

# Multiphase Extraction: Rapid Phase-Transfer of [ $^{18}\text{F}$ ]Fluoride Ion for Nucleophilic Radiolabeling Reactions

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In multiphase extraction [ $^{18}\text{F}$ ]fluoride ion for radiolabeling is recovered from target water by passage through a small column of microporous polymer impregnated with a lipophilic cryptand or quaternary ammonium salt. The  $^{18}\text{O}$  enriched water can be recovered for reuse. The [ $^{18}\text{F}$ ]fluoride ion-pair is eluted from the column by a small volume of acetonitrile or other organic solvent. Evaporation of the acetonitrile removes traces of water to yield a reactive ion pair for nucleophilic radiofluorination reactions. A wide range of ion-pairs based on  $\text{K}^+$  or  $\text{NH}_4^+$  cryptands or quaternary ammonium salts can be employed. The method was applied to the synthesis of [ $^{18}\text{F}$ ]FDG.

## Introduction

Solubilization of [ $^{18}\text{F}$ ]fluoride ion for nucleophilic radiolabeling is usually accomplished by evaporation of aqueous fluoride in the presence of a tetraalkylammonium salt (Brodack *et al.*, 1986) or  $\text{K}^+$  cryptand (Hamacher *et al.*, 1986) to form an ion-pair soluble in organic solvent. Commonly some means of recovering the evaporated [ $^{18}\text{O}$ ]target water is employed. Recently, Schlyer *et al.* (1987) demonstrated a simple way to remove [ $^{18}\text{F}$ ]F $^-$  from [ $^{18}\text{O}$ ]water by a small column of anion exchange resin. The [ $^{18}\text{F}$ ]F $^-$  was eluted from the column by dilute aqueous potassium carbonate, and the water was evaporated in the presence of a quaternary ammonium hydroxide or  $\text{K}^+$  cryptand to obtain a reactive ion-pair. Here we describe a method which accomplishes extraction and ion-pairing in a single step, eliminating the need to evaporate several hundred microliters of water in the remotely operated nucleophilic radiolabeling of PET radiopharmaceuticals. In this new method, which we call multiphase extraction, the [ $^{18}\text{F}$ ]F $^-$  is extracted from the [ $^{18}\text{O}$ ]target water by a small column containing a microparticulate polymer impregnated with a quaternary ammonium salt or lipophilic  $\text{K}^+$  or  $\text{NH}_4^+$  cryptand. The [ $^{18}\text{O}$ ]water is recovered, and the [ $^{18}\text{F}$ ]F $^-$  ion-pair is eluted from the column by a small amount of organic solvent such as acetonitrile. Evaporation of the solvent removes traces of water to

yield reactive anhydrous [ $^{18}\text{F}$ ]F $^-$ . The method allows considerable latitude in the choice of ion-pairing systems, which may facilitate nucleophilic reactions of [ $^{18}\text{F}$ ]F $^-$  with base-sensitive substrates. We describe the use of the method in the synthesis of [ $^{18}\text{F}$ ]fluorodeoxyglucose (FDG) in addition to preliminary experiments to establish its possible limitations and advantages.

## Materials and Methods

### Extractions

For most experiments a  $\text{C}_{18}$ -derivatized polystyrene resin (Macrophase MP-1 for HPLC, Interaction Chemicals) was slurry packed in EtOH in a polyethylene column. Dimensions of the resin bed were  $16 \times 3$  mm. The resin was held in place by small plugs of fine polypropylene wool (Phillips Fibers). A dilute solution of Kryptofix 222-D ( $\text{C}_{10}\text{H}_{21}$ -2,2,2-cryptand, Parish Chemical Co.) in MeOH (8 mg in  $200 \mu\text{L}$ ) was passed through the column. The excess liquid was forced out by gently purging with  $\text{N}_2$ , and gas flow was continued for about a minute to evaporate most of the MeOH. Aqueous KOH (0.3 mL, 1 N) was then passed through the column to convert the adsorbed cryptand to the  $\text{K}^+/\text{OH}^-$  adduct. The column was purged with  $\text{CO}_2$ -free deionized distilled

water (boiled and stored under N<sub>2</sub>; approx 3 mL) until the pH of the effluent was neutral. Residual free water was blown out of the column with N<sub>2</sub>. Aqueous [<sup>18</sup>F]F<sup>-</sup> solutions were prepared by irradiating [<sup>18</sup>O]water with protons in a silver target (1 mL, 20 μA, 10–20 min). In some cases unenriched distilled, deionized water was similarly irradiated and later labeled with a trace amount of [<sup>18</sup>F]F<sup>-</sup>. This was done to obtain water chemically similar to the enriched water without consuming [<sup>18</sup>O]water. After allowing sufficient time for radioactivity from <sup>13</sup>N to decay, the water (1 mL) from the target was passed through the column to extract the [<sup>18</sup>F]F<sup>-</sup>. The column was purged for 1 min with N<sub>2</sub> to remove remaining free water and then eluted with a total of 1.0 mL dry MeCN. The radioactivity in the various fractions was measured either by an ionization chamber or NaI detector. Other ion-pairing agents and solid phases were handled in the same way. Quaternary ammonium halides were applied to the columns as dilute solutions in MeOH and subsequently converted to the hydroxide form by washing with aqueous KOH followed by distilled, deionized, CO<sub>2</sub>-free water until the pH of the effluent was neutral. In some cases the hydroxides were further exchanged with other anions. The amount of ion-pairing agent retained on a column was determined by extracting with MeCN into a tared tube and weighing the residue remaining after evaporation of the solvent. Microporous glassy carbon was prepared by the method of Knox and Kaur (1986). The C<sub>18</sub>-silica was in the form of a Sep-Pak cartridge (Waters, Inc.)

For the synthesis of [<sup>18</sup>F]FDG Macrophase MP-1 polymer was suspended in MeCN and slurry packed in a stainless steel column (1.6 × 20 mm) with 2 μm stainless steel frits. The column was installed on a 10-port HPLC injection valve (Valco). A solution of

Kryptofix 222-D in methanol (8 mg, 0.3 mL) was passed through the column followed by N<sub>2</sub> to blow out the excess liquid and evaporate the MeOH. Aqueous KOH (1 N, 0.2 mL) was passed through the column, which was then rinsed with about 3 mL distilled, deionized CO<sub>2</sub>-free water until the pH of the effluent was neutral. Excess water was removed from the column by a flow of N<sub>2</sub> for 10 min. The irradiated target water was passed through the column at a pressure of about 5 atm. The [<sup>18</sup>O]water was recovered. Dry MeCN, 1 mL was then passed through the column to elute the [<sup>18</sup>F]fluoride ion pair. After evaporation of the MeCN under a stream of N<sub>2</sub> at 80°C, two additional 1 mL volumes of dry MeCN were added and evaporated to remove all traces of water. The subsequent procedure for nucleophilic incorporation of [<sup>18</sup>F]fluoride into 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose was identical to that of Padgett *et al.* (1988), which is in turn based on that of Hamacher *et al.* (1986).

## Results and Discussion

Results of a preliminary survey of materials and conditions for extraction of [<sup>18</sup>F]F<sup>-</sup> are summarized in Table 1. For most experiments C<sub>18</sub>-derivatized microparticulate polystyrene was used because it was readily available as an HPLC packing. The packed columns were reused indefinitely with low levels of radioactivity without loss of extraction efficiency. It is not known whether radiolytic degradation may occur with repeated extractions at the level of several hundred mCi. The high reported capacity factor and satisfactory HPLC performance of underivatized microparticulate styrene-divinylbenzene (PRP-1, Hamilton Co.) indicates that it may be a satisfactory and less expensive polymeric phase when it again

Table 1. Summary of extraction and recovery of [<sup>18</sup>F]fluoride ion

Expt No.	Solid phase <sup>a</sup>	Organic cation	Counter anion	Adsorbed phase wt (mg)	Source of water <sup>b</sup>	% [ <sup>18</sup> F]fluoride extracted	% Total [ <sup>18</sup> F]F recovered
1	M	(C <sub>4</sub> ) <sub>4</sub> N <sup>+</sup>	OH	0.8	P	69	69
2	M	(C <sub>4</sub> ) <sub>4</sub> N <sup>+</sup> Me	OH	-	P	94	93
3	M	(C <sub>6</sub> ) <sub>4</sub> N <sup>+</sup>	OH	0.3	P	>99	90
4	M	(C <sub>6</sub> ) <sub>4</sub> N <sup>+</sup>	OH	-	P	>99	98
5	M	(C <sub>7</sub> ) <sub>4</sub> N <sup>+</sup>	OH	0.6	P	>99	>99
6	M	(C <sub>7</sub> ) <sub>4</sub> N <sup>+</sup>	OH	-	T	>99	72
7	M	Perfluoroalkyl N <sup>+</sup>	OH	0.8	P	60	59
8	M	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub> N <sup>+</sup>	OH	-	P	>99	87
9	M	Crypt 222-K <sup>+</sup>	OH	0.6	P	88	77
10	M	Crypt 222/NH <sub>4</sub> <sup>+</sup>	OH	-	P	76	75
11	M	Crypt 222D-K <sup>+</sup>	OH	2.5	P	>99	>99
12	C	Crypt 222D-K <sup>+</sup>	OH	<0.1	P	97	2
13	S	Crypt 222D-K <sup>+</sup>	OH	6.2	P	>99	<1
14	M	Crypt 222-K <sup>+</sup>	OH	-	T	87	81
15	M	Crypt 222-K <sup>+</sup>	CO <sub>3</sub> <sup>2-</sup>	-	T	80	80
16	M	Crypt 222-K <sup>+</sup>	C <sub>2</sub> O <sub>4</sub> <sup>2-</sup>	-	T	64	60
17	M	Crypt 222-K <sup>+</sup>	isoC <sub>4</sub> COO	-	T	29	26
18	M	Crypt 222D-K <sup>+</sup>	acetate	-	P	>99	88
19	M	Crypt 222D/NH <sub>4</sub> <sup>+</sup>	acetate	-	T	>99	98

<sup>a</sup>Solid phase: M = macrophase MP-1; C = microporous glassy carbon; S = C<sub>18</sub>-silica.

<sup>b</sup>P: Deionized distilled water labeled with a trace amount of [<sup>18</sup>F]F<sup>-</sup>; T: <sup>18</sup>O-enriched water from silver target.

<sup>c</sup>Methylbenzethonium<sup>+</sup>.

becomes commercially available in the form of disposable miniature extraction cartridges. A number of observations are possible from these limited results which are likely to apply as well to other microporous solid phases, and which should aid in the design of practical systems. First, octadecyl silica, while it retains the ion-pair well, irreversibly binds [ $^{18}\text{F}$ ]F $^-$ . This occurred even when the quaternary ammonium hydroxide was formed prior to adsorption on the solid phase to minimize exposure to strong aqueous base. This was also true for the sample of microporous glassy carbon, probably because of our inability to remove completely the silica matrix used for preparing this material. Secondly, a minimum degree of lipophilicity is required of the cryptand or quaternary ammonium salt in order for it to be completely retained on the polymeric phase. With the less lipophilic phases, the aqueous washes never reached neutrality after conversion of the adsorbed phase to the hydroxide form. Thus, unlike the C $_{10}$ -substituted cryptand, the unsubstituted 2,2,2-cryptand continued to elute from the column into the aqueous phase making complete extraction of [ $^{18}\text{F}$ ]F $^-$  impossible. Similarly, tetrabutylammonium hydroxide did not permit complete extraction of [ $^{18}\text{F}$ ]F $^-$ , whereas the tetraheptyl ammonium hydroxide permitted quantitative extraction. Thirdly, NH $_4^+$  can substitute for K $^+$  as the guest ion in a 2,2,2-cryptand. Finally, at trace levels, fluoride ion can displace a number of anions in addition to hydroxide, allowing the formation of less basic ion pair systems. This may be useful where substrates for nucleophilic radiofluorination are labile toward hydroxide ion.

In nucleophilic exchange reactions a buffer such as K $_2\text{CO}_3$  is employed with sensitive substrates such as the mannose triflate precursor of [ $^{18}\text{F}$ ]FDG to minimize attack on the substrate by hydroxide ion (Hamacher *et al.*, 1986; Padgett *et al.*, 1988). We tested the feasibility of converting cryptand-KOH (pH in aqueous solution = 10.5) to the cryptand-KHCO $_3$  (pH = 7.5) directly on the column by exposure to CO $_2$ . The adsorbed phase reacted readily with CO $_2$  labeled with [ $^{14}\text{C}$ ]CO $_2$ . Measurement of the pH after elution of the ion pair into water indicated conversion to the bicarbonate form.

When eluting the  $^{18}\text{F}$  ion-pair from the column, collection of the MeCN in 100  $\mu\text{L}$  fractions revealed that almost all of the radioactivity eluted with the first 100  $\mu\text{L}$ . The radioactivity could also be eluted with THF. Impregnation of the C $_{18}$ -derivatized polystyrene resin with organic ion pairs in the hydroxide form caused it to become hydrophilic, making it difficult to dry the column completely before extraction of the [ $^{18}\text{F}$ ]F $^-$  from the target water. After removal of all visible water from the column by purging for 5 min with N $_2$ , 20 mg of water still remained adsorbed on the resin. Approximately 4 mg of water was strongly retained on the column even after purging with N $_2$  for 30 min. This resulted

in some contamination of the target water with [ $^{16}\text{O}$ ] water. For the same reason, some water was always eluted along with the ion pair by the organic solvent making one or more azeotropic distillations necessary for complete drying.

Multiphase extraction is presently the method of [ $^{18}\text{F}$ ]F $^-$  solubilization in use in our laboratory for the routine production of FDG for human studies. After the extraction step, the method is the same as that already in use by Padgett *et al.* (1988) which is in turn based on that of Hamacher *et al.* (1986). It has the advantages of mechanical simplicity, ease of miniaturization and recovery of [ $^{18}\text{O}$ ]water inherent in the method of Schlyer *et al.* (1987) without the need for evaporating several hundred microliters of water. With further optimization it thus promises to be faster than other methods. However, since the subsequent deblocking step still requires about 20 min, this advantage will only be fully realized if the latter can be accomplished more quickly. The recovery of [ $^{18}\text{F}$ ]F $^-$  as the kryptofix 222-D adduct is usually between 88 and 95%, and the nucleophilic incorporation of the [ $^{18}\text{F}$ ] activity into tetraacetyl-FDG is approximately 80%. No radioactive contaminants other than fluoride can be detected, even with a triflate substrate known to be susceptible to side reactions caused by excess base, excessively high temperatures or the presence of water. It can thus be expected that the method will be readily applicable to more robust substrates such as aromatic nitro compounds. It should be noted that, as with the method of Hamacher *et al.* (1986), excess cryptand and base (as K $_2\text{CO}_3$ ) are still required to achieve a useful degree of  $^{18}\text{F}$ -incorporation in the substrate.

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