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Al[^{18}F]F-HBED-CC-radiolabeling in a media of protic alkanolammonium ionic liquids

Dmitrii O. Antuganov,[a] Michail A. Nadporojskii[a] and Yulia A. Kondratenko*[b]

[a] *A. M. Granov Russian Research Center of Radiology & Surgical Technologies, 197758 Pesochny, St. Petersburg, Russian Federation*
[b] *I. V. Grebenshchikov Institute of Silicate Chemistry, Russian Academy of Sciences, 199034 St. Petersburg, Russian Federation. E-mail: kondratencko.iulia@yandex.ru*

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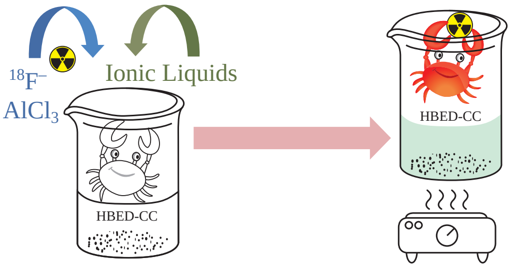
The possibility of Al^{18}F-radiolabeling of molecules with the *N,N'*-bis[2-hydroxy-5-(2-carboxyethyl)benzyl]ethylenediamine-*N,N'*-diacetic acid motif in the medium of protic alkanolammonium ionic liquids is reported for the first time. Ionic liquids containing triethanolammonium or triisopropanolammonium cations proved to be the most promising. High values of radiochemical conversion were achieved using the minimum amount of the precursor.



Keywords: protic ionic liquids, Al^{18}F-radiolabeling, positron emission tomography, fluorine-18, radiopharmaceutical.

Positron emission tomography is a promising medical imaging technique that provides information on physiological and biochemical changes at the molecular level *in vivo*. Among a wide range of positron-emitted radionuclides, the most used isotope is fluorine-18 (^{18}F, half-life 109.7 min, 97% decays by positron emission, maximum positron energy 0.635 MeV). The most valuable ^{18}F-labelled radiopharmaceuticals are the biological analog of glucose, [^{18}F]fluorodeoxyglucose, and ^{18}F-labelled amino acids.[1,2]

Radiolabeling of peptides with the easy-to-handle and abundantly available fluorine-18 is an attractive imaging strategy as a counterbalance to ^{68}Ga-radiolabeling.[3] There are two main approaches to obtain ^{18}F-labelled peptides based on covalent and non-covalent radiofluorination. The synthesis of peptides with the covalent C–^{18}F bond is a multi-step process that may involve quite harsh reaction conditions. The non-covalent strategy[4] is based on the strength of the bond between the fluoride anion [^{18}F]F$^-$ and the aluminum cation Al^{3+}. Typically, the [Al^{18}F]$^{2+}$ cation forms stable complexes *in vivo* with polydentate N$_2$O$_3$-donor ligands under mild labeling conditions (pH 4.5–5).[5,6] Thus, Al^{18}F-radiolabeling of peptides can occur through a one-step reaction similar to other radiometals such as ^{68}Ga and ^{177}Lu. This approach leads to both a reduction in synthesis time and an increase in the possibility of large-scale production in automated synthesis modules. One of the best-known examples of [Al^{18}F]$^{2+}$ binding is Al^{18}F-PSMA-11, used in positron emission tomography for diagnosing prostate cancer.[4,7] In the cited works,[8,9] the possibility of obtaining the Al^{18}F-PSMA-11 complex with high radiochemical conversions (RCCs) up to 84% in 0.5 M acetate buffer (pH 4.2) and in a small reaction volume (from ~150 to ~250 μl) was demonstrated.

In this study, we aimed to optimize the Al^{18}F-radiolabeling of the chelator *N,N'*-bis[2-hydroxy-5-(2-carboxyethyl)benzyl]ethylenediamine-*N,N'*-diacetic acid (HBED-CC) and the peptide PSMA-HBED-CC (PSMA-11) (Figure S1, see Online Supplementary Materials) in the presence of protic alkanolammonium ionic liquids (PAAILs). We compared the effect of PAAIL composition and concentrations, cosolvents and precursor mass on reaction yields.

Salts of triethanolammonium (TEA), tris(hydroxymethyl)methylammonium (TRIS), *N*-phenyldiethanolammonium (PDA) and triisopropanolammonium (TPA) cations with carboxylate anions such as benzoate (Benz), cinnamate (Cin), salicylate (Sal), acetylsalicylate (AcSal), 2-methylphenoxyacetate (Crez), 4-chloro-2-methylphenoxyacetate (ClCrez), hydrogen oxalate (HOx), oxalate (Ox), hydrogen malonate (HMal), malonate (Mal), hydrogen succinate (HSuc), succinate (Suc), hydrogen malate (HMlt) and malate (Mlt) were chosen as PAAILs buffers for the Al^{18}F-radiolabeling reactions.[†]

The first stage of the work was to determine the optimal amount of the HBED-CC chelator and the buffer system. The general method of Al^{18}F-radiolabeling used in our work reproduces the previously described approaches.[‡,8,9] We compared the efficiency of well-described buffers, 0.9% NaCl and 0.2 M sodium acetate (pH 4.0),[6,10–12] with 0.1 M [TEA]Benz solution. For this, reactions were carried out with different amounts of the precursor (Figure 1). The efficiency of [TEA]Benz as a reaction buffer has been proven in our recent studies on ^{68}Ga-radiolabeling of bifunctional chelating agents and peptides.[13–15] Besides, based on the published data, ethanol (~50 vol%) was used as a cosolvent in all cases.[12,16,17]

According to our data, normal saline is not a good buffer for Al^{18}F-radiolabeling reactions. RCCs in sodium acetate buffer gradually increased from nearly 10% to 60% using 2.5 and 30 μg of HBED-CC, respectively. In contrast, almost 60% RCC was obtained in [TEA]Benz buffer using only 10 μg of the HBED-CC precursor, while only 30% was obtained in the commonly used

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[†] For details, see Online Supplementary Materials.

[‡] Briefly, a 2 mM AlCl$_3$ solution (3 μl) in 0.1 M sodium acetate (pH 4.0) was loaded into 2 ml microcentrifuge tubes, and then buffer (100 μl) and EtOH (100 μl) were added. Afterward, aqueous [^{18}F] fluoride (10 μl) and a solution of the HBED-CC precursor (1 mg ml^{-1}, 2.5–30 μl) in deionized water were added to the reaction tube. The mixture was incubated at 50 °C for 20 min, shaking at 1000 rpm. Each experiment was repeated in triplicate. RCC was measured using radio-TLC. For details, see Online Supplementary Materials.

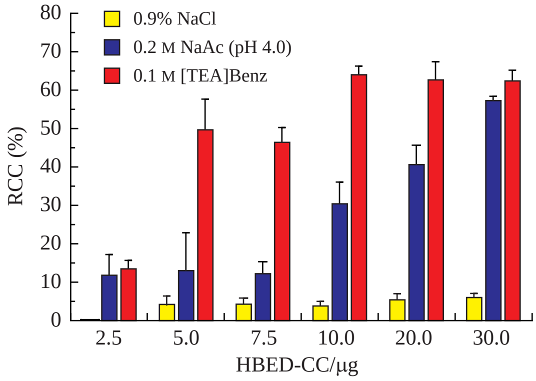

Figure 1 Screening of optimal conditions for Al^{18}F-radiolabeling in terms of the amount of HBED-CC precursor and the buffer system (n = 3 in all cases).

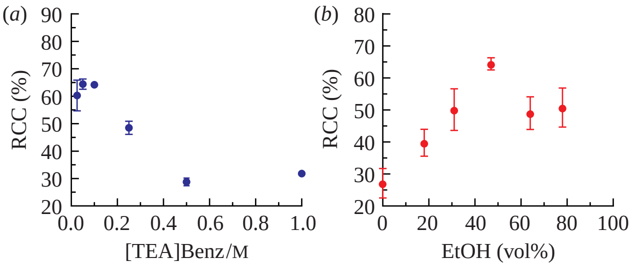

Figure 2 Dependence of the RCC of Al^{18}F-radiolabeling of HBED-CC on (*a*) the [TEA]Benz buffer concentration and (*b*) the EtOH cosolvent concentration (n = 3 in all cases).

sodium acetate buffer. Thus, the first results showed that the [TEA]Benz buffer is a promising buffer system for radiolabeling HBED-CC with the [Al^{18}F]$^{2+}$ cation. Subsequently, all reactions were carried out with 10 μg of the precursor.

The next step was to determine the optimal value of the molar concentration of the buffer. For this, reactions were carried out with [TEA]Benz concentrations from 0.025 to 1 м. Figure 2 shows that the optimal buffer concentration is in the range of 0.025–0.1 м. The data obtained differ from the data for ^{68}Ga-radiolabeling reactions in PAAILs buffers, where the optimal buffer concentration was 1 м.[13–15] A high buffer concentration (more than 0.25 м) in Al^{18}F-radiolabeling reactions leads to a significant decrease in RCC (see Figure 2). Later, the effect of various amounts of EtOH on the RCC of the Al^{18}F-radiolabeling reaction in the [TEA]Benz buffer medium was studied (see Figure 2). From published data,[12,16,17] it is widely known that organic solvents accelerate metal-based radiolabeling reactions. It can be seen that the absence of ethanol as a cosolvent results in the RCC of almost 27%. Adding ethanol to the reaction in an amount of about 50 vol% led to an almost twofold increase in the RCC (64%). A further increase in ethanol concentration from 50 to 80 vol% reduces the RCC up to 50%.

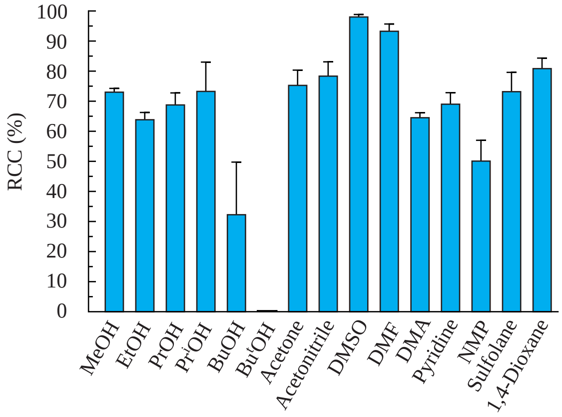

Figure 3 Dependence of the RCC of Al^{18}F-radiolabeling of HBED-CC on the nature of the cosolvent (n = 3 in all cases).

Afterward, the influence of organic solvents on the RCC of the Al^{18}F-radiolabeling reaction was investigated (Figure 3). It was found that alcohols such as MeOH, PrOH and Pr^{i}OH have an even more significant effect on the RCC of the Al^{18}F-radiolabeling reaction than EtOH and BuOH. Unexpectedly, Bu^{t}OH passivated the Al^{18}F-radiolabeling reaction. To our surprise, we found that aprotic solvents have a much more significant effect on the RCC of Al^{18}F-radiolabeling, and in the case of DMSO and DMF, the RCC exceeded 90%. Thus, DMSO proved to be the most effective in Al^{18}F-radiolabeling reactions among all the studied organic solvents. By analogy with EtOH, we optimized the amount of DMSO in the reaction mixture (Figure S2 and Table S4). As a result, DMSO appeared to promote the Al^{18}F-radiolabeling reaction much more strongly, peaking at 40–50 vol%. It is noteworthy that a further increase in the amount of DMSO in the reaction mixture to 75 vol% does not lead to a decrease in RCC. Therefore, DMSO was used as a cosolvent in further reactions instead of EtOH.

Thus, the optimal conditions for carrying out the Al^{18}F-radioactive labeling are 0.1 м [TEA]Benz as a buffer and DMSO as a cosolvent in an amount of ~50 vol%. Having chosen the optimal conditions, we screened the synthesized PAAILs based on TEA, TRIS, PDA and TPA salts of carboxylic acids to find the most optimal buffers for Al^{18}F-radiolabeling reactions. The pH values of the reaction mixtures are presented in Table S7. When using salts of dicarboxylic (oxalic, malonic and malic) acids as buffers, the RCCs were less than 10%. This effect may be related to the side complex formation of Al with dicarboxylic acid anions.[18,19] Similar low RCCs were observed for succinates, except for TPA succinate. Buffer screening results (Figure 4) show that RCC is greater than 95% with the following buffers: [TPA]Crez, [TPA]ClCrez and [TEA]Benz. It is noteworthy that for these buffers, the pH of the reaction mixture was in the range from ~5.9 to ~6.2, which is

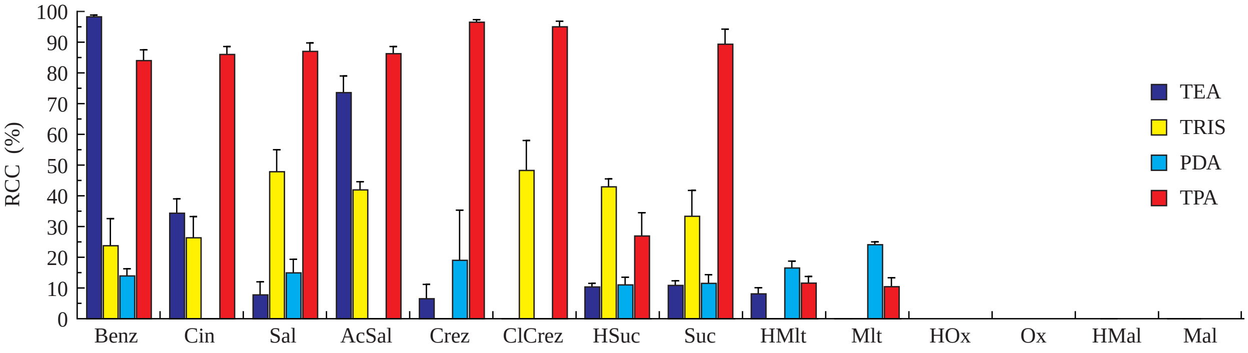

Figure 4 Effects of PAAILs based on TEA, TRIS, PDA and TPA carboxylates on the RCC of Al^{18}F-radiolabeling reactions (n = 3 in all cases).

slightly higher than for the previously described approaches.[8,9] For the [TEA]Benz buffer, we investigated the reaction of obtaining the $Al^{18}F$-HBED-CC complex in the pH range from 2 to 11 (Figure S3 and Table S8). As a result, lowering the pH to 5.0, which is optimal according to the published data,[8] has virtually no effect on RCC. A further decrease in pH to 2.0 or an increase to 11.0 leads to a gradual decrease in the RCC value to almost zero. Thus, for the investigated PAAILs, the optimal pH value of the reaction mixture is the range of 5.0–6.5.

Further, the $Al^{18}F$-PSMA-11 complex was prepared using [TPA]Crez, [TPA]ClCrez and [TEA]Benz as buffers. As a result, the RCC for these reactions was 84.9 ± 2.2% in [TPA]Crez, 84.0 ± 3.0% in [TPA]ClCrez and 77.5 ± 2.1% in [TEA]Benz ($n = 3$ in all cases). Since the reaction volume in our case was only 233 μl, the results obtained are consistent with the data for the sodium acetate buffer.[8,9]

Later we tried to adapt our method from 'aliquot-type' radiolabeling to radiofluorination by concentrating ^{18}F-fluoride on a disposable Sep-Pak QMA Light cartridge. This step is crucial for the automated synthesis of radiopharmaceuticals. In this case, an increase in reaction volumes is unavoidable. The sorbent was preconditioned in three different ways[§] (Table S9). According to the results obtained, the best RCCs for the $Al^{18}F$-HBED-CC complex were achieved when the cartridges were prepared with water only. At the same time, the RCC decreased slightly to 81.9 ± 5.7% ($n = 3$) in [TEA]Benz buffer compared to 'aliquot-type' radiolabeling.

Finally, the 'concentration-type' method for preparing $Al^{18}F$-PSMA-11 in a media of PAAILs buffers [TEA]Benz, [TPA]Crez and [TPA]ClCrez was tested. In each case, we used only 20 μg of the PSMA-11 peptide. As a result, we obtained nearly the same RCCs for each of the PAAILs buffers: 46.6 ± 3.6%, 39.2 ± 1.6% and 43.9 ± 1.6% for [TEA]Benz, [TPA]Crez and [TPA]ClCrez, respectively ($n = 3$ in each case). With an increase in the mass of PSMA-11 to 40 μg, the RCC remains the same, 44.3 ± 1.2% ($n = 3$) for [TEA]Benz. The resulting $Al^{18}F$-PSMA-11 complex can be purified using RP-SPE cartridges and converted into an injectable form.

It should be noted that a similar RCC for $Al^{18}F$-PSMA-11 (~45%) was achieved using a larger amount of the peptide (50 μg) in a reaction volume of ~1.5 ml on a SynthraFCHOL automated synthesis module using 0.05 M sodium acetate buffer (pH 4.5).[17] RCCs up to 90% can only be achieved using hundreds of micrograms of the peptide.

Thus, we investigated the influence of the nature of PAAIL and the organic cosolvent on the reactions of $Al^{18}F$-radiolabeling of the HBED-CC chelator and the PSMA-11 peptide. The combination of TEA and TPA salts with DMSO cosolvent turned out to be the most promising. A study of the cosolvent/buffer concentration showed that the highest reaction yields were achieved at 50 vol% of cosolvent and in 50 mM buffer. Work is underway to automate the synthesis of $Al^{18}F$-PSMA-11.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.05.040.

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[§] For details, see Online Supplementary Materials. Briefly, $[^{18}F]F^-$ was loaded from the 'male side' and eluted with [TEA]Benz solution back into the reaction tube preloaded with the required precursors. The total volume of the reaction mixture increased from 0.2 to 1.6 ml.