Journal of Radioanalytical and Nuclear Chemistry (2019) 322:249–254
https://doi.org/10.1007/s10967-019-06700-9

Influence of metal ions on the ^{44}Sc-labeling of DOTATATE

Rafał Walczak[1] · Weronika Gawęda[1] · Jakub Dudek[1] · Jarosław Choiński[2] · Aleksander Bilewicz[1]

Received: 24 December 2018 / Published online: 9 August 2019

Abstract
The aim of the study was to evaluate the labeling yield of ^{44}Sc-DOTATATE radiobioconjugate when the labeling is performed in the presence of various amounts of competing metallic impurities. In the case of Ca^{2+} and Al^{3+} the effect is irrelevant, which is understandable considering the low stability constant of Ca^{2+}-DOTA and Al^{3+}-DOTA complexes. However, the presence of $Fe^{2+/3+}$, Zn^{2+} and Cu^{2+} cations very strongly influences the efficiency of the ^{44}Sc-DOTATATE formation. Surprisingly, while the Zn^{2+}-DOTA stability constants is the smallest, Zn^{2+} cations competes more strongly with Sc^{3+} than $Fe^{2+,3+}$ and Cu^{2+} at the DOTATATE coordination site.

Keywords Scandium radionuclides · DOTATATE · Radiolabeling

Introduction

The use of ^{68}Ga labeled peptide in positron emission tomography (PET and PET/CT) to visualize various type of tumors allows better planning of the therapeutic strategy and effective prediction of the treatment outcomes [1]. For example DOTATOC labeled with ^{68}Ga shows high binding affinity for the human somatostatin receptor subtype 2, improving this way tumor imaging capabilities and offering the possibility of low dose imaging, followed by higher dose treatment by DOTATOC labeled with ^{177}Lu or ^{90}Y [2].

However, because of their short half-life ($T_{1/2}$ = 67.71 min), ^{68}Ga-based radiopharmaceuticals are synthesized and used in-house. Furthermore, the relatively high cost of the generators render this isotope of limited utility in a clinical setting. These circumstances lead to high overall cost and render ^{68}Ga radiopharmaceuticals of limited interest for centralized production and commercial distribution.

Recently there has been steadily growing interest in the medical applications of Sc radioisotopes. The longer half-life of the scandium β^+ emitters, ^{44g}Sc and ^{43}Sc ($T_{1/2}$ = 3.97 h and $T_{1/2}$ = 3.89 h, respectively) compared to ^{68}Ga potentially permits of a centralized production of ^{44}Sc-labeled peptides and their shipment over several hundred kilometers to hospitals with PET centers that do not have a radiopharmacy on site. [3, 4]. In addition, images could be acquired over longer periods. Another advantage in the use of ^{43,44}Sc as diagnostic radioisotopes lies in the other scandium radioisotope, i.e. ^{47}Sc ($T_{1/2}$ = 3.35 d) which is a promising low-energy β^- emitter for targeted radionuclide radiotherapy, and therefore represents an theranostic pair with the β^+ emitting ^{44g}Sc or ^{43}Sc radioisotopes. The potential of ^{44}Sc/^{47}Sc as a theragnostic pair has been demonstrated in a preclinical pilot study with tumor-bearing mice [5, 6].

Additionally, it has been shown that Sc^{3+}, like Y^{3+} and Lu^{3+}, forms in solution DOTA complexes with coordination number 8, whereas Ga^{3+} forms octahedral complexes. Because the difference in the coordination of complexes influences on the lipophilicity of conjugates, Sc-DOTATATE has nearly identical lipophilicity as that of Lu- and Y-DOTATATE, whereas Ga-DOTATATE is more hydrophilic [7]. The difference in the chelates structure determinates the receptor affinity of labeled conjugates. For example, the IC50 value of Ga-DOTATOC for the hsst2 receptor is 2.5 nmol, while that for Y-DOTATOC is 11 nmol [8]. Due to the chemical similarity of Sc^{3+} to the Lu^{3+} and Y^{3+} cations ^{44}Sc-DOTA-bioconjugates will likely demonstrate similar properties in vivo (i.e. receptor affinity, kidney clearance) to the ^{177}Lu- and ^{90}Y-conjugates currently applied in therapy, therefore ^{44}Sc and ^{43}Sc can form theranostic pairs with ^{177}Lu and ^{90}Y.

✉ Aleksander Bilewicz
a.bilewicz@ichtj.waw.pl

[1] Institute of Nuclear Chemistry and Technology, Dorodna 16, 03-195 Warsaw, Poland

[2] Heavy Ion Laboratory, University of Warsaw, Pasteura 5A, 02-093 Warsaw, Poland

For receptor radionuclide diagnosis and therapy radiolabeling and molar activity i.e., radionuclide–ligand molar ratio should be as high as possible to minimize receptor occupancy of unlabeled conjugates. Therefore in radiolabeling process the presence of trace amounts of carrier or other cations that form strong complexes with the ligand can significantly reduce the degree of labeling yield and the molar activity of the bioconjugate. In radiopharmaceutical practice, the influence of transition metal contaminants on the labeling of bioconjugates with ^{177}Lu, ^{90}Y and ^{68}Ga has been repeatedly observed since many of the used chelates form strong complexes with a range of metal ions [9]. Particularly, this effect was observed for the cations such as Fe^{3+}, $Zn^{2+}$and Cu^{2+}. This effect was also observed in our studies on the labeling of bioconjugates with radionuclides ^{43}Sc, ^{44}Sc and ^{47}Sc. While in the case of ^{68}Ga, this problem has been extensively studied [10–12], in the case of scandium radionuclides studies have not been conducted. Only in a number of studies, the effect of transition metal cations on ^{44}Sc-DOTA radiobioconjugates stability were studied [13–15]. However, due to the kinetic inertness of the Sc-DOTA complexes, both processes are completely different.

This article details investigations into the influence of a variety of metal ions on ^{44}Sc radiolabeling. We have chosen to studies the clinically relevant somatostatin receptor ligand, DOTATATE (DOTA^0-Tyr^3-octreotate), and tested labeling yield in the presence of varying amounts metal ion impurities. The cation impurities were selected taking into account the possibility of their occurrence in the labeling solution and forming stable complexes with the DOTA ligand, namely $Fe^{2+/3+}$, Cu^{2+}, Zn^{2+}, Al^{3+} and Ca^{2+}. If DOTA-functionalized bioconjugates labeled with scandium radionuclides should be used for clinical practice, it will be important to determine the maximum concentration of metal contaminants which would still allow high specific and reproducible labeling. The obtained results can be used to optimize the labelling conditions of clinical radiopharmaceutical production.

Experimental

Materials and methods

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 (DOTA^0-Tyr^3-octreotate), was purchased from Polatom (Poland). Since the hydrochloric acid may contain traces of iron ions we decided for its further purification on DistillacidTM BSB-939-IR instrument specially designed for preparation of high purity acids. The distilled HCl contains metallic impurities like $Fe^{2+/3+}$, Zn^{2+} in concentrations below 5 ppb. The target was prepared from 100 mg of natural $CaCO_3$ (99.999%, Sigma Aldrich) pressed in graphite.

Irradiation of calcium target

Irradiations of the calcium targets were performed using the GE PETtrace cyclotron at the Radiopharmaceuticals Production and Research Centre put into operation by the Heavy Ion Laboratory, University of Warsaw a few years ago. This cyclotron was recently equipped with an external beam line for solid sample irradiations, also allowing a good cooling conditions for these samples [16]. A 2-h proton irradiation at the energy 16 MeV and 15 µA current were performed. During irradiation process the front side and the back side of the target were cooled.

Separation of ^{44}Sc from calcium target

For separation of ^{44}Sc from calcium target the method recently elaborated by Minegishi et al. [17] in combination with purification on Dowex 50 resin was applied. The separation process is presented in Fig. 1.

The irradiated $CaCO_3$ target was dissolved in 3 mL 1 M HCl solution during 10 min. Then the dissolved target solution was alkalized to pH 10 by aq ammonia solution (25%) In this condition Sc^{3+} forms $Sc(OH)_3$ which is quantitatively separated from the solution by passed through the Teflon 0.2 µm filter. Subsequently, 5 mL of pure water were passed through the filter to wash out residual Ca^{2+} and NH_4^+

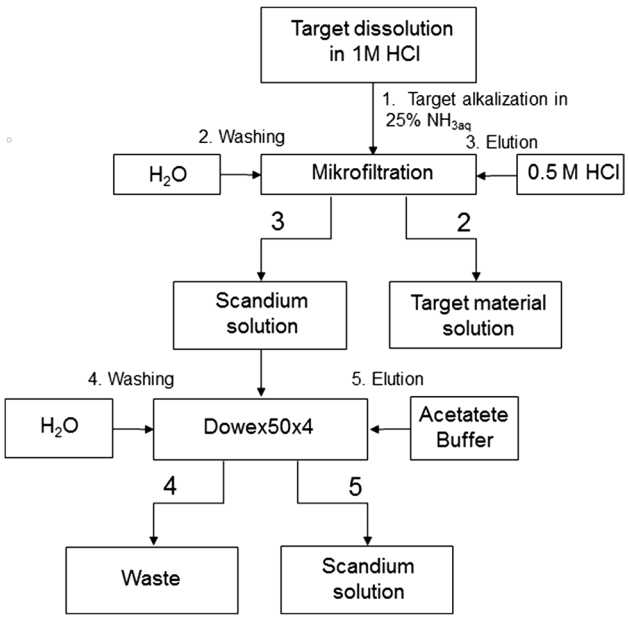

Fig. 1 Separation scheme of the isolation of ^{44}Sc from the $CaCO_3$ targets

cations. ^{44}Sc trapped on the filter was eluted by 0.5 M aqueous HCl (2 mL).

For additional purification of the ^{44}Sc and change pH to appropriate for labeling the eluate from filter was adsorbed on the column filled with DOWEX 50Wx4 cation exchange resin. The elution of ^{44}Sc was performed using 1.5 mL 1 M ammonium acetate aq. solution (pH 4.5). The activity of the eluted fractions was monitored and 0.75 mL fraction with maximum activity of eluate was selected for labeling experiments. The concentration of metallic impurities in labeling solutions was measured by the ICP-MS instrument Elan DRC II from Perkin Elmer (USA).

DOTATATE labeling

DOTATATE was labeled with obtained ^{44}Sc using various amounts of the peptide. The stock solution was prepared by dissolution of 100 µg DOTATE (69 nmol) in 100 ul 1 M ammonium acetate buffer. Next, the most active fraction of ^{44}Sc solution (80 µL, 20 MBq) was combined with 100 µL 1 M ammonium acetate containing 3, 5, 10 and 15 nmol of DOTATATE. Peptide was labeled for 30 min at 95 °C.

Radiochemical yield was estimated by instant thin-layer chromatography (ITLC) using silica gel 60 TLC plates (Merck) in citrate buffer (1.5 M, pH 5). The 2 µL of the solution was spotted on the ITLC plate. Free ^{44}Sc moved with the front boundary of the solution, whereas the labeled bioconjugate stayed at the starting point. The activity on the plate was measure by cyclone Plus Phosphor Imager (Perkin-Elmer, USA). The radiochemical yield of reaction was calculated as the ratio of activity of the plate application part to the whole plate activity.

DOTATATE labeling in presence of metal cations carried out as follows. Different amounts of metal salts was dissolved in 1 M HCl and alkalized to pH ~ 4,5 with ammonium acetate buffer. Then 20 MBq (80 µL) of ^{44}Sc and 10 nmol (14.5 µL) DOTATATE was added. After mixing all components the reaction solution was then heated to 95 °C for 30 min. After that radiochemical yield was checked by ITLC method in citrate buffer as described above. Labeling was repeated 3–4 times in separate experiments.

Results and discussion

Production and separation of ^{44}Sc

After 2 h of proton irradiation of natural $CaCO_3$ target at 16 MeV current gives 419 MBq of ^{44}Sc, 11.4 MBq of ^{43}Sc, 7.3 MBq of ^{44m}Sc, 5,6 MBq of ^{47}Sc and 6.5 of ^{48}Sc.

The separation process was completed within 50 min from the end of bombardment (EOB) and involved the following steps: dissolution of target, alkalization with NH_3, trapping of ^{44}Sc on the filter, washing of filter, elution of ^{44}Sc, adsorption of eluent on the Dowex 50 column and elution ^{44}Sc with ammonium acetate buffer. Finally we obtained 270 MBq of ^{44}Sc in 0.7 mL of 1 M ammonium acetate buffer (pH ~ 4,5). Scandium recovery after filtration step was: 94.1 ± 1.0% and after additional purification on Dowex 50 column 89.8 ± 1.2%. Separation of ^{44}Sc is relatively simple and can be quickly implemented and automated.

The solution after dissolution of the target and final product after separation process was analyzed with ICP-MS for trace metal contaminants. The results of analysis is presented in Table 1.

The obtained results indicate that the concentration of calcium, iron, zinc, copper and aluminum was relatively low. In the case of Ca^{2+} and Fe^{3+} impurities their concentration after separation process decrease by a factor of 10^3 and 40 respectively, but in the case of Zn^{2+} by only 1.5.

Table 1 Concentration of cationic impurities in solution after dissolution of the target and after separation steps

Cations	Initial concentration (µg/mL)	After separation (µg/mL)
Al^{3+}	< 0.3	< 0.3
Zn^{2+}	3.3	2.1
Cu^{2+}	0.9	0.11
$Fe^{2+/3+}$	42.5	1.1
Ca^{2+}	18,190	18.6

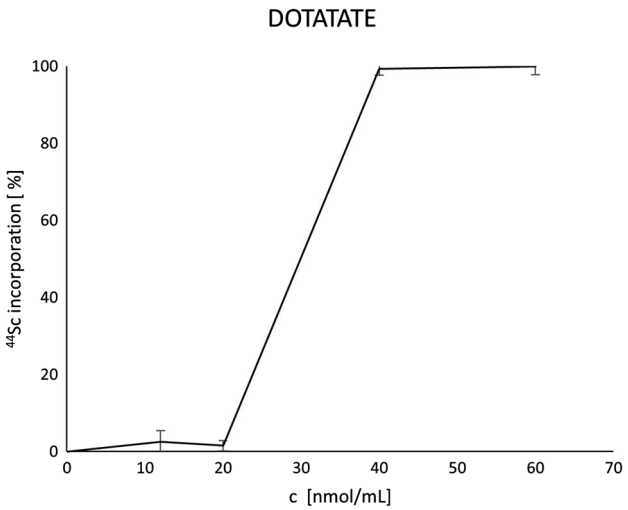

Fig. 2 Influence of DOTATATE concentration on its labeling with ^{44}Sc. Volume of solution = 0.25 ml, ^{44}Sc radioactivity 30 MBq

Metal ion competition on labeling DOTATAE with ^{44}Sc

The obtained ^{44}Sc solution was tested for labeling with DOTATATE bioconjugate. As show in Fig. 2 the DOTATATE concentration of 40 nmol is sufficient to achieve the full labeling.

The obtained molar activity of ^{44}Sc-DOTATATE does not exceed 3 MBq/nmol. This is related to the using a natural calcium target, from which we obtained relatively low ^{44}Sc activity, which caused a relatively high ratio of metallic impurity concentrations to ^{44}Sc. Therefore, the molar activity was small. Similar molar activity was obtained in others works where natural Ca targets were used [18].

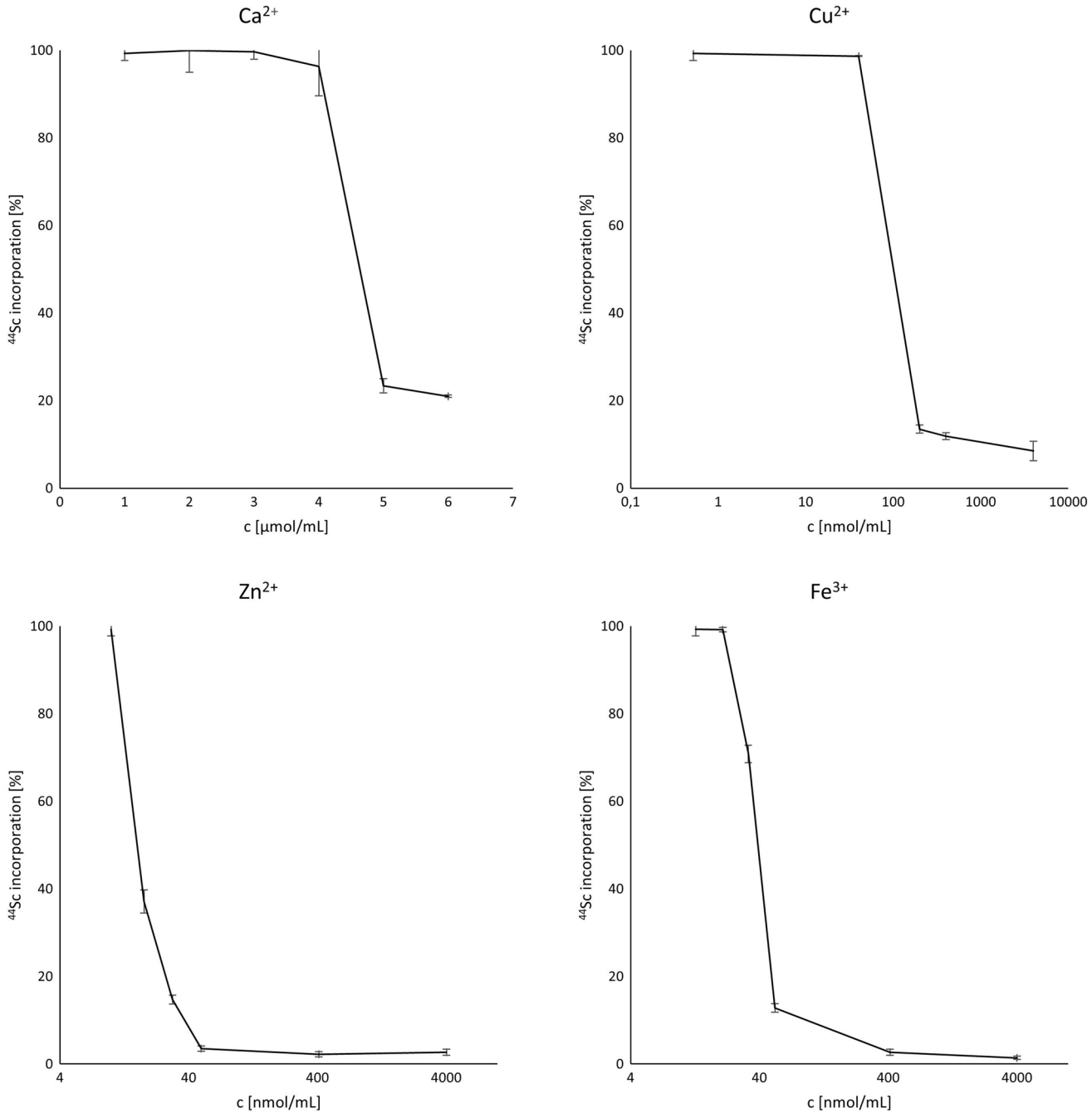

Fig. 3 Influence of Ca^{2+}, $Fe^{2+/3+}$, Zn^{2+} and Cu^{2+} concentration on DOTATATE labeling with ^{44}Sc. Concentration of DOTATATE—40 nmol/ml, volume of solution = 0.25 ml

Table 2 Log *K* values for the M + DOTA = M(DOTA) reactions for selected cations

Cation	M-DOTA stability constant
Sc^{3+}	27.2 [7]
Ca^{2+}	17.2 [19]
Al^{3+}	17.0 [19]
Cu^{2+}	22.44 [19]
Fe^{3+}	29.4 [19]
Fe^{2+}	20.22 [19]
Zn^{2+}	20.52 [19]

To evaluate the influence of metal cations contaminant on the radiolabeling of DOTATE with ^{44}Sc solutions, the formation of ^{44}Sc-DOTATATE was investigated in the presence of metal cations added to the solution. The cations were selected taking into account formation of stable complexes with the DOTA ligand ($Fe^{2+/3+}$, Cu^{2+}, Zn^{2+}) and possibility of their occurrence in the labeling solution (Ca^{2+} from target material, Al^{3+} from aluminum foil and holder used for irradiation). The results are shown in Fig. 3.

We compared obtained results to relevant stability constants for formation of M(DOTA) complexes presented in Table 2. In M^{3+}-DOTA molecule complexation occurs through four N donor atoms in the ring and four carboxylic groups. In the case of the DOTATATE conjugate, the coordination sphere of M^{3+} cations is filled by four donor nitrogen atoms, three carboxyl groups and one carbonyl group. To the best of our knowledge no stability or formation constants for [M(DOTATATE)] type complexes have been determined. More data is for DOTA-amides derivatives, but they are usually for DOTA-tetraamides derivatives. Due to the similarity of complex structures we decided to use stability constants determined for the DOTA ligand. We believe, that the stability constants values for monoamide complexes may be slightly smaller, but the same trends in stability constants should be maintained.

For Ca^{2+} and Al^{3+} no effect was detectable up to 10^4 nmol of $Ca^{2+}$and Al^{3+}. Similar results were obtained also by Pruszyński et al. [13], where transmetallation reactions were not observable for ^{44}Sc labeled DOTA-peptides, even at high Ca^{2+} concentrations of 400 ppm. This is understandable considering the low stability constant of Ca^{2+}-DOTA and Al^{3+}-DOTA complexes (17.2 and 17.0 respectively). In addition, in the case of Al^{3+} the formation of solid $Al(OH)_3$ is observed. However for Cu^{2+}, Zn^{2+} and $Fe^{2+/3+}$ cations, we can see a much higher impact on labeling efficiency.

Analyzing the results obtained it is clear that there is no correlation between the stability constants of [M(DOTA)] complexes and influence M cations on radiolabeling. In particular, on the basis of the stability constants values it is not possible to predict the stronger effect of the Zn^{2+} on the DOTATATE labeling with the ^{44}Sc radionuclide. On the contrary the stability constants of DOTA complexes suggest less impact of Zn^{2+} than Cu^{2+} on DOTATE labeling. In fact, since the stability constant of the Fe^{3+}-DOTA is more than hundred times higher than those of Sc^{3+} we expected also a much greater influence of iron than Zn^{2+} cations. Lower impact of iron for labeling can be explained by the reduction of Fe^{3+} to Fe^{2+} in the labeling conditions. As see in Table 2 the DOTA complex with Fe^{2+} are 9 orders of magnitude less stable than those of Fe^{3+} and Fe^{2+} much less compete for coordination sites of the DOTA ligand. Such effect was also observed by Asti et al. [9] studying the influence of metals cations on the labeling of DOTATATE conjugate with ^{90}Y and ^{177}Lu, and Šimeček et al. [11] examining DOTA complex formation in presence of competing cations. It should be noted that while the $Fe^{2+,3+}$, Zn^{2+} and Cu^{2+} cations strongly influence on the labeling process of DOTA radiobioconjugates with ^{44}Sc, their impact on the stability of the formed complexes is negligible [13–15]. This is associated with kinetic inertness of DOTA complexes.

Conclusions

While studying the influence of $Fe^{2+/3+}$, Cu^{2+}, Zn^{2+}, Al^{3+} and Ca^{2+} on formation ^{44}Sc-DOTATATE conjugate we found that labeling efficiency strongly decreases in presence of trace concentration (> 2 nmol) of iron and zinc cations. Cu^{2+} cations reduce the labeling to a lesser extent (at concentrations greater than 10 nmol) and in the case of Ca^{2+} and Al^{3+} we do not observe any effect on labeling up to 2 µmol. Since ^{44}Sc labeled radiopharmaceuticals started to be used on the patients the obtained results can be transferred to radiopharmaceutical preparation and used to optimize labeling conditions.

Acknowledgements The authors warmly thank the PETtrace operation team for running a number of the beam hours necessary for the research described in this paper. The authors also thank dr. Anna Stolarz for targets preparation for proton irradiation. This study was financed by Ministry of Science and Higher Education of Poland from funding for science in the years 2016-2019 (cofinanced international program) and by IAEA Research Contract No: 20488 and also partly supported by the Polish Funding Agency NCBiR, Grant No. DZP/PBS3/2319/2014.

References

1. Velikyan I (2015) ^{68}Ga-based radiopharmaceuticals: production and application relationship. Molecules 20:12913–12943
2. Liu F, Zhu H, Yu J, Han X, Xie Q, Liu T, Xia C, Li N, Yang Z (2017) ^{68}Ga/^{177}Lu-labeled DOTA-TATE shows similar imaging and biodistribution in neuroendocrine tumor model. Tumour Biol 39:1010428317705519
3. Krajewski S, Cydzik I, Abbas K, Bulgheroni A, Simonelli F, Holzwarth U, Bilewicz A (2013) Cyclotron production of ^{44}Sc for clinical application. Radiochim Acta 101:333–338
4. Walczak R, Krajewski S, Szkliniarz K, Sitarz M, Abbas K, Choiński J, Jakubowski A, Jastrzebski J, Majkowska A, Simonelli F, Stolarz A, Trzcinska A, Zipper W, Bilewicz A (2015) Cyclotron production of ^{43}Sc for PET imaging. EJNMMI Phys 2:1–10
5. Müller C, Bunka M, Haller S, Köster U, Groehn V, Bernhardt P, van der Meulen N, Türler A, Schibli R (2014) Promising prospects for ^{44}Sc/^{47}Sc-based theragnostics: application of ^{47}Sc for radionuclide tumor therapy in mice. J Nucl Med 55:1658–1664
6. Müller C, Domnanich KA, Umbricht CA, van der Meulen NP (2018) Scandium and terbium radionuclides for radiotheranostics: current state of development towards clinical application. Br J Radiol 91:20180074
7. Majkowska A, Bilewicz A (2011) Macrocyclic complexes of scandium radionuclides as precursors for diagnostic and therapeutic radiopharmaceuticals. J Inorg Biochem 105:313–320
8. Antunes P, Ginj M, Zhang H, Waser B, Baum RP, Reubi JC, Maecke H (2007) Are radiogallium-labeled DOTA-conjugated somatostatin analogues superior to those labeled with other radiometals? Eur J Nucl Med Mol Imag 34:982–993
9. Asti M, Tegoni M, Farioli D, Iori M, Guidotti C, Cutler CS, Mayerd P, Versaria A, Salvoa D (2012) Influence of cations on the complexation yield of DOTATATE with yttrium and lutetium: a perspective study for enhancing the ^{90}Y and ^{177}Lu labeling conditions. Nucl Med Biol 39:509–517
10. Velikyan I, Beyer GJ, Bergström-Pettermann E, Johansen P, Bergström M, Långström B (2008) The importance of high specific radioactivity in the performance of ^{68}Ga-labeled peptide. Nucl Med Biol 35:529–536
11. Šimeček J, Hermann P, Wester H-J, Notni J (2013) How is ^{68}Ga labeling of macrocyclic chelators influenced by metal ion contaminants in ^{68}Ge/^{68}Ga generator eluates? ChemMedChem 8:95–103
12. Oehlke E, Le So V, Lengkeek N, Pellegrini P, Jackson T, Greguric I, Weiner R (2013) Influence of metal ions on the ^{68} Ga-labeling of DOTATATE. Appl Radiat Isotop 82:232–238
13. Pruszyński M, Majkowska-Pilip A, Loktionova NS, Eppard E, Roesch F (2012) Radiolabeling of DOTATOC with the long-lived positron emitter ^{44}Sc. Appl Radiat Isot 70:974–979
14. Domnanich KA, Müller C, Farkas R, Schmid RM, Ponsard B, Schibli R, Türler A, van der Meulen NP (2016) ^{44}Sc for labeling of DOTA-and NODAGA-functionalized peptides: preclinical in vitro and in vivo investigations. EJNMMI Radiopharm Chem 1:8
15. van der Meulen NP, Bunka M, Domnanich KA, Müller C, Haller S, Vermeulen C, Türler A, Schibli R (2015) Cyclotron production of ^{44}Sc: from bench to bedside. Nucl Med Biol 42:745–751
16. Choiński J, Bracha T, Radomyski B, Świątek Ł, Antczak M, Jakubowski A, Jastrzębski J, Kopik R, Miszczak J, Nassar S M, Pietrzak A, Stolarz A, Tańczyk R (2015) Accelerator production of ^{99m}Tc-an external, well cooled, target holder for the PETtrace cyclotron. Heavy Ion Laboratory annual report, pp 39–41
17. Minegishi K, Nagatsu K, Fukada M, Suzuki H, Ohya T, Zhang MR (2016) Production of scandium-43 and -47 from a powdery calcium oxide target via the (nat/44)Ca(α, x)-channel. Appl Radiat Isot 116:8–12
18. Hernandez R, Valdovinos HF, Yang Y, Chakravarty R, Hong H, Barnhart TE, Cai W (2014) ^{44}Sc: an attractive isotope for peptide-based PET imaging. Mol Pharm 11:2954–2961
19. Martell A, Smith R, Motekaitis R (2004) NIST critically selected stability constants of metal complexes database. http://wwwnist.gov/srd/nist46.cfm
20. Chaves S, Delgado R, DaSilva JJ (1992) The stability of the metal-complexes of cyclic tetra-aza tetraacetic acids. Talanta 39:1873–3573