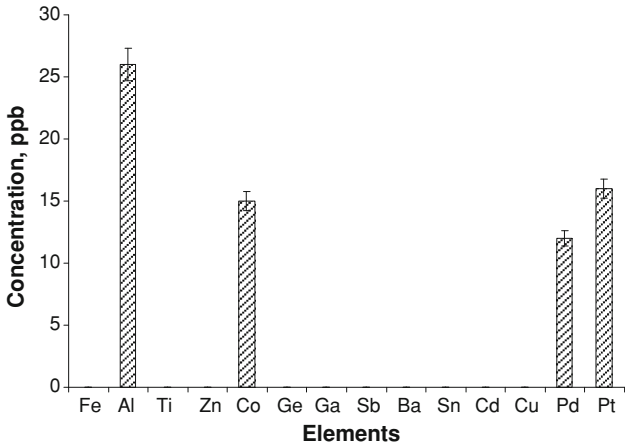

Fig. 2 Different elemental impurities in the MilliQ fraction containing ^{68}Ga eluted from the anion exchange resin

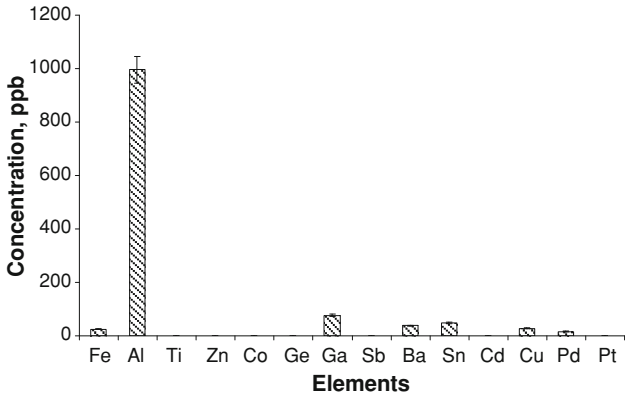

Fig. 3 Different elemental impurities in the HCl solution before passing through the anion exchanger for further purification

decreases. The distribution of Ga, Na, Cl and the pH of the fractions are tabulated in Table 1. The variation in the dissolved species of Ga is shown in Fig. 1. It could be seen that the Ga^{3+} slowly grows with increase in pH while the $GaCl^{2+}$ decreases. The estimations of the above elements along with the pH of the total eluted volume initially in the (2 mL) with MilliQ indicate that Ga is mostly present as $GaCl^{2+}$ (Table 2). However, just before labeling to DOTA the pH of the Ga eluate is adjusted to ~3.5 using HEPES buffer and at that condition also Ga is expected to remain as Ga^{3+} species. The pH of the medium is raised to 3.5 before labeling because, actual labeling occurs at an optimum pH depending on the radiotracer and the peptide used [17]. Labelling with ^{68}Ga was found to be optimum at pH ~3.5. This result is also in agreement with earlier speciation of Ga in blood plasma by Jackson and Byrne where the medium contained a number of different metal ions and a myriad of low molar mass ligands [11]. A computer model simulated that the major portion of Ga was present as Ga^{3+} at pH 3.

Very low-level elemental impurities were found to be present in the MilliQ eluate according to the results of ICP measurements (Fig. 2). Al, Co, Pd and Pt at a few (15–25) ppb concentrations can be expected from side products of nuclear reactions in the ^{68}Ge generation step or from the chemicals used at various stages of elution and purification. There was certainly a reduction in the total amounts of contaminants when compared with the mother solution that was loaded to the anion exchanger for purification of ^{68}Ga (Fig. 3). However, these impurities do not participate in labeling of DOTA peptides. The radionuclide purity (RNP, expressed as the activities of ^{68}Ge over ^{68}Ga) of ^{68}Ge was found nearly 10^{-2}%. In the labeling step, solid phase extraction by C_{18} reversed phase provides an extra "safety net" for clinical applications since it reduces ^{68}Ge and RNP alters to a level of 10^{-4}% [18].

Conclusion

Processing of ^{68}Ga radionuclide after eluting from the ^{68}Ge column involves a number a chemical steps before actual labeling of DOTA is done with it. An efficient and optimized method of radiopharmaceutical preparation should include a vivid understanding of the actual chemistry and speciation of the radionuclide involved. The present method directs towards the fact that, an experimentalist may use any generator material and develop any separation scheme but the ultimate species of the ^{68}Ga radionuclide just before the labeling experiment should preferably be Ga^{+3}. The findings of the radionuclide speciation in our present study offer a "master key" in the development of radiopharmaceuticals. Not only ^{68}Ga but such speciation of other radionuclides before actual tagging might come out to be a directive to an optimized labeling in development of radiopharmaceuticals.

Acknowledgments The authors gratefully acknowledge Martijn Visser, Erasmus MC, Rotterdam, for his help in collection of samples and Anneke-Koster Ammerlaan of TU Delft for her help in neutron activation analysis of the samples.

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