http://dx.doi.org/10.5012/bkcs.2012.33.7.2177

High Yielding [18F]Fluorination Method by Fine Control of the Base

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Received January 25, 2012, Accepted March 26, 2012

New [18F]F-fluorination methods using a minimized amount of precursor has been developed by controlling the base concentration. In the first method, pre-conditioning of the anion exchange cartridge with K_2CO_3 solution or water was carried out. The trapped [18F]fluoride on the cartridge was then eluted by KOMs or KOTf solution. [18F]F-Fluorination could be performed without additional base. In the second method, the QMA cartridge was preconditioned with KOMs solutions. Trapped [18F]fluoride on the QMA was then eluted with KOMs and additional base, such as KOH, K_2CO_3, and $KHCO_3$, was added into the reaction vessel. Method 1 showed a [18F]F-incorporation yield of 20.9% for [18F]FLT synthesis with 5 mg of precursor. Unlike method 1, a [18F]F-incorporation yield of 91.4% was achieved from the same amount of precursor in method 2.

Key Words : [18F]Fluoride, [18F]Fluorination, KOMs, [18F]FLT, Nucleophilic substitution

Introduction

[18F]Fluoride was the most frequently used radioisotope for positron emission tomography (PET) because of its manageable half-life ($t_{1/2}$ = 109.7 min) and easy production by the cyclotron through the $^{18}O(p,n)^{18}F$ nuclear reaction.[1] It was also produced in an aqueous [18F]fluoride ion form from 18O-enriched water with high specific activity (generally exceeding 40 GBq/μmol).[2,3]

The [18F]fluoride was usually separated from 18O-water using an anion exchange cartridge such as QMA or Chromafix® (PS-HCO_3) to remove 18O-water and to recover expensive 18O-water. Trapped [18F]fluoride on the anion exchange cartridge was released with a basic aqueous solution mixture such as K_2CO_3/K_{222} or $TBAHCO_3$. At least 0.8 mg of K_2CO_3 or 5 μL of 40% $TBAHCO_3$ is needed for complete elution of the trapped [18F]fluoride from the PS-HCO_3 cartridge.[4] A basic environment is preferable for obtaining a high radiochemical yield, but large amounts or strong bases such as K_2CO_3, KOH, and TBAOH may cause side reactions such as elimination and hydroxylation.[5] Such precursor decomposition is one of the major drawbacks of low radiochemical yield and leads to multiple purification steps, such as high performance liquid chromatography (HPLC).

In our previous reports, we obtained a [18F]F-incorporation yield of 90% with 10 μL of 40% $TBAHCO_3$ and 20 mg (24 μmol) of FLT precursor.[6,7] However, when the amount of precursor was reduced from 20 mg to 10 mg (12 μmol), [18F]F-incorporation yield was also reduced to 50%.[8]

There was a report investigating the role of base in the synthesis of [18F]FLT and [18F]F-incorporation of 70% was obtained with 10 mg of precursor under a precursor-to-base ratio (P/B ratio) of 1.2-1.5.[9] However, [18F]fluoride and base were directly added into the reaction vessel without applying an anion exchange cartridge in this study. In general, there is an unknown amount of basic anion in the reactor after elution of trapped [18F]fluoride, even though the exact amount of basic solution needed to elute the trapped [18F]fluoride is used. To prevent this problem, an exact amount of base must be added to the reactor without application of an anion exchange cartridge.

Direct base addition without an anion exchange cartridge may control the exact amount of base added, but this method has some problems with controlling the 18O-water volume and metallic impurities in the irradiated 18O-water from the cyclotron target. Large amounts of 18O-water need a long drying time for [18F]fluorination under anhydrous conditions and metallic impurities lead to low radiochemical yield in [18F]F-labeled radiopharmaceutical production.[10,11]

In this report, new elution methods for [18F]fluoride are described, using the anion exchange cartridge with inert potassium salts instead of applying basic solution to ensure an exact base concentration and to reduce the amount of precursor.

Experimental

5'-*O*-DMTr-2'-deoxy-3'-*O*-nosyl-β-D-threo-pentofuranosyl)-3-*N*-BOC-thymine as a precursor for [18F]FLT synthesis was purchased from FutureChem (Seoul, Korea). 2-Methyl-2-butanol, potassium methanesulfonate (KOMs), potassium trifluoromethanesulfonate (KOTf), and other solvents and reagents were purchased from Sigma-Aldrich (Seoul, Korea) and used as supplied.

Amount of Inert Potassium Salts Required to Elute [18F]Fluoride. PS-HCO_3 and QMA as conventional anion exchange cartridges were preconditioned with 10 mL of water and 10 mL of 0.2 M K_2CO_3, respectively, and the preconditioned QMA was then washed with 10 mL of water to remove the excess K_2CO_3 on the cartridge. 370 MBq of

[^{18}F]fluoride was trapped on each cartridge.

A continuous 0.1 mL aliquot of 0.025 M of KOTf or KOMs aqueous solution was applied to elute [^{18}F]fluoride and the residual radioactivity on the anion exchange cartridge was measured by a dose calibrator after every 0.1 mL addition. This procedure was repeated until the trapped [^{18}F]fluoride was eluted completely. After complete elution of [^{18}F]fluoride, the amount of inert salt required for complete elution of [^{18}F]fluoride from the anion exchange cartridge was decided.

[^{18}F]Fluorination Using Conventional Anion Exchange Cartridge and Inert Potassium Salt (Method 1). PS-HCO_3 and QMA were preconditioned using the conventional method described in section 2.1. 370 MBq of [^{18}F]fluoride was trapped on the anion exchange cartridges. It was eluted using 700 μL of MeOH with 20 μmol of KOMs or KOTf and 40 μmol of Kryptofix 222(K_{222}). After elution, the water residue was completely evaporated by azeotropic distillation with 3 × 500 μL of CH_3CN under a mild N_2 stream at 120 °C.

5-10 mg of [^{18}F]FLT precursor was added to the reaction vessel with 100 μL of CH_3CN and 500 μL of 2-methyl-2-butanol. [^{18}F]Fluorination was performed at 120 °C for 10 min and the [^{18}F]F-incorporation yield was evaluated by radio-TLC.

Base Controlling Method with Modified Anion Exchange Cartridge and Optimized Base Addition (Methods 2-1 and 2-2). QMA was preconditioned with 10 mL of 0.2 M KOMs solution and washed with 10 mL of water to exchange the Cl^- counter anion on QMA with OMs^-. [^{18}F]Fluoride (370 MB) was trapped on the cartridge and eluted with the same elution buffer described in section 2.2. In this method, no basic chemical species such as OH^- or KCO_3^- existed in the reaction vessel. Therefore, additional base was added for [^{18}F]fluorination, as shown in Figure 1. Two base addition methods, direct and indirect, were also developed as methods 2-1 and 2-2, respectively.

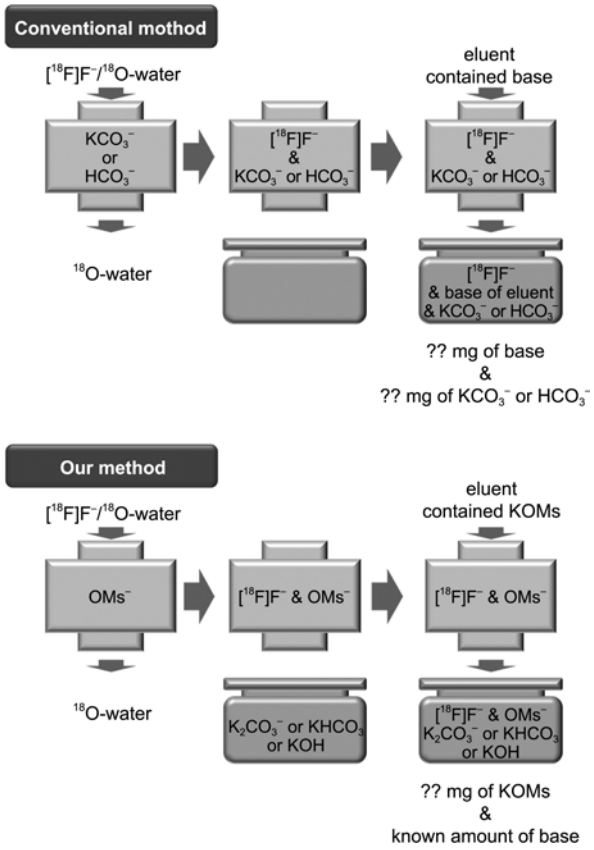

Figure 1. Schematic comparison of conventional method with our method.

Direct Addition as Method 2-1. Before the elution of [^{18}F]fluoride from the QMA cartridge, 7.3 μmol of base (KOH, 0.4 mg/100 μL water; K_2CO_3, 1.0 mg/100 μL water; $KHCO_3$, 0.73 mg/100 μL water) was already added into the reaction vessel. After elution of [^{18}F]fluoride, the residual water was completely dried with added base and then [^{18}F]-fluorination was performed with 5 mg of [^{18}F]FLT precursor dissolved in 100 μL of CH_3CN and 500 μL of *t*-amyl alcohol at 120 °C for 10 min. [^{18}F]F-Incorporation yield was analyzed by radio-TLC.

Indirect Addition-CH_3CN Containing Inorganic Base as Method 2-2. After elution of [^{18}F]fluoride, azeotropic distillation was carried out using 500 μL of CH_3CN containing 7.3 μmol of inorganic base and 2 × 500 μL of anhydrous CH_3CN. Preparation of CH_3CN stock solution containing inorganic base followed. The base (0.14 mmol) consisted of KOH (8.1 mg), $KHCO_3$ (14.5 mg), and K_2CO_3 (20.0 mg) dissolved in 1.0 mL of water and K_{222} (105 mg, 0.28 mmol) dissolved in 2 mL of CH_3CN. The mixture was completely dried by azeotropic distillation with CH_3CN at 120 °C under the mild N_2 stream. The residual base-K_{222} complex was then re-dissolved in 10 mL of CH_3CN. The same [^{18}F]fluorination conditions as in section 2.4 applied.

Results and Discussion

Amount of Inert Potassium Salt Required to Elute [^{18}F]Fluoride. As illustrated in Figure 2, > 97% of trapped [^{18}F]fluoride was eluted using 800 μL of KOMs and KOTf (20 μmole), regardless of cartridge, and this amount was applied to further experiments.

Conventional Anion Exchange Cartridge and Inert

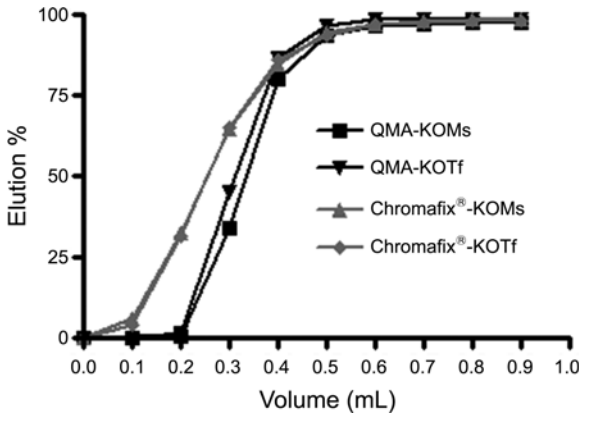

Figure 2. Percent radioactivity eluted from QMA and Chromafix® by 0.025 M potassium salt solutions.

Table 1. [^{18}F]F-incorporation yield with normal anion exchange cartridge (method 1)

Entry	Additive 20 μmol	Precursor	mg (μmol)	[^{18}F]F-incorporation yield[a]	
				QMA	Chromafix
1	KOMs	[^{18}F]FLT	10.0 (12.1)	79.61	62.02
2	KOMs	[^{18}F]FLT	5.0 (6.0)	17.79	20.85

[^{18}F]Fluorination condition: 0.5 mL of *t*-amyl alcohol, 120 °C, 10 min. [a]Radio-TLC yield.

Table 2. [^{18}F]F-incorporation yield with modified QMA and KOMs solution (method 2)

Entry	Adding method	Base[a]	[^{18}F]F-incorporation yield (%)		
			5 min	10 min	15 min
1	Direct	KOH	61.9±13.4	63.6±14.7	63.2±16.1
2		K_2CO_3	24.1±14.2	24.5±15.8	21.6±11.4
3		$KHCO_3$	69.3±7.3	72.9±14.2	78.1±10.8
4	Indirect	KOH	25.7±7.6	26.1±5.2	28.0±8.3
5		K_2CO_3	42.3±2.3	46.3±5.5	46.9±6.0
6		$KHCO_3$	71.9±10.6	73.2±4.0	91.4±4.3

[^{18}F]Fluorination condition: 5 mg of precursor, 0.5 mL of *t*-amyl alcohol, 120 °C. n = 3, each reaction

Potassium Salt without Base (method 1). 79.61% and 62.02% of 10 mg of [^{18}F]F-incorporation yield were obtained with the QMA and PS-HCO_3 cartridges in [^{18}F]FLT synthesis. However, the [^{18}F]F-incorporation yields with 5 mg of FLT precursor were only 17.79% and 20.85% for QMA and Chromafix, respectively.

Modified Anion Exchange Cartridge and KOMs with Small Amount of Base (Methods 2-1 and 2-2). $KHCO_3$ (entry 3, Table 2) as a mild base showed the best results, such as a [^{18}F]F-incorporation yield of 78.1 ± 10.8% for [^{18}F]FLT synthesis using method 2-1 as a direct addition method. KOH as a strong base also gave a 63.2 ± 16.1% yield using method 2-1.

$KHCO_3$ (entry 6, Table 2) also showed the highest [^{18}F]F-incorporation yield of 91.4 ± 4.3% using method 2-2 as an indirect addition method, but strong bases such as KOH and K_2CO_3 showed lower labeling yields than $KHCO_3$ using method 2-2. The results are summarized in Table 2.

In this study, we tried to use the minimum amount of base, such as the counter anion of the conventional anion exchange cartridge that was applied to [^{18}F]fluorination by the first method. Lee *et al.* reported that after preconditioning of QMA and Chromafix, both cartridges already had 13.2 μmol of KCO_3^- and 31.0 μmol of HCO_3^- as counter anions, respectively.[5] Therefore, we assumed the same amount of basic component on the cartridge for [^{18}F]F-fluorination after elution of [^{18}F]fluoride with 20.0 μmol of KOMs (method 1).

The [^{18}F]F-incorporation yield was 62.02-79.61% with 10 mg of precursor for [^{18}F]FLT synthesis. This result was a 10% higher [^{18}F]F-incorporation yield than in our previous report.[8] In that report, a [^{18}F]F-incorporation yield of 53% was obtained using 10 μL of $TBAHCO_3$ and Chromafix with same amount of precursor. However, the amount of precursor could not be reduced below 10 mg. Method 1 showed a [^{18}F]F-incorporation yield of just 17.79-20.85% with 5 mg of precursor. The reason for the low [^{18}F]F-incorporation yield may be a lower precursor/base ratio than 10 mg of precursor. These results demonstrate that the amount of precursor could not be reduced below 10 mg without anion exchange cartridge modification.

Hayashi *et al.* tried to reduce the size of the anion exchange cartridge to optimize the synthesis conditions of [^{18}F]FAZA and reported a high radiochemical yield with 1.3-2.5 μmol of precursor.[12] This method showed good results, but it is inconvenient because it needs cartridge modification.

Therefore, we changed the cartridge counter anion from a basic anion to an inert anion to trap and release [^{18}F]fluoride without basic components. The QMA counter anion was exchanged from Cl^- to OMs^- using 0.2 M of KOMs solution in the preconditioning step. Using the second method, [^{18}F]fluoride was obtained from the anion exchange cartridge without any basic component. The exact amount of base or wanted precursor/base ratio can additionally be directly or indirectly added.

$TBAHCO_3$ or $KHCO_3$ as a mild base led to mild reaction conditions and these were preferred for the synthesis of [^{18}F]F-labeled radiopharmaceuticals over TBAOH or K_2CO_3 as a strong base. Interestingly, KOH shows higher [^{18}F]F-incorporation yield than K_2CO_3 in the direct adding method. From these results, it was concluded that even a strong base may produce good [^{18}F]F-incorporation yield if the amount of base is exactly controlled. Unfortunately, the complexes of strong bases such as K-K_{222}-OH and K-K_{222}-KCO_3 in CH_3CN may produce the acetate by reaction between CH_3CN and the strong base. After the preparation of this mixture, the color of the mixture changed from colorless to yellow. Therefore, the indirect adding method with strong base showed lower [^{18}F]F-incorporation yields than the direct adding method.

In conclusion, a new [^{18}F]fluoride elution method without base and control of the base concentration on [^{18}F]fluorination were described. Thereby, the first and second methods produced high [^{18}F]F-incorporation yields on the synthesis of [^{18}F]FLT with minimal precursor.

Acknowledgments. This work was supported by National Research Foundation of Korea Korea (NRF) grant funded by the Korean government (MEST). (grant code: 2011-0030162) and by the Converging Research Center Program through the Ministry of Education, Science and Technology (2010K001051).

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