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Alternative solvents for electrophilic synthesis of 6-[^{18}F]fluoro-L-DOPA

Sarita Forsback,[a]* Olli Eskola,[a] Jörgen Bergman,[a] Merja Haaparanta,[b] and Olof Solin[a]

Owing to the ozone layer-depleting properties of chlorofluorocarbon compounds, alternative solvents for electrophilic fluorination reactions are desirable. Chloroform, dichloromethane, acetone or their deuterated analogues were examined as substitutes for Freon-11 in the electrophilic synthesis of 6-[^{18}F]fluoro-L-DOPA ([^{18}F]FDOPA). $CDCl_3$, CD_2Cl_2 and C_3D_6O were found to be suitable solvents in this reaction, with the deuterated solvents providing significantly higher yields than Freon-11. There were no differences among the solvents in the specific radioactivity, the radiochemical purity, the chemical purity or the microbiological quality of the final product. However, the radiochemical yield of [^{18}F]FDOPA was increased when acetic acid was added to the precursor solution prior to the fluorination reaction.

Keywords: [^{18}F]FDOPA; electrophilic fluorodestannylation; [^{18}F]F_2; Freon-11

Introduction

Freon-11 (CCl_3F) is a widely used solvent in electrophilic fluorination reactions in PET tracer chemistry, because it is inert and can be easily removed by evaporation.[1–6] Chlorofluorocarbons are harmful to the atmosphere, and thus their availability is restricted. At the same time, the demand for electrophilically fluorinated PET tracers, such as 6-[^{18}F]fluoro-L-DOPA ([^{18}F]FDOPA), has increased.

Earlier attempts to replace Freon-11 were undertaken by De Vries *et al.* using either chloroform ($CHCl_3$) or acetonitrile (CH_3CN) as a solvent in the synthesis of [^{18}F]FDOPA.[3] During the evaporation of both solvents, however, a radical loss of radioactivity occurred. The radiochemical yields (RCY) (corrected for decay) were lower with $CHCl_3$ ($5 \pm 4\%$) or CH_3CN ($17 \pm 2\%$) than with Freon-11 ($33 \pm 4\%$). The quality of the solvents was also found to be important. Deuterated chloroform ($CDCl_3$) stabilized with silver gave slightly better yields of [^{18}F]FDOPA than Freon-11,[7] (25 ± 3 vs. $21 \pm 2\%$, respectively, decay-corrected), with no significant loss of radioactivity during the evaporation. With the non-deuterated chloroform stabilized with ethanol or amylene, 25–40% of the radioactivity was lost as volatile substances.[7]

We considered that the lower recovery of [^{18}F]FDOPA in previous studies was due to the loss of volatile radioactivity caused by the presence of stabilizing reagents or trace impurities in the commercially prepared solvents. With the goal of preparing PET imaging tracers in adequate yield with satisfactory specific activity, we investigated the possibility of using chloroform, dichloromethane, acetone and their deuterated analogues as solvents for electrophilic radiofluorination reactions for the synthesis of [^{18}F]FDOPA using [^{18}F]F_2, post-target produced from [^{18}F]F^-.

Results and discussion

For all solvents used in the radiosyntheses to prepare [^{18}F]FDOPA, we compared RCY, specific radioactivities (SAs) (see Table 1) and radiochemical purities (RCP).

Freon-11 was a suitable solvent for electrophilic fluorination in our reaction system, and our control studies with Freon-11, performed in the presence of acetic acid (AcOH), gave a product with nearly quantitative RCP ($\geqslant 98\%$), acceptable SA ($\geqslant 18.5$ MBq/μmol) and $\sim 6\%$ RCY. Total synthesis time was 50 min. The RCYs, SAs and RCPs of products achieved by using different deuterated solvents with added AcOH were comparable to those obtained with Freon-11 (Table 1). Indeed, all reactions in deuterated solvents with AcOH gave significantly better RCYs of [^{18}F]FDOPA than reactions in Freon-11 ($p < 0.0028$, Figure 1(A)).

The RCYs obtained with deuterated CD_2Cl_2 and $CDCl_3$ with AcOH were 8- and 15-fold higher, respectively, than the RCYs obtained with the corresponding non-deuterated solvents ($p < 0.0001$). However, the RCY obtained with acetone was comparable to that obtained with its deuterated analogue C_3D_6O. As expected, the SA of the product showed little variation

[a]*Radiopharmaceutical Chemistry Laboratory and Accelerator Laboratory, Turku PET Centre Porthaninkatu 3, FI-20500 Turku, Finland*

[b]*MediCity/PET Preclinical Imaging, Turku PET Centre, Tykistökatu 6A, FI-20520 Turku, Finland*

**Correspondence to: Sarita Forsback, Radiopharmaceutical Chemistry Laboratory and Accelerator Laboratory, Turku PET Centre Porthaninkatu 3, FI-20500 Turku, Finland.*
E-mail: sarita.forsback@utu.fi

Table 1. The effect of experimental conditions [solvent, volume of the solvent (V), solvent evaporation temperature (T_{evap}) and amount of acetic acid] on the radiochemical yield (RCY) and the specific radioactivity (SA) of [^{18}F]FDOPA

Solvent	AcOH (μl)	RCY[a] (%)	SA[b] (GBq/imol)
Freon-11, $V=700$ μl, $T_{evap}=25°C$	25, $n=7$	6.0±1.5	2.4±1.0
CD_2Cl_2, $V=750$ μl, $T_{evap}=40°C$	0, $n=1$	4.6	2.9
	25 or 50, $n=6$	7.8±0.6	2.7±0.4
$CDCl_3$, $V=700$ μl, $T_{evap}=60°C$	0, $n=1$	6.5	2.1
	25 or 50, $n=3$	7.5±0.7	2.6±1.1
C_3D_6O, $V=750$ μl, $T_{evap}=55°C$	0, $n=1$	5.2	3.4
	25 or 50, $n=2$	8.5±0.9	3.1±0.03
CH_2Cl_2, $V=700$ μl, $T_{evap}=40°C$	25, $n=1$	0.9	3.4
$CHCl_3$, $V=700$ μl, $T_{evap}=60°C$	25, $n=1$	0.5	2.8
C_3H_6O, $V=750$ μl, $T_{evap}=55°C$	25, $n=1$	5.2	2.8

[a]Radiochemical yields (RCY) are calculated from the initial amount of [^{18}F]F^- and decay corrected to EOB.
[b]Specific radioactivities (SA) are corrected to EOS.

between different solvents, see Table 1. This demonstrates the reproducibility of the [^{18}F]F^- to [^{18}F]F_2 conversion process.

In our method, [^{18}F]F_2 is post-target produced from [^{18}F]F^-[8] and the RCYs are calculated from the initial [^{18}F]F^-. The RCYs in the previous study of Füchtner *et al.* are calculated from in-target produced [^{18}F]F_2.[7] Therefore, our percentage RCYs are lower compared to those of Füchtner. The SA of the final product, in this case [^{18}F]FDOPA, achieved by using post-target produced [^{18}F]F_2 is higher compared to in-target production methods.[6]

In our modification of Namavari's electrophilic fluorodestannylation,[1] a small amount of AcOH is added to the precursor solution prior to the labeling reaction and Freon-11 is evaporated to dryness before acid hydrolysis of the protecting groups.[6] Freon-11 is inert and easily evaporated to dryness without heating. To evaporate the solvents with boiling points higher than Freon-11 in a reasonable time, heating of the reaction vessel was required. If the evaporation temperature was too high, near to or above the boiling point of the solvent, the amount of radioactivity in the reaction vessel decreased radically during the evaporation in our experiments. Probably due to the relatively high boiling point, studies performed in $CDCl_3$ were the most sensitive with respect to loss of radioactivity due to elevated evaporation temperature. Two experiments with observed loss of radioactivity gave significantly lower RCYs (4.2±0.9%) when compared to experiments without radioactivity loss (7.5±0.7%). However, increasing the evaporation temperature of $CDCl_3$ to 130°C did not further decrease the RCY (4.0%). With CD_2Cl_2, the evaporation was performed at lower temperature and the RCY was reproducible in all six trials included in this study. With $CHCl_3$ and CH_2Cl_2, [^{18}F]F_2 reacted with the double bond in the amylene stabilizer and unidentified volatile radioactive by-products were formed, decreasing the overall radiochemical yield.

Owing to the high reactivity of [^{18}F]F_2, additives (such as stabilizers) and impurities in commercially available reagents and solvents will decrease the RCY of the final product during electrophilic fluorination. Because of the low RCYs, the number of experiments with amylene stabilized solvents was kept small; only one test synthesis with $CHCl_3$ or CH_2Cl_2 was performed. Commercially available acetone does not contain stabilizers, which can react with [^{18}F]F_2, and thus, the RCY using C_3H_6O was higher compared to $CHCl_3$ and CH_2Cl_2. In terms of RCP, all deuterated solvents used in this study gave nearly quantitative results (⩾98%).

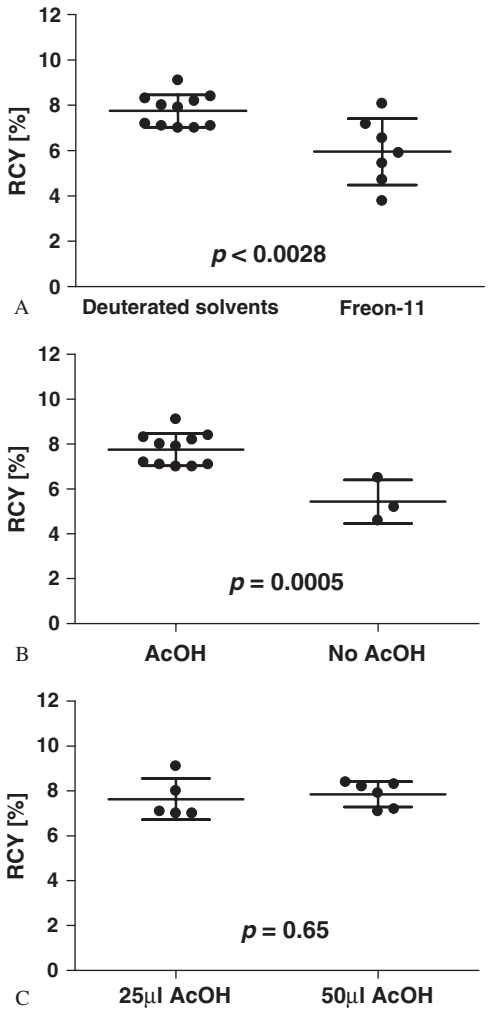

Figure 1. The effect of different reaction conditions on radiochemical yields (RCY) of [^{18}F]FDOPA. Panel A: the radiochemical yields (RCY) from experiments with Freon-11 and with all deuterated solvents. All reactions contained AcOH. Panel B: the RCYs from experiments with deuterated solvents, with AcOH and from experiments with deuterated solvents, without AcOH. Panel C: the RCYs from experiments with deuterated solvents, with 25 μl AcOH or with 50 μl AcOH.

AcOH is used in electrophilic fluorination in order to make F–F bond more susceptible towards the substitution reaction.[9,10] In this work, we studied the influence of the amount of AcOH on

the RCY of [^{18}F]FDOPA. We found out that absence of AcOH decreased significantly the RCY of [^{18}F]FDOPA ($p = 0.0005$, Figure 1(B)), but increasing the amount of AcOH from 25 μl (430 μmol) to 50 μl (860 μmol) did not further improve the RCY ($p = 0.65$, Figure 1(C)). The addition of more AcOH had no further benefit, because used amounts were both in great molar excess to the reactants.

All deuterated solvents gave better RCY of [^{18}F]FDOPA than Freon-11. This may be due to the higher surface tension of CD_2Cl_2, $CDCl_3$ and C_3D_6O as compared to Freon-11. Higher surface tension affects the formation of bubbles and thus increases the contact time between [^{18}F]F_2 and the dissolved precursor.

Our results are in good agreement with earlier studies using $CHCl_3$ or $CDCl_3$ as a solvent in electrophilic fluorination[3,7] and we have demonstrated that CD_2Cl_2 and C_3D_6O are also suitable solvents for electrophilic fluorination. CD_2Cl_2 is preferred due to good reproducibility using our methods.

Experimental

[^{18}F]FDOPA was synthesized starting from post-target produced [^{18}F]F_2[8] using previously described methods.[6]

The stannylated precursor (4,5-di-[(1,1-dimethylethoxy-carbonyl)oxy]-*N*-formyl-2-trimethylstannyl-L-phenylalanine ethyl ester; 3.1 ± 0.1 mg, ~5 μmol) was dissolved in 700–750 μl of one of the following solvents: CCl_3F, $CDCl_3$, CD_2Cl_2, C_3D_6O, $CHCl_3$ stabilized with amylene, CH_2Cl_2 stabilized with amylene or C_3H_6O. All the solvents, except CCl_3F, were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). CCl_3F was from Fluka (Fluka Chemie AG, Buchs, Switzerland). Dry acetic acid (25 or 50 μl, 430 or 860 μmol, respectively) was added to the precursor solution a few minutes before the labeling reaction. Three control experiments were done without acetic acid. [^{18}F]F_2 was bubbled through the precursor solution at room temperature. Solvents were evaporated using neon flow and by heating the reaction vessel in an oil bath. Volume of the solvent (V) and of acetic acid (AcOH) and the evaporation temperature (T_{evap}) are presented in Table 1. Hydrolysis of protection groups and semi-preparative HPLC purification were performed as previously described.[6]

A sample from the semi-preparative fraction containing [^{18}F]FDOPA was analyzed using a Merck-Hitachi L-7100 HPLC pump (Merck AG, Darmstadt, Germany) combined with a Merck-Hitachi L-7400 UV-absorption detector ($\lambda = 280$ nm) and a $2 \times 2'$ NaI-crystal for radioactivity detection. An Atlantis dC18 (5 μm; 3.9 × 150 mm, Waters Corp., Milford, MA, USA) was used as a column and it was eluted with 0.07 M KH_2PO_4 (aq) at flow rate 1.25 ml/min.

Chemical purity, RCP and SA were determined by comparisons of HPLC retention times and peak intensities using a reference compound of known concentration. RCYs were calculated from the initial amount of [^{18}F]F^-, not from [^{18}F]F_2, and they were decay corrected to the end of bombardment (EOB). SAs were decay corrected to the end of synthesis (EOS), i.e. to the end of the semi-preparative HPLC separation.

Microbiological quality of the final product was confirmed by testing sterility and bacterial endotoxins from seven selected experiments. The tests were performed by Turku University Hospital Pharmacy.

Statistical analyses were performed using the program Graph Prism, version 5.01 (GraphPad Software, San Diego, CA, USA). Comparison of RCY were tested using unpaired two-tailed Student's *t* test. Results are expressed as means ± SD for the indicated number of observations. Means were considered significantly different when $p < 0.05$.

Conclusion

CD_2Cl_2, $CDCl_3$ and C_3D_6O are all suitable solvents for the electrophilic synthesis of [^{18}F]FDOPA. They gave better yields than Freon-11 and the final product produced by using any of those solvents fulfilled our quality requirements for patient injection. Including AcOH (25 μl) in the reaction mixture significantly increased the radiochemical yield, but no additional benefit was achieved when a larger volume (50 μl) of AcOH was supplied.

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