Nucl. Med. Biol. Vol. 17, No. 3, pp. 273–279, 1990
Int. J. Radiat. Appl. Instrum. Part B
Printed in Great Britain. All rights reserved

0883-2897/90 $3.00 + 0.00
Copyright © 1990 Pergamon Press plc

Routine Production of 2-Deoxy-2-[^{18}F]fluoro-D-glucose by Direct Nucleophilic Exchange on a Quaternary 4-Aminopyridinium Resin

STEVEN A. TOORONGIAN, G. KEITH MULHOLLAND,*
DOUGLAS M. JEWETT, MICHAEL A. BACHELOR
and MICHAEL R. KILBOURN

Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, U.S.A.

(*Received* 11 *October* 1989)

Resin-supported [^{18}F]fluoride ion has been prepared and applied to a rapid, convenient synthesis of [^{18}F]FDG. "No-carrier-added" [^{18}F]fluoride ion is collected on a quaternary 4-(*N,N*-dialkylamino)-pyridinium functionalized polystyrene anion exchange resin directly from a [^{18}O]water target, dried by rinsing with acetonitrile, and then reacted with 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyrannose. Acidic hydrolysis yields [^{18}F]FDG in a synthesis time of 40 min with overall yields presently averaging above 50%.

Introduction

2-Deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) is the most widely used radiopharmaceutical for positron emission tomography (PET). Considerable effort has been expended in the development and refinement of methods of preparing this radiopharmaceutical (Fowler and Wolf, 1986; Coenen *et al.*, 1987). The original methods of synthesis using electrophilic fluorination have, in most institutions, been supplanted by the nucleophilic synthesis utilizing [^{18}F]fluoride ion displacement of the 2-triflate ester of tetra-*O*-acetyl-β-mannose (Hamacher *et al.*, 1986). This procedure is a stereospecific synthesis which gives FDG in moderate and consistent yield. Most recently, methods for separation of the [^{18}F]fluoride ion from [^{18}O]water (Schlyer *et al.*, 1987; Jewett *et al.*, 1988) have improved the economy of [^{18}F]FDG syntheses by allowing simple recovery of the expensive enriched target material.

We have recently described custom-synthesized 4-aminopyridinium anion exchange resins which allow the combination of the steps of collection of [^{18}F]fluoride (with separation and recovery of the [^{18}O]water), drying and nucleophilic substitution reactions into a single and very simple procedure (Mulholland *et al.*, 1989b). Here we describe the implementation of the resin synthetic approach to the routine delivery of [^{18}F]FDG and analyze its performance in 104 clinical syntheses.

*All correspondence should be addressed to: G. Keith Mulholland, Ph.D., Division of Nuclear Medicine, Cyclotron/PET Facility, 3480 Kresge III, University of Michigan, Ann Arbor, MI 48109, U.S.A.

Experimental

General

[^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F reaction on [^{18}O]H_2O (95–97% enrichment, Isotec) using a pressurized all-silver target as previously described (Mulholland *et al.*, 1989a). All target irradiations were carried out at 20 μA. Irradiations ranged between 10 and 30 min duration with most occurring for 15 or 20 min. Acetonitrile (99+%, anhydrous) and 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyrannose were obtained from Aldrich Chemical Co. and used without further purification. 4-(4-Methyl-1-piperidino)pyridine was obtained from Reilly Tar and Chemical Co., Indianapolis, Indiana. Chloromethylated 2% crosslinked polystyrene beads ("Merrifield's resin") were obtained from Bio-Rad (S-X-2) or Aldrich. The fibrous cation exchange resin Toray TIN-100, H^+ form (Yoshioka, 1983) was obtained from C.I. Specialty Chemicals Inc., New York. Mixed bed resin columns

were slurry packed (ethanol–water) in short sections of 1.59 mm i.d. Teflon tubes with a 4:1 or 6:1 mixture of aminopyridinium (CO_3^{2-} form): TIN-100 (H^+) exchange resins (total resin weight of 15 or 20 mg: column dimensions approx. 1.6 × 50 mm) and dried with a flow of nitrogen. Columns may be prepared in advance and stored at 0–5°C. Radio-thin layer chromatography was done using plastic-backed silica gel plates (Merck) and a Berthold linear TLC analyzer. Capintec CRC745 ionization chambers were used for radioactivity measurements.

Preparation of 4-(4-methyl-1-piperidino)pyridinium functionalized polystyrene resins (4-aminopyridinium or "4-AP" resins)

4-(4-Methyl-1-piperidino)pyridine (3.18 g, 18 mmol), chloromethylated 2% crosslinked polystyrene beads ("Merrifield's resin" 200–400 mesh, 1.2 m-equiv Cl/g, 10 g) and acetonitrile (50 mL) were combined in a flask. The flask was sealed under N_2 atmosphere and heated (50–60°C) for 24 h, with occasional gentle swirling. The resin was then collected by filtration and sequentially washed with CH_3CN (3 × 100 mL), CH_2Cl_2 (3 × 100 mL) and methanol (3 × 100 mL) and then vacuum dried (1 torr, 50°C, 12 h). The weight of product resin was 12.01 g. Analysis: N = 3.22%, Cl = 4.18%. The resin was converted to the carbonate form by extensive rinsing in a fritted glass funnel with 1.8 M K_2CO_3, followed by deionized water until the washings were neutral, then washed with 50% aqueous methanol and finally 100% methanol. Analysis C = 68.8%, H = 7.41%, N = 2.64%, Cl = trace or none; no ash detected (no significant potassium). The 4-aminopyridinium resin (CO_3^{2-} form) is indefinitely stable at room temperature. Synthesis of the resin is shown in Fig. 1.

Synthesis of [^{18}F]FDG

The overall procedure is outlined in Fig. 2. The apparatus for the synthesis of [^{18}F]FDG is shown in Fig. 3. The [^{18}O]water containing [^{18}F]fluoride solution is transferred from the target through 25 m of 0.86 mm i.d. polyethylene or Teflon tubing to the synthesis apparatus by means of helium gas pressure and slowly (5–7 min) passed through the mixed bed resin column. The [^{18}F]fluoride is trapped on the resin column and the [^{18}O]H_2O is recovered downstream for later reuse. After the [^{18}F]fluoride has been collected the outflow of the resin column is diverted by means of a 3-way distribution valve to a waste reservoir. The resin-bound [^{18}F]fluoride is then dried by passing 2 mL of anhydrous acetonitrile through the column while gradually (2–3 min) heating it to 100°C. Very little ^{18}F-activity (< 1%) is lost from the column during this solvent treatment. The outlet valve is then rotated to the next position and the outflow is directed to the hydrolysis vessel. A solution of 20 mg of the precursor 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyrannose in 2 mL of anhydrous acetonitrile is then passed through the heated column (100°C) over a period of 5–7 min (manual push, using a 2 cm^3 syringe). The progress of the labeling reaction is followed by observing the loss of radioactivity from the ionization chamber. To ensure elution of all product material from the column, the precursor solution is followed by 1–3 mL of additional anhydrous acetonitrile.

The hydrolysis vessel consists of a test tube situated in a hot air heating block apparatus. This heating block has been previously warmed to ~130°C and contains nitrogen purges at top and bottom to facilitate evaporation of acetonitrile. As the acetonitrile reaction solution reaches the vessel the solvent quickly evaporates, leaving behind a residue of tetra-*O*-acetyl-[^{18}F]FDG. For the hydrolysis step 2 mL of 1 N HCl is added and this solution is allowed to reflux at 100°C for 15 min with nitrogen purge flows off. Upon completion of hydrolysis the product solution is drawn through a C_{18} Sep-Pak, an ion-retardation column (Bio-Rad AG 11A8) and finally a neutral alumina column. Two rinses of sterile water (5 mL each) are then passed through the hydrolysis system and the combined final product solution is sterilized through a 0.22 μm membrane filter into a multidose vial. Radiochemical purity is checked by TLC (95:5 acetonitrile:water).

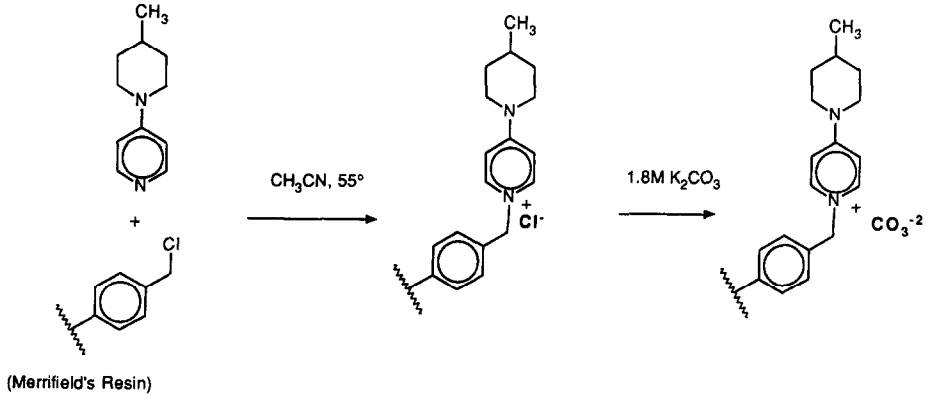

Fig. 1. Synthesis of 4-aminopyridinium resin.

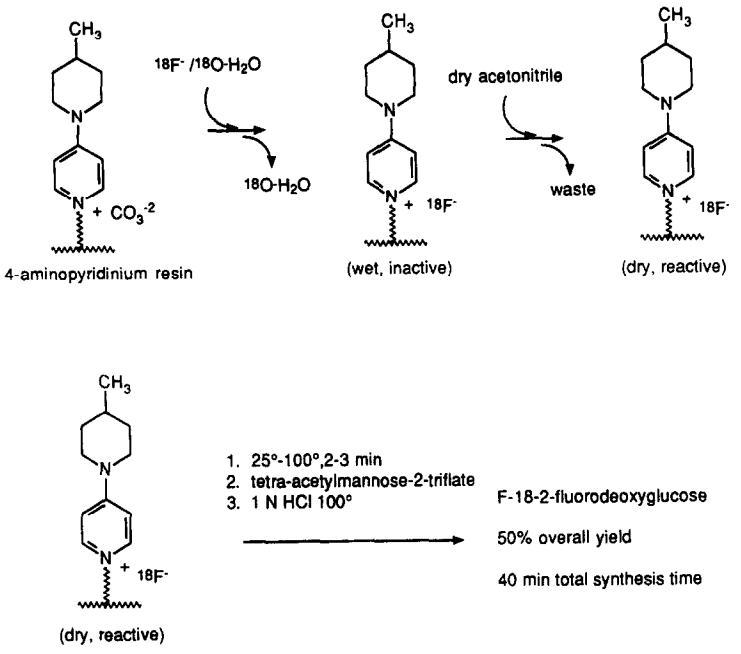

Fig. 2. Resin-supported [^{18}F]FDG synthesis procedure.

Results and Discussion

More than 100 [^{18}F]FDG syntheses have been performed using this resin based nucleophilic [^{18}F]fluorination technique. Radiochemical purity was uniformly >97%. The highest end-of-synthesis FDG yield attained to date is 291 mCi from a 30 min/20 μA irradiation. Figure 4 shows graphs of (A) the total production of ^{18}F activity from the target, (B) the trapping efficiency of the resin column for activity from the target, (C) the covalent incorporation of resin trapped [^{18}F]fluoride (percentage of resin bound activity eluting from column when the mannose triflate solution is passed through the resin), and (D) the overall decay corrected yields of [^{18}F]FDG based on total ^{18}F radioisotope from the target for 104 consecutive clinical synthesis runs. Only one run (No. 35) failed to produce enough FDG for a patient dose. Although there is a substantial degree of variability in these data, steady upward

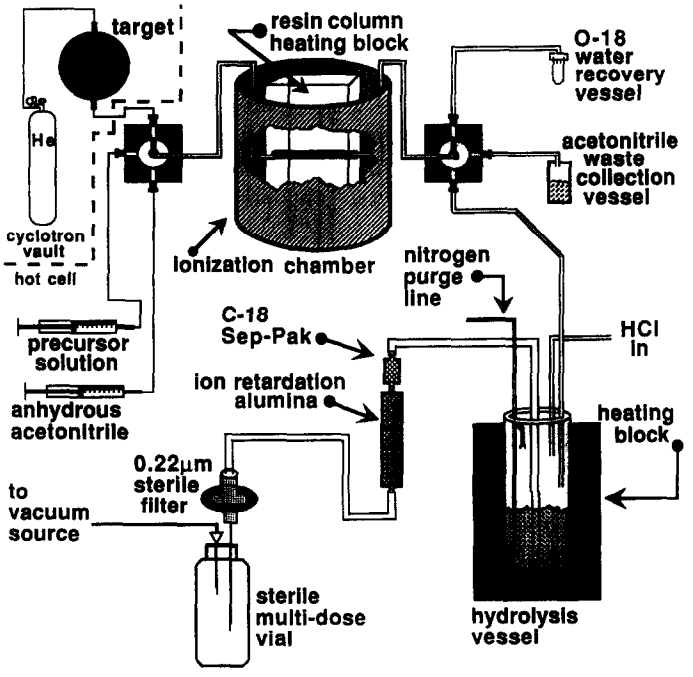

Fig. 3. Apparatus for synthesis of [^{18}F]FDG using resin-supported method.

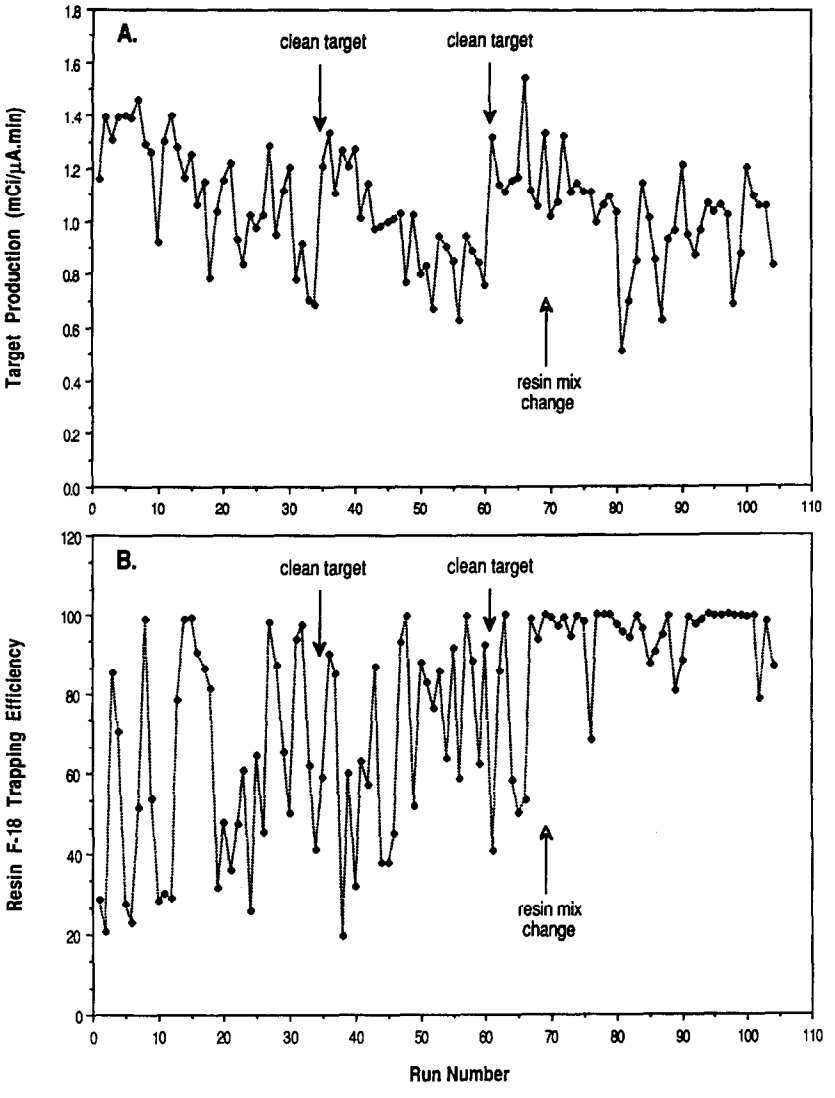

Fig. 4(A, B)

trends in resin trapping efficiencies and overal FDG yields over the entire series of runs are apparent. The average yield of FDG for all 104 syntheses was $41 \pm 15.6\%$, based on aqueous fluoride. Several factors appear to contribute to the fluctuations in the [^{18}F]FDG yield. Among them are variability in the amount of [^{18}F]fluoride collected on the resin column. Because target water makes only a single pass through the resin in this technique, the column trapping efficiency is sensitive to flow rate, packing defects, column geometry and the total amount of 4-aminopyridinium resin in the bed. These factors are interdependent and therefore difficult to examine separately. Slurry packing the columns by suction seems to produce the most uniform beds and consistent results. We have not attempted a systematic evaluation of resin column geometry effects on the yields of the [^{18}F]fluoride ion trapping and nucleophilic displacement steps, using the single pass technique described here. However, using an alternate, mechanically more complex modification (reciprocating action through column) we have occasionally obtained overal yields of tetra-*O*-acetyl-[^{18}F]FDG ([^{18}F]fluoride trapping plus nucleophilic labeling) of 84% (corrected, 10 min synthesis time).

Effect of mixed resin bed

The Toray TIN-100 cation exchange fiber resin was included in these columns to improve flow characteristics over the 4-aminopyridinium resin alone. Its fibrous nature appears to prevent channeling of liquids and decreases back pressure. The sulfonic acid residues may also help to remove potential interfering metal ions or basicity from the target water. The first 69 runs were carried out using the 4:1 (12 mg 4-AP:3 mg TIN-100) resin mixture and the subsequent 35 runs used the ~6:1 (17 mg 4-AP:3 mg TIN-100) resin mixture. The performances of the two mixtures are compared in Table 1. Increasing the quantity of 4-aminopyridinium resin in the column

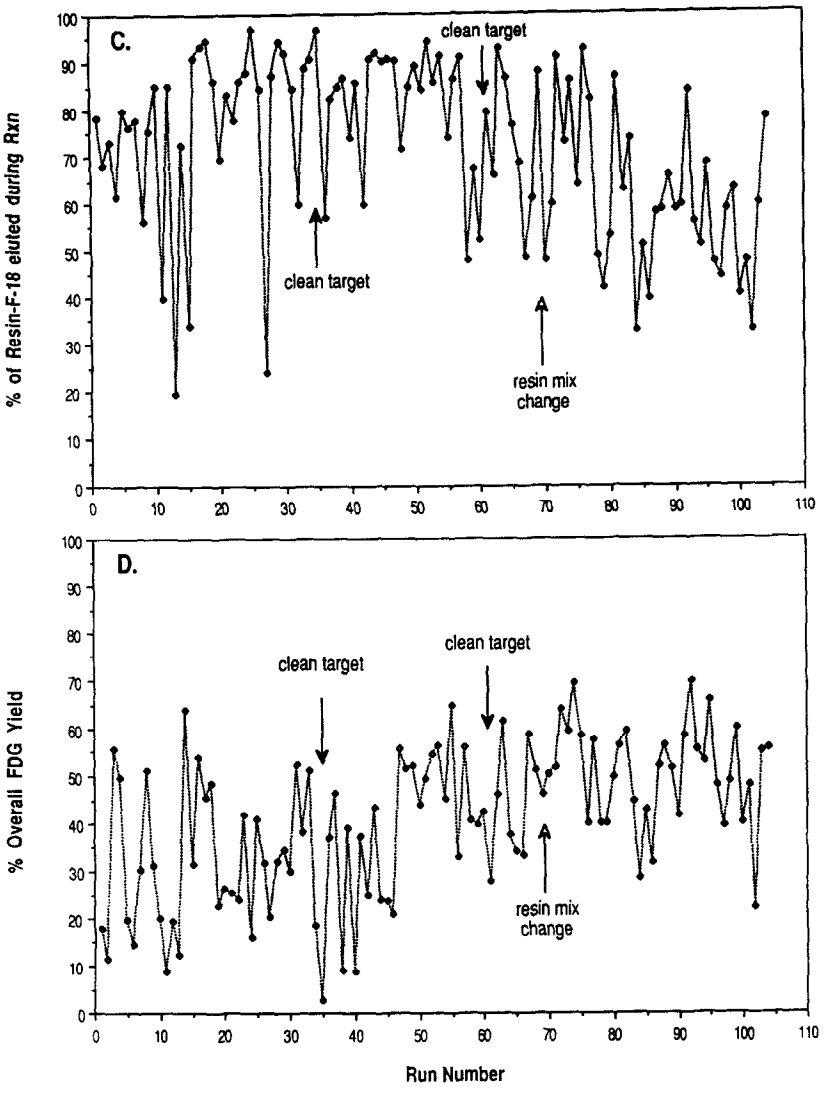

Fig. 4(C, D)

Fig. 4. Production data for 104 consecutive resin-supported clinical [^{18}F]FDG synthesis runs.

to ~6:1 improved both the [^{18}F]fluoride trapping efficiency and overall [^{18}F]FDG yield without adversely affecting flow through the column. Paradoxically, the average percentage of the total resin bound [^{18}F]fluoride *not* reacting with (not eluted from the column by) the mannose triflate precursor solution was significantly greater when the 6:1 mixture was used. Using either the 4:1 or the 6:1 resin mix, essentially all the activity that eluted from the column on passage of precursor solution was in the form of tetra-*O*-acetyl-[^{18}F]FDG upon TLC analysis (silica gel, 1:1 hexane:ethylacetate). Negligible amounts of [^{18}F]fluoride ion or other polar labeled materials were present in the column eluent solution.

Table 1. Effect of changing resin mixture on clinical production results

	4:1 Resin mix (15 mg total)	6:1 Resin mix (20 mg total)
Trapping efficiency	66% ± 25	95% ± 5.5
Fraction of resin bound ^{18}F activity eluting during reaction	77% ± 17	61% ± 16
[^{18}F]FDG yield based on total ^{18}F activity from target	36% ± 15.3	50.4% ± 11
Number of syntheses	69	35

Temperature effects

The extent of reaction of resin bound [^{18}F]fluoride with mannose triflate precursor is also a source of variability in overall FDG yields. The thermal sensitivity of the 4-aminopyridinium resin is likely a contributing factor here. Maximal rates of nucleophilic [^{18}F]fluoride labeling on the resin occur within a relatively narrow temperature window between 90 and 105°C. Higher temperatures result in rapid loss of the resin's heterogeneous [^{18}F]fluoride donating activity. The resin columns themselves are not reusable, and are discarded after the synthesis is

complete. During normal reaction conditions the resin becomes strongly discolored. The mode of resin decomposition is not understood clearly at this time. Since our present system is not automated, precise control and reproducibility on the resin column heating rate and the timing for passing the precursor solution through the column for each run are difficult to achieve. These factors may be critical and a systematic examination of them is warranted once the system is automated. Interestingly, most of the residual [^{18}F]fluoride left on the column after nucleophilic displacement reaction of the mannose triflate solution is complete can be eluted with dilute aqueous K_2CO_3 and is still useable in solution phase nucleophilic labeling reactions using Kryptofix 2.2.2 or tetraalkylammonium catalysts. This behavior suggests that the nonreacting portion of the resin bound [^{18}F]fluoride is either physically inaccessible within the polymer matrix to precursor during heterogeneous labeling conditions or it is bound in some sort of inert chemical complex that can be disrupted by aqueous base/catalyst treatment to release reactive [^{18}F]fluoride ion again. Generally, the radioisotope which is not initially retained by the resin during the flow of the target water through the column is still suitable for other radiochemical syntheses and is used as source of [^{18}F]fluoride ion for other applications, including collection on a second resin column.

As with many [^{18}F]fluoride ion reactions described previously, the success of this resin-based approach is dependent on starting the synthesis with [^{18}F]fluoride ion of reasonable quality. Although our current all-silver target may reduce or eliminate problems with metal ion interferences (similar to experiences of others: Tewson *et al.*, 1988; Berridge and Kjellstrom, 1989), after extended use particulate residues and impurities derived from radiolytic attack on plastic fittings and tubing accumulate in the target system, the production declines (less [^{18}F]fluoride is recovered from target, Fig. 4A) and the [^{18}F]FDG synthesis suffers from reduced total yields. However, no clear correlations between declines in total extracted target activity and either resin trapping efficiency (Fig. 4B) or overall yield of FDG, expressed as a percentage of the total extracted target activity (Fig. 4D) are apparent from the clinical synthesis data. Simple periodic cleaning of the target and transfer lines to remove residues, or in our case replacement with an alternate identical target (for radiation safety reasons, to allow radionuclide decay before cleaning) restores both [^{18}F]fluoride ion and [^{18}F]FDG production levels (this has been performed twice in the course of this work).

Due to the small overall size of the resin column and heating block, the assembly fits easily into a shielded ionization chamber. The processes of [^{18}F]fluoride ion trapping and nucleophilic radiolabeling are therefore continuously monitored and quantified during the synthesis. Reagents are transferred into the apparatus via 0.86 mm i.d. Teflon tubing from outside of the hot cell. All radioactive solution transfers within the apparatus are done by means of helium pressure, remote syringes, or vacuum, with no operator handling necessary except to remove the final product vial: these factors keep the radiation exposure to personnel at a minimum. The amount of training required and effort involved in operating this system also are minimal. Although we have not as yet automated this procedure it is clearly adaptable to either a dedicated apparatus (Padgett *et al.*, 1989; Alexoff *et al.*, 1989; Korpela *et al.*, 1989) or a robotics (Brodack, 1988) approach. The time required to perform a complete synthesis was 55 min (average), *including* a 15 min target irradiation. This short synthesis time for an [^{18}F]FDG dose was found to be very useful in short-notice situations such as with cardiac patients. This synthetic method is suitable either for production of large daily batches of [^{18}F]FDG (the overall percent yields remain essentially constant over a 10–30 min irradiation range) or preparation on demand. Most importantly, we have experienced a failure rate of less than 1% in the delivery of [^{18}F]FDG doses for human studies.

The use of a resin column for the nucleophilic [^{18}F]fluorination step eliminates some problems which have arisen in other reactions with resolubilized [^{18}F]fluoride ion. Solubilities of counterions (Kryptofix/K^+ tetraalkylammonium salts, rubidium or cesium) need not be considered. Concerns over loss of [^{18}F]fluoride on reaction vessel walls which have led, in the past, to the use of such materials as platinum (Brodack *et al.*, 1986), siliconized glass (Brodack *et al.*, 1986), pyrolytic carbon (Hamacher *et al.*, 1986), Pyrex and polycarbonate (Lemaire *et al.*, 1987) are also not important: all steps are conducted in the resin held in Teflon tubes, and there is no evidence of "sticking" of [^{18}F]fluoride to these surfaces (we have, alternatively, used stainless steel columns, also without problems of [^{18}F]fluoride ion sticking to them). Finally, the use of a polymeric reagent eliminates the need to remove phase transfer reagents (tetraalkylammonium salts), cryptands (Kryptofix 2.2.2) or crown ethers (18-crown-6), an important consideration given that such reagents are usually toxic chemicals.

Summary

The synthesis of [^{18}F]FDG has been simplified by the application of a solid phase [^{18}F]fluorination reagent. Collection of [^{18}F]fluoride ion from aqueous solution, drying, and nucleophilic reaction with 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose are all rapidly performed on a single quaternary 4-aminopyridinium substituted polystyrene resin. This new method allows for the collection and recycling of [^{18}O]water, and complete synthesis of [^{18}F]FDG in 40 min.

Acknowledgements—This work was supported by Grants from the National Institutes of Health (NS 15655) and the Department of Energy (DE-FG02-87ER60561).

References

Alexoff, D. L.; Russell, J. A.; Shiue, C-Y.; Wolf, A. P.; Fowler, J. S.; MacGregor, R. R. Modular automation in PET tracer manufacturing: application of an autosynthesizer to the production of 2-deoxy-[^{18}F]fluoro-2-D-glucose. *Appl. Radiat. Isot.* 37: 1045; 1987.

Berridge, M. S.; Kjellstrom, R. Fluorine-18 production: new designs for O-18 water targets. *J. Labeled Compd. Radiopharm.* 26: 188; 1989.

Brodack, J. W.; Dence, C. S.; Kilbourn, M. R.; Welch, M. J. Robotic production of 2-deoxy-2-[^{18}F]fluoro-D-glucose: a routine method of synthesis using tetrabutylammonium [^{18}F]fluoride. *Appl. Radiat. Isot.* 39: 699; 1988.

Brodack, J. W.; Kilbourn, M. R.; Welch, M. J.; Katzenellenbogen, J. A. NCA 16α-[^{18}F]fluoroestradiol-17β: the effect of reaction vessel on fluorine-18 resolubilization, product yield, and effective specific activity. *Appl. Radiat. Isot.* 37: 217; 1986.

Coenen, H. H.; Pike, V. W.; Stocklin, G.; Wagner, R. Recommendations for a practical production of [2-^{18}F]-fluoro-2-deoxy-D-glucose. *Appl. Radiat. Isot.* 38: 605; 1987.

Fowler, J. S.; Wolf, A. P. 2-Deoxy-[^{18}F]fluoro-D-glucose for metabolic studies: Current status. *Appl. Radiat. Isot.* 37: 663; 1986.

Hamacher, K; Coenen, H. H.; Stocklin, G. Efficient stereospecific synthesis of NCA 2-[^{18}F]fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. *J. Nucl. Med.* 27: 235; 1986.

Jewett, D. M.; Toorongian, S. A., Mulholland, G. K.; Watkins, G. L.; Kilbourn, M. R. Multiphase extractions: rapid phase-transfer of [^{18}F]fluoride for nucleophilic radiolabeling reactions. *Appl. Radiat. Isot.* 39: 1109; 1988.

Korpela, H.; Autio, T.; Tikkinen, J.; Kamarainen, E. L. Automated computer controlled synthesis of 2-^{18}F-FDG. *J. Labeled Compd. Radiopharm.* 26: 467; 1989.

Lemaire, C., Guillame, M.; Christaens, L.; Palmer, A.; Cantineau, R. A new route to [^{18}F]fluoroaromatic substituted amino acids: no carrier added L-p-[^{18}F]fluorophenylalanine. *Appl. Radiat. Isot.* 38: 1033; 1987.

Mulholland, G. K.; Hichwa, R. D.; Kilbourn, M. R.; Moskwa, J. A reliable pressurized water target for F-18 production at high beam currents. *J. Labeled Compd. Radiopharm.* 26: 140; 1989a.

Mulholland, G. K.; Mangner, T. J.; Jewett, D. M.; Kilbourn, M. R. Polymer-supported nucleophilic radiolabeling reactions with [^{18}F]fluorine and [^{11}C]cyanide ion on quaternary ammonium resins. *J. Labeled Compd. Radiopharm.* 26: 378; 1989b.

Padgett, H. C.; Schmidt, D. G.; Luxen, A.; Bida, G. T.; Satyamurthy, N.; Barrio, J. R. Computer-controlled radiochemical synthesis: a chemistry process control unit for the automated production of radiochemicals. *Appl. Radiat. Isot.* 40: 433; 1989.

Schlyer, D. J.; Bastos, M.; Wolf, A. P. A rapid quantitative separation of fluorine-18 fluoride from oxygen-18 water. *J. Nucl. Med.* 28: 764; 1987.

Tewson, T. J.; Berridge, M. S.; Bolomey, L.; Gould, K. L. Routine production of reactive fluorine-18 fluoride salts from an oxygen-18 water target. *Nucl. Med. Biol.* 15: 499; 1988.

Yoshioka, T. The preparation and fundamental characteristics of polystyrene-based ion exchange fiber. *Bull. Chem. Soc. Japan* 3726; 1983.