Technical University of Denmark

DTU

Towards automated solid phase radiofluorination for dose-on-demand PET: retention of activity by solid support

Mathiessen, Bente Ingemann; Severin, Gregory; Zhuravlev, Fedor

DTU Library
Technical Information Center of Denmark

Bente Mathiessen, Gregory Severin, and Fedor Zhuravlev*

Towards automated solid phase radiofluorination for dose-on-demand PET: retention of activity by solid support

Abstract: On-column [18F]fluoride trapping and radiofluorination of 2-(naphthalen-1-yl)ethyl-4-methyl-benzenesulfonate ($C_{10}H_7(CH_2)_2OTs$), performed on polystyrene supported phosphazene base PS-P$_2^{tBu}$ yielded [18F]1-(2-fluoroethyl)naphthalene ([18F]$C_{10}H_7(CH_2)_2F$) in 50% radiochemical yield but left up to 43% of activity unreacted on the resin. This activity could be eluted with Kryptofix/K_2CO_3 and then used for conventional radiofluorination of the same substrate, suggesting that the column-retained activity was present in the form of [18F]fluoride entrapped in polymer matrix. An approach to minimize the amount of entrapped [18F]fluoride by use of glass beads functionalized with alkylsilane-derivatized phosphazene residues was attempted but was stymied by fluorolysis/hydrolysis of the alkylsilane spacer. The results suggest that the key to high yield of on-column radiofluorination is to minimize the residual [18F]fluoride absorption in the matrix by the judicious choice of solid support.

Keywords: Dose-on-demand PET, [18F], radiofluorination, on-column, solid phase.

1 Introduction

The worldwide demand for PET scans is increasing at a dramatic rate. Over the time period from 2010 to 2012, the number of scans performed in Western Europe increased by 19.6%, with over one million scans performed annually in the region [1]. The supply of radioisotopes, particularly fluorine-18, has appropriately grown to meet the increasing demand. The most cost effective supply chain for fluorine-18 has been through daily, high activity, single-batch radiopharmaceutical production at a regional facility with distribution to several PET centers within a reasonable distance. While this model has effectively provided the population with PET tracers, it has led to a one-size-fits-all clinical implementation, where 95% of all PET scans performed use [18F]FDG. [18F]FDG is an important general purpose tracer, and its value should not be ignored. But, with the development of disease specific tracers, the important opportunity for personalized medicine is presented in dose-on-demand (DoD) PET.

The basic aims of DoD PET are to consider the needs of a given patient, and produce the tracer that is best suited for them as necessary. One way to implement DoD PET is to shift radiopharmaceutical production from the large regional centers, which depend upon large batches matching large patient populations, to small hospital based radiopharmacies. These laboratories would have to be equipped with robust multi-use, single-platform, automated radiochemistry systems that could be supplied with fluorine-18, for example, either from a small in-house cyclotron or from [18O]H_2O that was irradiated at regional facility. Any such radiochemical system should involve rapid chemical processes, eliminate or simplify time-consuming steps like solvent evaporations, and be capable of producing many tracers from the same platform. One way to realize these improvements to current procedure, and thereby make DoD PET more tenable, is through use of on-column, solid-supported radiofluorination methodology.

Practically, the majority of radionuclide extractions and purifications are now performed on solid phase anion exchange cartridges. Conventional solvent-related operations such as solvent wash and solvent exchange, which could be rather difficult to perform on radioactive samples, become straightforward and amenable to automation when on-column radionuclide recovery is used. However, the incorporation of PET radionuclides into PET tracers also requires desorption of the radionuclide from the solid support followed by the conventional liquid

*Corresponding author: Fedor Zhuravlev, Hevesy Laboratory, Center for Nuclear Technologies, Technical University of Denmark, Risø Campus, Frederiksborgvej 399, P.O. Box 49, 4000 Roskilde, Denmark, e-mail: fezh@dtu.dk
Bente Mathiessen, Gregory Severin: Hevesy Laboratory, Center for Nuclear Technologies, Technical University of Denmark, Risø Campus, Frederiksborgvej 399, P.O. Box 49, 4000 Roskilde, Denmark

phase radiochemistry. This approach is implemented in emerging microfluidic radiofluorination devices. While taking advantage of microreactor-based chemistry the microfluidics still requires the use of "macro-sized" pre-concentration and solid phase extraction modules [2]. We believe that combining isotope recovery and radiosynthesis into one inexpensive and robust on-column process can be especially attractive for the emerging DoD PET technology if implemented as a tracer-specific self-shielded compact cartridge module. The possibility of direct on-column [18F]fluoride recovery and radiofluorination has been demonstrated by Toorongian, Mulholland and co-workers as early as in 1989 [3, 4]. Unfortunately, their aminopyridinium-based resin suffered from low stability, inconsistent trapping and poor synthetic reproducibility. We have recently reported a new method which allowed for solid phase extraction of [18F]fluoride from [18O]H_2O on a polystyrene-based phosphazene resin followed by radiofluorination of a wide variety of substrates on the same resin [5]. The method is characterized by uniformly high trapping efficiency (>98%), moderate yields (40%–69%, substrate dependent) and column reusability. However, numerous on-column radiofluorination runs indicated a persistent deficiency of the method: up to 43% of activity remained absorbed on the column after reaction. In this work we further investigated the nature of the activity retained on the solid phase.

2 Experimental

2.1 General

Unless otherwise noted, all synthetic steps were performed under an inert atmosphere of argon or helium. Glassware and reaction vessels were dried in an oven at 160 °C overnight or flame-dried on the Schlenk line before use. All reagents were used as received without additional purification; solvents were dried over activated molecular sieves. All radiochemical yields were decay-corrected. Polystyrene-supported P_2^{tBu} **PS-P_2^{tBu}** (loading: 1.6 mmol/g), was obtained from Sigma Aldrich; the aminoalkylsilane-functionalized glass beads were purchased from Polysciences Inc. The 2-(naphthalen-1-yl)ethyl-4-methylbenzenesulfonate ($C_{10}H_7(CH_2)_2OTs$) was synthesized as described elsewhere.

2.2 Instrumentation

^{1}H, ^{13}C and ^{31}P-NMR spectra were recorded on a Bruker Avance II 500 instrument operating at 500, 126 and 202 MHz, respectively. ^{19}F-NMR was recorded on a Bruker Avance DPX 250 instrument at 235 MHz. The mass spectrometry was performed on a Bruker Esquire 4000 ion-trap (IT) spectrometer equipped with electrospray ionization (ESI) interface. Thin-layer chromatography (TLC) was run on pre-coated plates of silica gel 60, F254 (Merck). Radio-TLC was performed with a Raytest MiniGita TLC scanner. The aqueous solutions of [18F]fluoride were prepared by the $^{18}O(p,n)^{18}F$ reaction in a GE PETtrace cyclotron using a Ag or Nb target containing 2.5 mL of 95%–98% enriched [18O]H_2O irradiated by a 16.5 MeV proton beam at 55 µA for around 100 min. A small fraction of the irradiated water was diluted with unenriched water, and used as the source of fluorine-18 for radiochemistry. For automated radiosynthesis we used custom-made module controlled by LabView software.

2.3 Column preparation and on-column radiofluorination followed by Kryptofix/K_2CO_3/[18F]fluoride labeling

The representative procedures are as follows:

2.3.1 Column preparation

A borosilicate glass tube (OD 0.6 mm, length 12 cm) was packed with **PS**-supported P_2^{tBu} (150 µmol base, loading 1.6 mmol/g, 93.75 mg) mixed with glass beads (212–300 µm, 100–500 mg) and placed in a column oven in a LabView controlled automation apparatus.

2.3.2 [18F]fluoride trapping and column drying

[18F]fluoride (in water, 2.5 mL, 684 MBq, flow rate 1 mL/min) was passed through the column followed by acetonitrile (MeCN) : water (1 : 1, 1 mL, flow rate 1 mL/min). MeCN (dry, 1 mL) was passed through the column at room temperature followed by MeCN (dry, 5 mL, flow rate 1 mL/min) while heating the column at 50 °C. Then He was passed through the column until excess of solvent was removed. [18F]Fluoride trapping was 100%.

2.3.3 On-column radiofluorination followed by Kryptofix/K$_2$CO$_3$ elution and radiofluorinaton

MeCN (dry, 5 mL) was passed through the column (flow rate 1 mL/min) at 80 °C followed by C$_{10}$H$_7$(CH$_2$)$_2$OTs (19.64 mg, 58 µmol) dissolved in MeCN (dry, 3 mL, flow 0.55 mL/min) at 80 °C. Then MeCN (dry, 2 mL, flow rate 0.55 mL/min) was passed through the column to elute the remaining product. The fluorinated product was analyzed by radio-TLC (eluent heptane : EtOAc 80 : 20). The conversion to the desired product [^{18}F]C$_{10}$H$_7$(CH$_2$)$_2$F was 91%. 43% of the [^{18}F]fluoride (291 MBq) was retained on the column at this point. The column was then washed using MeCN (dry, 12 mL, flow rate 750 µL/min) and dried using He while cooling from 80 °C to 50 °C. Kryptofix (K$_{2.2.2}$) solution (1.0 mL solution of a mixture containing 22.0 mg of Kryptofix, 7.0 mg K$_2$CO$_3$, 3.0 mL MeCN and 3.0 mL H$_2$O) was passed through the washed and dried column (flow rate 0.55 mL/min) at room temperature in order to elute the remaining [^{18}F]fluoride still trapped on the column after the previous on-column radiofluorination. Only 6%–7% [^{18}F]fluoride (48 MBq) was retained on the column at this point. The resulting Kryptofix/K$_2$CO$_3$/[^{18}F]fluoride mixture was collected in a glass reactor vial and the solvents were removed by repeated azeotropic evaporation steps (vacuum and He applied, 1st time at 60 °C for 3 min, 2nd to 4th time at 90 °C at 3–4 min, 0.5–1.0 mL dry MeCN added between each evaporation step). C$_{10}$H$_7$(CH$_2$)$_2$OTs (18.60 mg, 57 µmol) dissolved in MeCN (dry, 3 mL) was added to the reactor vial and radiofluorination was carried out at 80 °C for 10 min. After cooling to room temperature the product was analyzed by radio-TLC (eluent heptane : EtOAc, 80 : 20); conversion to the desired product [^{18}F]C$_{10}$H$_7$(CH$_2$)$_2$F was 66%.

2.3.4 Phosphazene-functionalized glass beads G-P_2

The aminoalkylsilane-functionalized glass beads were reacted with P$_2^{Cl}$ · BF$_4$ (1-chloro-1,1,3,3,3-pentakis(dimethylamino)-1λ^5-diphosphazen-3-ium tetrafluoroborate) by mixing the beads (200–1000 mg) with P$_2^{Cl}$ · BF$_4$ (3 eq to amine) and Et$_3$N (dry, 9 eq to amine) in dichloromethane (DCM, dry, 5 mL) in a glass vial, which was sealed under vacuum. The reaction mixture was then heated with slight agitation at 90 °C for three days. This coupling procedure was repeated two times in order to ensure good coupling. The resulting functionalized glass beads were treated with a mixture of KOMe (1 eq to amine) in MeOH (dry, 5 mL) for one hour at room temperature in order to give the desired glass bead-supported phosphazene base.

3 Results and discussion

The on-column [^{18}F]fluoride recovery and radiofluorination was performed in the following way (Figure 1a). First,
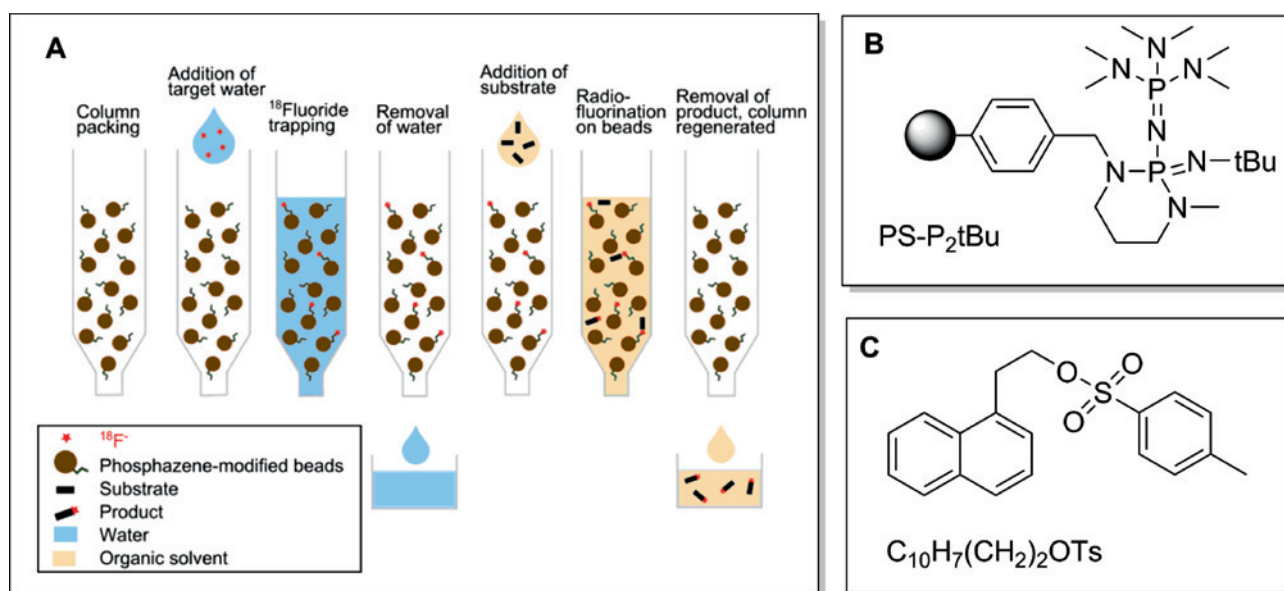

Fig. 1: (**A**) Conceptual representation of on-column [^{18}F]fluoride recovery and radiofluorination; (**B**) the structure of polystyrene-based phosphazene base **PS-P$_2^{tBu}$**, and (**C**) the structure of model substrates 2-(naphthalen-1-yl)ethyl alkylsulfonate C$_{10}$H$_7$(CH$_2$)$_2$OTs used in this study.

the cyclotron target water, containing [18F]fluoride was passed through the column containing **PS-P$_2^{tBu}$** beads (Figure 1b). The [18F]fluoride was retained on the column, most likely as a counter anion to phosphazenium cation initially formed by deprotonation of water with highly basic **PS-P$_2^{tBu}$**. The target water was then released from the column and the column was dried by rinsing with dry acetonitrile. Then, a solution of $C_{10}H_7(CH_2)_2OTs$ (Figure 1c), a model substrate used previously by us [6] and others [7], in acetonitrile was slowly (10–15 min) passed through the column while the column was heated at 80 ˚C. This resulted in radiofluorination of the substrate directly on the phosphazene-modified polymer beads. The solution containing the radiolabeled substrate was then released from the column. Regardless of radiofluorination conditions (flow rate, temperature, substrate concentration), a substantial amount of radioactivity (up to 43%) remained trapped on the column.

Conceivably, the retained activity can be either physically entrapped in the polymer matrix in form of free or loosely bound [18F]fluoride, or chemically bound to the impurities in the polymer support. In the first case, [18F]fluoride encapsulated in the cavernous pockets of cross-linked polymer matrix might not be accessible to the larger molecules of the substrate. However, smaller ions, such as hydroxide or carbonate might still be able to substitute fluoride and release the entrapped activity. Interestingly, the absorption of activity was also reported in case of on-column radiofluorination of mannose triflate on aminopyridinium resin in an earlier work by Toorongian and Mulholland [3]. The authors were able to elute the retained activity with aqueous solution of potassium carbonate and use the eluate for radiofluorination. To test the entrapment hypothesis, we modified our automatic radiofluorination apparatus to include a module for elution of retained activity and subsequent radiofluorination (Figure 2).
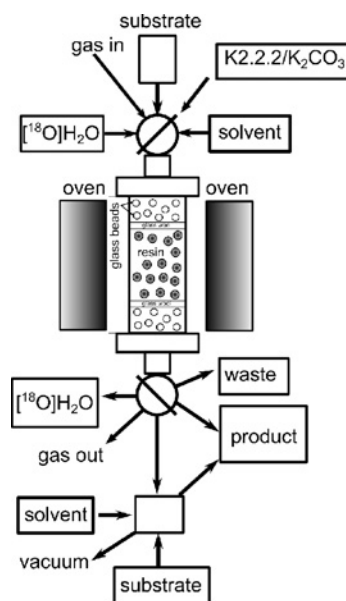
Fig. 2: The schematics of apparatus for automatic on-column radiofluorination of $C_{10}H_7(CH_2)_2OTs$. The apparatus is equipped with a module for elution of column-retained activity with Kryptofix/K_2CO_3 and subsequent radiofluorination of the same substrate in the liquid phase.

After on-column radiofluorination of $C_{10}H_7(CH_2)_2OTs$ a solution of Kryptofix/K_2CO_3 in acetonitrile-water was passed through the column containing **PS-P$_2^{tBu}$** and the resulting activity was dried by azeotropic evaporation of water. The radiofluorination ability of the eluted [18F]fluoride activity was accessed by reacting it with the model substrate $C_{10}H_7(CH_2)_2OTs$ in acetonitrile at 80 ˚C. The results are presented in Table 1.

The results show that approximately half of the activity loaded on the column as target water reacted with the substrate directly on the column (51%–56%, Table 1, column 4). The rest (41%–43% of activity, column 3) remained attached to the resin. The subsequent elution with

Table 1: Automated on-column radiofluorination of $C_{10}H_7(CH_2)_2OTs$ and subsequent elution/radiofluorination of the same substrate with the column-retained activity using Kryptofix/K_2CO_3 elution. All activities and radiochemical yields (RCY) were calculated relative to the initial radioactivity of [18F]fluoride loaded onto the column. Radiochemical conversion (RCC) was calculated relative to the total activity in a given solution.[1]

Entry	Activity trapped on column (%)	Activity remaining on column after on-column radio-fluorination (%)	On-column radio-fluorination RCC (%)/RCY(%)	Activity remaining on column after K$_{2.2.2}$/K$_2$CO$_3$ wash (%)	Radio-fluorination with K$_{2.2.2}$/[18F]KF, RCC (%)/RCY (%)	Combined RCY from on-column and K$_{2.2.2}$/[18F]KF radio-fluorinations (%)
1	99	42	97/56	6	42/14	70
2	100	43	91/51	7	66/21	72
3	100	41	92/52	6	41/13	65

[1] For details on the reaction conditions see Section 2.

Scheme 1: Synthesis of phosphazene-functionalized glass beads **G**-P$_2$.

Scheme 2: Possible pathway for fluorolysis of **G**-P$_2$ in the presence of [^{18}F]fluoride.

the solution of Kryptofix/K$_2$CO$_3$ does remove most of this activity leaving behind only 6%−7% (column 5). Based on the consistency of retained values across different runs and resins, and on the control experiment, where the resin was pre-treated with cold fluoride and yielded the same column activity retention value after on-column radiofluorination, we believe that the 6%−7% we are seeing is due to the system's dead volume. The Kryptofix/K$_2$CO$_3$ wash/radiofluorination experiment demonstrated that after on-column radiofluorination 30%−35% of fluorine-18 activity is retained on the column in the form of nucleophilically active, but latent [^{18}F]fluoride. Thus, the results of these experiments appear to be in line with the entrapment hypothesis.

One way to minimize the influence of solid support on [^{18}F]fluoride retention and reactivity is to substitute the polymer-based matrix for a non-porous solid support, such as glass beads. To that end, aminoalkylsilane functionalized glass beads were treated with P$_2^{Cl}$ · BF$_4$ giving the glass-supported **G**-P$_2$ (Scheme 1).

Unfortunately, the column containing glass-supported **G**-P$_2$ trapped no activity upon passing the target water. Instead, all activity came through the column in a cloudy aqueous solution. This can be explained by instability of Si−O$_{glass}$ bond with respect to fluorolysis

(Scheme 2) and/or hydrolysis (not shown). Therefore, at present, glass appears to be an unlikely candidate for solid support compatible with on-column radiofluorination.

4 Conclusions

In our effort to develop an automated, solid phase based radiofluorination method for dose-on-demand PET we have investigated and quantified the fluorine-18 activity retained on the polystyrene supported phosphazene base **PS**-P$_2^{tBu}$ after reaction with the model substrate C$_{10}$H$_7$(CH$_2$)$_2$OTs. After on-column radiofluorination, proceeding with up to 56% yield, as much as 43% of fluorine-18 activity was retained on the resin. All of it (less 5%−6%, corresponding to the dead volume of the apparatus) could be eluted with Kryptofix/K$_2$CO$_3$ and then used for conventional radiofluorination of the same substrate, suggesting the activity was present in the form of [^{18}F]fluoride entrapped in polymer matrix. An approach to minimize the amount of entrapped [^{18}F]fluoride by use of glass beads functionalized with alkylsilane-derivatized phosphazene residues was attempted but was stymied by fluorolysis and/or hydrolysis of the alkylsilane spacer. The results suggest that the key to high yield of

on-column radiofluorination is to minimize the residual [^{18}F]fluoride absorption by the judicious choice of solid support. The future directions include development of stable, low fluoride-absorbing phosphazene-functionalized solid support.

On-column synthesis of fluorine-18 radiotracers could represent a major step forward in the development of DoD PET. This method for rapid, general-purpose, nucleophilic radiofluorination allows production of many different tracers from the same hot cell. Coupling this with the abundance of available radioactivity from small proton accelerators brings a wide variety of tracers closer to the patient population. Ideally this platform will eventually provide single patient doses, as needed, in the new era of personalized medicine.

References

1. Stevens, A.: Molecular and Multimodality Imaging: Hybrid Imaging – Image Processing and Quantitation. Annual Congress of the European Association of Nuclear Medicine, OP104 (2013).

2. Arima, V., Pascali, G., Lade, O., Kretschmer, H. R., Bernsdorf, I., Hammond, V., *et al.*: Radiochemistry on chip: towards dose-on-demand synthesis of PET radiopharmaceuticals. Lab Chip **13**, 2328–2336 (2013).

3. Toorongian, S. A., Mulholland, C. K., Jewett, D. M, Bachelor, M. A., Kilbourn, M. R.: Routine production of 2-deoxy-2-[^{18}F]fluoro-D-glucose by direct nucleophilic exchange on a quaternary 4-aminopyridinium resin. Int. J. Radiat. Appl. Instrum. B **17**, 273–279 (1990).

4. Mulholland, C. K, Mangner, T. J., Jewett, D. M., Kilbourn, M. R.: Polymer-supported nucleophilic radiolabeling reactions with [^{18}F]fluoride and [^{11}C]cyanide ion on quaternary ammonium resins. J. Lab. Comp. Radiopharm. **26**, 378–380 (1989).

5. Mathiessen, B., Zhuravlev, F.: Automated solid-phase radiofluorination using polymer-supported phosphazenes. Molecules **18**, 10531–10547 (2013).

6. Mathiessen, B., Jensen, A. T. I., Zhuravlev, F.: Homogeneous nucleophilic radiofluorination and fluorination with phosphazene hydrofluorides. Chemistry Eur. J., **17**, 7796–7805 (2011).

7. Jadhav, V. H., Jang, S. H., Jeong, H. J., Lim, S. T., Sohn, M.-H., Chi, D. Y., Kim, D. W.: Polymer-supported pentaethylene glycol as a facile heterogeneous catalyst for nucleophilic fluorination. Org. Lett. **12**, 3740–3743 (2010).