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Comparison of synthesis yields of 3′-deoxy-3′-[^{18}F]fluorothymidine by nucleophilic fluorination in various alcohol solvents

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[^{18}F]Fluorothymidine ([^{18}F]FLT) is synthesized with a high radiochemical yield by nucleophilic substitution in protic solvent. In this study, we compared [^{18}F]fluorination yields of [^{18}F]fluorothymidine ([^{18}F]FLT) in various alcohol solvents: 3,3-dimethyl-1-butanol, 2-trifluoromethyl-2-propanol, *t*-BuOH (2-methyl-2-propanol), *t*-amyl alcohol (2-methyl-2-butanol), thexyl alcohol (2,3-dimethyl-2-butanol) and 3,3-dimethyl-2-butanol. We used 5′-*O*-DMTr-2′-deoxy-3′-*O*-nosyl-*β*-D-threo-pentofuranosyl)-3-*N*-BOC-thymine as a precursor for [^{18}F]fluorination. [^{18}F]F$^-$ was eluted with TBAHCO$_3$ solution after trapping [^{18}F]F$^-$ on a PS-HCO$_3$ cartridge. [^{18}F]fluorination was performed at 100°C for 5–30 min using 20 mg of the precursor. [^{18}F]fluorination and radiochemical yields of [^{18}F]FLT were evaluated by radioTLC. [^{18}F]fluorination yields were dependent on the solvent used. All tertiary alcohol solvents, except 2-trifluoromethyl-2-propanol, showed >85% of [^{18}F]fluorination yields, whereas primary and secondary alcohols showed 26.3–71.8%. The highest yield of 94.1±4.4% was obtained with thexyl alcohol after [^{18}F]fluorination for 5 min. Automated synthesis with *t*-amyl alcohol resulted in high synthetic yields of 64.6±6.1% after high-performance liquid chromatography purification (*n*=43). The use of tertiary alcohol as a solvent provides high radiochemical yields of [^{18}F]FLT.

Keywords: [^{18}F]FLT; protic solvent; nucleophilic substitution; tumor; cell proliferation

Introduction

3′-Deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]FLT) is a promising radiopharmaceutical for assessing cellular proliferation on account of its metabolic trapping in proliferating cells, its resistance to degradation *in vivo* and the favorable half life of [^{18}F]fluoride for PET imaging.[1] Recent clinical studies have indicated that [^{18}F]FLT may be a promising tumor therapy response marker for lung cancer and other malignancies.[2–5]

Previously, [^{18}F]FLT was obtained with a moderately low radiochemical yield of <40% using a large amount of precursor (up to 40 mg) at high [^{18}F]fluorination temperature (up to 150°C).[6–10] Recently, our group showed that use of a protic solvent (*t*-BuOH) instead of conventional polar aprotic solvent such as CH_3CN increased the [^{18}F]fluorination yields even with a smaller amount of precursor at lower reaction temperatures.[11,12] Compared with [^{18}F]fluorination using ionic liquids,[13] the use of protic solvent provided higher yields without an additional separation procedure before high-performance liquid chromatography (HPLC) purification. Furthermore, alcohol was removed easily by evaporation due to lower boiling temperature (~100°C).

Primary, secondary and tertiary alcohols have different chemical properties, and therefore may have different solvent effects on [^{18}F]fluorination. We assessed the effect of various alcohol solvents on [^{18}F]FLT synthesis and developed an automated procedure for [^{18}F]FLT synthesis using one of these solvents.

Results and discussion

[^{18}F]fluorination yields were dependent on the alcohol solvent used (Table 1). All tertiary alcohol solvents, except for 2-trifluoromethyl-2-propanol, showed >85% [^{18}F]fluorination yields. In contrast, the primary and secondary alcohols showed lower [^{18}F]fluorination yields, ranging from 26.3 to 71.8%. The highest [^{18}F]fluorination yield, 94.1±4.4%, was obtained using thexyl alcohol for 5 min. Unlike the other tertiary alcohols, 2-trifluoromethyl-2-propanol had the lowest [^{18}F]fluorination yield, 19.7±15.0%. The more bulky tertiary alcohols, such as thexyl alcohol, showed a slightly higher yield than less bulky tertiary alcohols, such as *t*-BuOH. Secondary alcohols showed higher [^{18}F]fluorination yields than primary alcohols.

Using our automated synthesis system, *t*-amyl alcohol showed 64.6±6.1% of average radiochemical yields after HPLC purification ($n = 43$). The highest radiochemical yield was 74.0% and the lowest radiochemical yield was 53.0%. After

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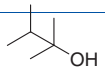
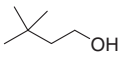
Table 1. Radiochemical yield of [^{18}F] fluorothymidine with various alcohol solvents[a]

		[^{18}F]Fluorination yield[b] (%) ($n = 3$)				
		Reaction time				
Solvent		5 min	10 min	20 min	30 min	Hydrolysis
Thexyl alcohol		94.1 ± 4.4	92.4 ± 0.7	94.3 ± 2.7	94.5 ± 3.0	88.8 ± 1.1
3,3-Dimethyl-2-butanol		58.6 ± 20.7	71.0 ± 4.5	66.9 ± 11.2	75.6 ± 2.3	71.8 ± 14.8
3,3-Dimethyl-1-butanol		6.8 ± 1.3	11.0 ± 1.0	17.6 ± 1.7	23.5 ± 3.1	26.3 ± 3.3
2-Trifluoromethyl-2-propanol		9.0 ± 3.4	11.6 ± 0.9	30.0 ± 22.9	37.3 ± 29.3	19.7 ± 15.0
t-Amyl alcohol		88.6 ± 2.5	92.0 ± 1.8	93.7 ± 1.0	92.0 ± 1.8	87.3 ± 2.0
t-BuOH		90.9 ± 2.4	91.3 ± 3.0	90.8 ± 4.1	89.7 ± 2.2	86.3 ± 1.7

[a]Reaction conditions consisted of 20 mg of (5′-*O*-DMTr-2′-deoxy-3′-*O*-nosyl-β-D-threo-pentofuranosyl)-3-*N*-BOC-thymine as precursor, 100 μL of CH_3CN, and 500 μL of each solvent, at a [^{18}F]fluorination temperature of 100°C. Samples were hydrolyzed with 1 N HCl at 85°C for 5 min.
[b]By radioTLC analysis.

HPLC purification, the [^{18}F]FLT synthesized under each condition had the same retention time as cold FLT at the analytical HPLC chromatogram.

[^{18}F]Fluorination yields were in the order tertiary > secondary > primary alcohol. Of the tertiary alcohols, [^{18}F]fluorination yields were in the order thexyl alcohol > *t*-amyl alcohol > *t*-BuOH.[12] These findings suggest that more bulky tertiary alcohols have higher [^{18}F]fluorination yields than less bulky tertiary alcohols. The [^{18}F]fluorination yield using thexyl alcohol for 5 min was 94.1 ± 4.4% and they were 1.1 times higher than that of the *t*-BuOH system[12] and 3–6 times higher than in previous reports.[6–9] Interestingly, we found that 2-trifluoromethyl-2-propanol, as tertiary alcohol, resulted in much lower [^{18}F]fluorination yields than other tertiary alcohols. Although it has very similar chemical structure to that of *t*-BuOH, the trifluoromethyl group has a strong electron withdrawing effect, which may have reduced the reactivity of TBA^+ [^{18}F]F^-–alcohol complex.[11] Our previous results[11] indicated that the steric effects from bulky tertiary alcohols may increase [^{18}F]fluorination yields, a finding confirmed here. Moreover, secondary and primary alcohols did not have this positive effect.

Alcohol solvents such as *t*-BuOH and *t*-amyl alcohol may have hydroscopic properties and may contain moisture from air. We did not, however, use any protective methods to prevent moisture contamination of these solvents. Our finding that radiochemical yields were stable indicates that moisture contamination had no effect on the [^{18}F]fluorination yields of [^{18}F]FLT synthesis and that anhydrous conditions are not needed.

Before developing our automated procedure, we compared the [^{18}F]fluorination results, evaporation conditions and the costs using *t*-BuOH, *t*-amyl alcohol and thexyl alcohol. Although thexyl alcohol had the highest [^{18}F]fluorination yield during manual synthesis, it has a higher boiling point (120°C) than *t*-BuOH (83°C) and *t*-amyl alcohol (102°C), thus necessitating higher temperatures and stronger vacuum conditions for complete removal of residual solvents. In addition, the cost of thexyl alcohol is much higher than those of *t*-BuOH and *t*-amyl alcohol. Of these three solvents, *t*-amyl alcohol has an intermediary radiochemical yield after hydrolysis and a moderate cost. Compared with thexyl alcohol, *t*-amyl alcohol has a lower boiling point, enabling its easy removal under vacuum and helium flow. From these results, we considered *t*-amyl alcohol to be the optimal [^{18}F]fluorination solvent for automated [^{18}F]FLT synthesis.

In our previously described automated synthesis using *t*-BuOH, drying time after [^{18}F]fluorination was 3 min and included helium purging, vacuum and heating to 100°C. Under these conditions, we did not detect any residual solvent in the final product due to the low boiling point of *t*-BuOH (83°C) similar to CH_3CN. In contrast, using *t*-amyl alcohol under the same conditions, we detected < 150 ppm of residual solvent by gas chromatography analysis in the final product. This residual solvent may have been due to *t*-amyl alcohol (102°C) having a higher boiling point than *t*-BuOH. When we used a longer drying time of > 5 min, however, we did not detect any residual solvent in the final product.[12]

In the materials safety data sheet (MSDS) for *t*-BuOH,[14] we found that it has similar toxicity to CH_3CN. The LD_{50} value from rat intravenous toxicity tests is 1538 mg/kg for *t*-BuOH and 1680 mg/kg for CH_3CN.[15] Thus, the use of *t*-alcohol solvents for F-18 labeled PET radiopharmaceuticals production is as safe as CH_3CN.

Materials and methods

Precursor and chemicals

The precursor (5′-*O*-DMTr-2′-deoxy-3′-*O*-nosyl-*β*-D-threo-pentofuranosyl)-3-*N*-BOC-thymine was prepared as described previously.[8] All solvents and reagents were purchased from Sigma-Aldrich and used as received.

[^{18}F]Fluorination Methods

[^{18}F]Fluorination was performed as described previously.[12] Briefly, after collecting 185 MBq [^{18}F]F^- on a PS-HCO_3 cartridge (Macherey-Nagel, Germany), the trapped [^{18}F]F^- was eluted to the reactor with a solution containing 10 μL of tetrabutylammonium bicarbonate ($TBAHCO_3$), 300 μL of H_2O and 300 μL of CH_3CN. After completely drying the [^{18}F]F^- under N_2 gas flow, 20 mg of precursor, dissolved in 100 μL of CH_3CN and 500 μL of alcohol solvent, was added to the reactor. We used *t*-BuOH (2-methyl-2-propanol), *t*-amyl alcohol (2-methyl-2-butanol), thexyl alcohol (2,3-dimethyl-2-butanol), 3,3-dimethyl-2-butanol, 3,3-dimethyl-1-butanol and 2-trifluoromethyl-2-propanol as alcohol solvents. We only added CH_3CN to dissolve the precursor because we found low solubility of the precursor with only alcohol solvent. [^{18}F]Fluorination was performed at 100°C for 5–30 min with stirring, and [^{18}F]fluorination yield was evaluated with radioTLC using EtOH:ethyl acetate (1:1) for development. After [^{18}F]fluorination, the solvents were evaporated completely under a N_2 stream at 100°C and hydrolysis was performed with 500 μL of 1 N HCl and 100 μL of CH_3CN at 85°C for 5 min. Each solution was neutralized with 220 μL of 2 N NaOH and 200 μL of citrate buffer. [^{18}F]FLT synthesis yield was assessed by radioTLC using MeOH:dichloromethane (1:9; $R_f = 0.5–0.6$).

[^{18}F]FLT automatic synthesis with *t*-amyl alcohol

Commercially available TracerLab MX (GE Healthcare, Belgium) was used for the synthesis of [^{18}F]FLT with the same sequence program and reaction sequence as previously described.[12] Briefly, 7 mL of acetonitrile was added to the blue vial; 20 mg of precursor, 2 mL of *t*-amyl alcohol and 200 μL of acetonitrile to the red vial; 1.75 mL of 2 N NaOH and 0.7 mL of citrate buffer to the yellow vial; and 3 mL of 1 N HCl and 0.25 mL of acetonitrile to the green vial. After trapping of 37 GBq/mL [^{18}F]fluoride on a PS-HCO_3 cartridge, it was eluted to the reactor with a solution of 0.3 mL of H_2O, 0.3 mL of CH_3CN and 10 μL of $TBAHCO_3$. After complete drying, the precursor solution from the red vial was moved to the reactor. [^{18}F]Fluorination was performed at 120°C for 10 min.[12] The reaction solvent was removed under helium purging, vacuum and heating at 100°C for 2–8 min. Hydrolysis was performed at 85°C for 5 min with 1 N HCl from the green vial, and the solution was neutralized with the mixture solution from the yellow vial. The reaction mixture was automatically moved to the HPLC purification system (Waters, USA), and purified [^{18}F]FLT was eluted with EtOH:H_2O (10:90) at a flow rate of 5 mL/min. Quality control procedures were performed as described previously.[10]

Conclusion

We evaluated the effect of various alcohol solvents on radiochemical yields during [^{18}F]FLT synthesis. We found that bulky tertiary alcohols showed higher yields than primary or secondary alcohols. In automated synthesis, *t*-amyl alcohol showed high, stable yields during [^{18}F]FLT synthesis.

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[14] *t*-BuOH material safety data sheet (MSDS) from Sigma-Aldrich.
[15] CH_3CN material safety data sheet (MSDS) from Sigma-Aldrich.