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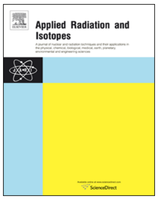

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Influence of metal ions on the ^{68}Ga-labeling of DOTATATE

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HIGHLIGHTS

- Evaluation of the influence of metal cations on the radiolabeling yield of [^{68}Ga(DOTATATE)].
- Detailed study of the impact of reaction conditions on the influence of metal ions.
- Stability of [^{68}Ga(DOTATATE)] towards transmetalation.

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ABSTRACT

The influence of metal cations (Fe^{3+}, Fe^{2+}, In^{3+}, Cu^{2+}, Ca^{2+}, Al^{3+}, Co^{2+}, Lu^{3+}, Ni^{2+}, Pb^{2+}, Ti^{4+}, Y^{3+}, Yb^{3+}, Zn^{2+}, and Zr^{4+}) on the radiolabeling yield of [^{68}Ga(DOTATATE)] was evaluated. Our most important observation was that, within our experimental limit, the metal ion/ligand ratio plays a critical role on the influence of most metal ions. More in-depth studies, with Cu^{2+} and Fe^{3+}, revealed that reaction temperature and concentration changes have little effect, but speciation changes with pH are crucial. Furthermore, we found that [^{68}Ga(DOTATATE)] is stable in the presence of high concentrations of Fe^{3+}, Zn^{2+} and Pb^{2+}, but transmetalates with Cu^{2+} at 95 °C.

1. Introduction

Gallium-68 (^{68}Ga) is a radionuclide of increasing clinical interest because of its favorable decay properties, easy access by a long-lived generator system and growing use in the PET imaging of malignant neuroendocrine disease (Breeman et al., 2011). This generator-produced radionuclide has the potential for wider use since it can be conveniently produced from the long-lived parent isotope ^{68}Ge ($t_{1/2}=271$ d) and has suitable properties with a half-life of 67.7 min and a positron yield of 89%. Several commercial ^{68}Ga generators have become available recently and they are increasingly being used in clinical settings to radiolabel a diverse set of biomolecules such as proteins, peptides and monoclonal antibodies.

The imaging of low density cell receptor sites, particularly those in the brain, require the production of radiopharmaceuticals with high specific activities (Velikyan et al., 2008). To produce high specific activity ^{68}Ga radiopharmaceuticals, the generator eluates must have low metallic contamination and high activity concentrations.

It has been widely postulated that metal ion contamination interferes, competitively, with $^{68}Ga^{3+}$ chelate binding, since many of the chelates currently used are promiscuous, forming stable complexes with a range of metal ions other than Ga^{3+}. Therefore, manufacturers of ^{68}Ge/^{68}Ga generators attempt to minimize the metal contaminant content in the final eluate. However, until recently, there has been no extensive study detailing the influence of individual metal ion contaminants on the radiolabeling performance of generator eluates. Velikyan et al. reported on the effect of Al^{3+}, Fe^{3+} and In^{3+} on the labeling of DOTATOC (1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid[DOTA]^0-Tyr^3-octreotide) with $^{68}GaCl_3$ (Velikyan et al., 2008) and Jeong et al. studied the labeling efficiency change of [^{68}Ga(NOTA)] (1,4,7-triazacyclononane-N,N′,N″-triacetic acid) in the presence of various metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, In^{3+}, Mg^{2+}, Ca^{2+}) (Jeong et al., 2009). Recently, Jakub Šimeček et al. published a study assessing the influence of the most relevant metal ions contained in generator eluates (Zn^{2+}, Cu^{2+}, Fe^{3+}, Al^{3+}, Ti^{4+}, Sn^{4+}) on incorporation of $^{68}Ga^{3+}$ into a

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range pendant-arm macrocyclic chelators such as NOTA, DOTA (1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid), TRAP (1,4,7-triazacyclononane-1,4,7-tris[methyl(2-carboxyethyl)phosphinic acid]) and NOPO (1,4,7-triazacyclononane-1-[methyl(2-carboxyethyl) phosphinic acid]-4,7-bis-[methyl(2-hydroxymethyl)phosphinic acid]) (Šimeček et al., 2012). Other likely metal contaminants (i.e. Zr^{4+}, Pb^{2+}) in generator eluates, reactant solutions or as surface contamination of reaction tubes have, to the best of our knowledge, not been studied at all. We were also not able to find any data on the impact of reaction conditions on the influence of metal ions.

This article details investigations into the influence of a variety of metal ions on $^{68}Ga^{3+}$ radiolabeling reactions, seeking to understand the influence of likely contaminant metal ions and to provide advice on the metal ions that should be monitored on a routine or semi-routine basis during the practical use of $^{68}Ga^{3+}$ generators in research laboratories and hospitals. With a clinical setting in mind, we have chosen to study the radiometal complexation reaction of ^{68}Ga with the clinically relevant somatostatin receptor ligand, DOTATATE (DOTA^0-Tyr^3-octreotate), in the presence of varying amounts of deliberately included metal ion impurities. The metal ion impurities were selected on the basis of likely presence in the eluates of a variety of commercial generators (i.e. Fe^{3+}, Cu^{2+}, Ti^{4+}, Al^{3+}, Zn^{2+}), from local and solution contamination (i.e. Fe^{3+}, Cu^{2+}, Pb^{2+}, Ca^{2+}), as well as metal ions (i.e. Y^{3+}, Yb^{3+}, Lu^{3+}) that may be present due to cross-contamination in radiochemical research laboratories. More detailed studies concerning reaction conditions and stability of the [^{68}Ga(DOTATATE)] complex were performed with Fe^{3+} and Cu^{2+} as these were shown to exert the greatest influence on the complexation of [^{68}Ga(DOTATATE)].

2. Materials and methods

2.1. Materials

All chemicals and solvents were of analytical or pharmaceutical grade unless otherwise specified. Metal salts (ultrapure grade; trace metal content) were obtained from Sigma-Aldrich. DOTATATE acetate (GMP-grade) was purchased from ABX Chemicals.

Reactions, up to 400 μL, were performed in 500 μL Eppendorf PCR tubes. For reaction volumes of 400–1100 μL, 1.5 mL Eppendorf micro-centrifuge tubes were used. All solvents and chemicals were stored in plastic bottles. All labware and reaction vials used were washed with 4 M HCl followed by ultrapure H_2O before use.

2.2. Radioactivity measurements and chemical analysis

Radioactivity was measured using an ionization chamber (Capintec Radioisotope Calibrator CRC 15R). Trace metal content was determined by inductive-coupled plasma optical-emission spectrometry (ICP-OES) using a Perkin-Elmer OPTIMA 5300DV instrument. Calibration was performed with single standard elements (high purity) obtained from Choice Analytical Pty Ltd.

High-performance liquid chromatography (HPLC) analyses were carried out using a Waters 600 system consisting of a UV detector (Linear UVIS Activon) and a radiation detector (Carroll & Ramsey Associates Model 105S) coupled in series. The column used was a 250 × 4.6 mm, Phenomenex Synergi, 4 μ, Hydro-RP, 80 Å. Separations were achieved employing a gradient with the eluents acetonitrile (A), H_2O (B) and 1% trifluoroacetic acid in H_2O (C) and a flow rate of 1.0 mL/min. Method: 0 min: 5% A, 85% B, 10% C; 1 min: 5% A, 85% B, 10% C; 9 min: 90% A, 10% C; 10 min: 5% A, 85% B, 10% C; 17 min: 5% A, 85% B, 10% C.

2.3. ^{68}Ga elution, purification, and concentration

^{68}Ga was obtained from a ^{68}Ge/^{68}Ga generator system (ANSTO, Australia) where ^{68}Ge was attached to a mixed metal oxide-based sorbent material (Le, 2013a; Le 2013c). Two generator columns were used for the experiments described, one loaded with 666 MBq (18 mCi) and one loaded with 16 MBq (0.32 mCi) of ^{68}Ge. Crude ^{68}Ga solution was eluted from the generator columns with 0.1 M hydrochloric acid. The elution volume (3–7 mL) depended on the activity adsorbed on the column.

The ^{68}Ga eluates were purified and concentrated using the following method (Le, 2013a; Le, 2013b): The solution eluted from the sorbent column, containing ^{68}Ga and possible metal contaminants, was directly applied to a cation exchange resin (AG 50W-X4). Undesirable metal ions were removed from the resin with an aqueous alcohol solution (0.5 M HCl + 0.1 M NaCl + 0.5% ascorbic acid + 30% methanol) while retaining ^{68}Ga. The column was then washed with 3 mL of water and any remaining liquid was purged from the resin using air (5 min). ^{68}Ga was eluted using 0.75 mL of 0.5 M KOH. Following the elution of ^{68}Ga, the cation exchange resin was regenerated for the next elution cycle by washing with 3 mL of 4 M HCl and 3 mL of H_2O.

For most experiments, the 16 MBq ^{68}Ge/^{68}Ga generator was used. Samples of the concentrated and purified eluate were regularly checked by ICP-OES over the period of the study (1 year). Typical results are illustrated in Fig. 1 (additional data can be found in the supplementary material). During this time no significant change of the metal ion content was observed. We found that only the duration between generator elutions had an influence on the metal ion content in any detectable manner. A minor rise in metal ion content of the purified solutions was observed when using freshly prepared cation exchange resin for the first time; therefore these eluates were not used for experiments and are not taken into account in Fig. 1. The eluate of the 666 MBq generator was not used directly, rather it was diluted with ultrapure water in a ratio of 1:6 to achieve ^{68}Ga concentrations similar to those of the 16 MBq generator.

To ensure that the observed effects resulted solely from the purposely added metal ions, we monitored the metal ion content of the generator eluates and all reagent solutions by ICP-OES before use. Apart from Al and Ca, the total background metal ion content in each reaction solution (calculated by adding the measured metal ion content in generator eluate and reagent solutions) was always less than 0.5 nmol. This value was well below the purposely added metal ion amounts. An exception was made for the Al and Ca content, since their interference level in our experimental set-up was very low (see results and discussion and Fig. 2).

2.4. Radiolabeling procedures

All experiments were repeated at least 3 and up to 10 times if significant deviation was observed. Tables containing the data illustrated in the figures in this paper can be found in the supplementary material. They also include the standard deviations (stdev) for all data points.

2.4.1. [^{68}Ga(DOTATATE)]

The following radiolabeling conditions were used as standard (de Blois et al., 2011): The pH of a solution of 50 μL of 1 M Hepes and 100 μL $^{68}Ga^{3+}$ solution was adjusted with aliquots of 4 M HCl to pH ~ 4.0 (as measured by PANPEHA® (pH range 0–14) and Acilit® (pH range 0–6) indicator strips). The ionic strength of the solutions was between 0.5 and 0.9 M depending on the amount of HCl needed. A 5 μL aliquot of 1 mM DOTATATE in 0.1 M HCl was added, the pH was checked again and if needed adjusted, and the