# COMPUTER AIDED SYNTHESIS (CAS) OF NO-CARRIER-ADDED 2-[$^{18}$F]FLUORO-2-DEOXY-D-GLUCOSE: AN EFFICIENT AUTOMATED SYSTEM FOR THE AMINOPOLYETHER SUPPORTED NUCLEOPHILIC FLUORINATION

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A microcomputer controlled automated synthesis of 2-[$^{18}$F]fluoro-2-deoxy-D-glucose is described. The modular designed set-up of the apparatus permits reliable and facile routine synthesis of fluorine-18 labelled radiopharmaceuticals based on the aminopolyether mediated nucleophilic fluorination. The uncorrected radiochemical yield is in the range of 40....55%. Batches up to 600 mCi of 2-$^{18}$FDG are prepared from 1,1 Ci[$^{18}$F] fluoride in less than 1 hour. The apparatus can also be used for other APE supported nucleophilic fluorination procedures.

## INTRODUCTION

2-[$^{18}$F]fluoro-2-deoxy-D-glucose is currently one of the most important radiopharmaceuticals and is being used for the measurement of glucose metabolism using Positron Emission Tomography (Reivich et al. 1979; Phelps et al. 1979). Since the first synthesis of 2-$^{18}$FDG in 1978 (Ido et al.) many synthesis have been developed using both electrophilic and nucleophilic fluorination. The disadvantages of electrophilic fluorination, e.g. limited radiochemical yield of 50% and formation of both epimerical forms led to extensive efforts to prepare 2-[$^{18}$F]FDG via nucleophilic substitution reactions (Levy et al. 1982; Tewson T.J. 1983). Presently, the most efficient method is based on the stereospecific aminopolyether (APE) mediated nucleophilic fluorination employing 1.3.4.6-tetra-0-acetyl-2-0-trifluoromethanesulfonyl-$\beta$-D-mannopyranose as a precursor (Hamacher et at. 1986). The increasing demand for routine production of 2-$^{18}$FDG accelerated developments in the design and construction of remote or automated procedures as well as laboratory robotic systems to synthesize this important radiopharmaceutical (Barrio et al. 1981; Fowler et al. 1981; Beeley et al. 1984; Iwata et al. 1984; Alexoff et al. 1986; DeJesus et al. 1986; Diksic et al. 1986; Brodack et al. 1988a; Brodack et al 1988b). Based on the convenient method of APE-mediated nucleophilic fluorination (Coenen et al. 1985) we now describe a modular apparatus which also includes a module for rapid separation of [$^{18}$F] fluoride and $H_2{}^{18}O$ (Schlyer et al. 1987). This apparatus can also be used for other nucleophilic fluorination procedures.

## MATERIALS AND METHODS

### Apparatus Design

The apparatus described was designed using a modular concept (Blessing G. 1987) with the general purpose to provide a modular draft which would allow the use of the system for radiosyntheses of FDG as well as other tracers with little or no modification, especially via aminopolyether supported nucleophilic fluorination.

The synthesis system consists of five connected modules, namely:

1. Fluoride separation unit
2. Single unit reactor
3. Purification unit
4. Filling unit
5. Computer control unit

A schematic set-up of the automated system and the connection of the modules 1 to 4 is shown in

Figure 1. The ensemble of Synthesis Unit Operations (SUO's) which are discussed in detail below are combined on the demands of shortest connections and smallest dead volumes. The pipes are made of teflon and the valves used are teflon membrane valves from: ANGAR SCIENTIFIC, type 368 or 190, 24 VDC.

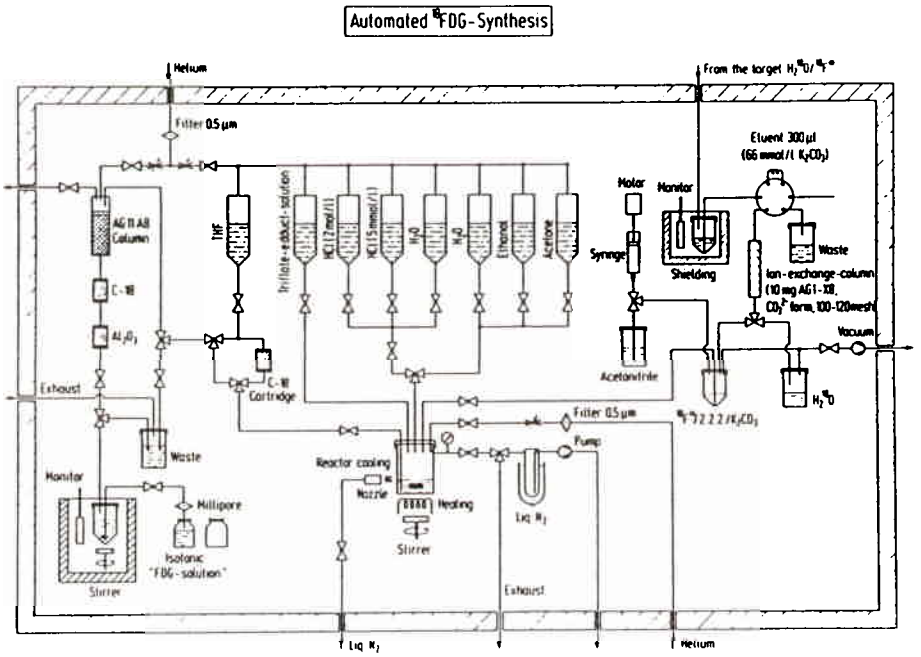


Figure 1 Schematic set-up of the automated system for $^{18}$FDG-synthesis, including the $^{18}F/H_2{}^{18}O$ separator unit. The adjacent solvent reservoirs containing water, ethanol and acetone are only used for the automated cleansing of the apparatus.

## Fluoride Separation Unit

The Fluoride Separation Unit is connected on-line with the $H_2{}^{18}O$ target via a polypropylene tube. Before the fluoride-18 and the O-18 water are separated, the water phase (2...2.5 ml) is collected in a degasing vessel and the total activity monitored by a System 414 Monitor (Genrich V. 1988).

The vessel is connected with a motor driven three-way valve LATEK HMV-P (LATEK, Heidelberg, FRG) that is combined with a small ion-exchange column (20x2 mm) and a loop containing potassium carbonate solution. The end of the column is formed as a capillary with a diameter smaller than the medium size of the ion exchange particles, so that the resin is held back without using a micro filter inside the column. The outlet-valve at the column is connected with two vials: vial a) to collect the $H_2{}^{18}O$ after fixation of the fluoride and vial b) to mix the fluoride-18 containing potassium carbonate solution with the appropriate amount of Kryptofix$^R$ 222 solubilized in acetonitrile by using a motor driven syringe. The transport of the solution through the resin is accelerated by decreasing the pressure to about 20 mbar at the end of the column using a vacuum pump.

## Single Unit Reactor

The central part of the SUO is a single unit reactor (Figure 2), for a one-pot reaction system that contains a single heated reaction vessel combined with a rectangular arrangement of teflon membrane valves and up to nine reagent reservoirs above the reactor. (For the FDG synthesis only 5 reservoirs are

used). The closed system allows reactions at isothermal and isometric conditions in the range of ~0 to 2 bar due to the teflon membrane valves. The cylindrical reaction vessel made of glassy carbon (Sigradur$^R$), 17 mm in diameter, 1 mm wall thickness and approximately 70 mm in height is surrounded by a copper cylinder with an integrated electric filament (thermocoax$^R$). The advantage of glassy carbon in comparison with glass is its extreme chemical resistance, low adsorption properties and a more efficient heat conduction (see Coenen et al. 1985). The top of the glassy carbon vessel was closed by an inconel cover which is connected by a thread with the cylinder. The small space between the glassy carbon and the copper cylinder was filled with about 1 ml silicon oil. Accordingly, the heat capacity is relatively low. The cylinder is surrounded by a steel coat with an inlet for cooling gas or liquid nitrogen at the bottom. (The injection of liquid nitrogen allows a rapid cooling of the reaction vessel and a temperature decrease from 100 to 20°C in about 30 s).

Purification Unit

The purification unit consists of a special glass column containing the AG11-A8 resin with a built-in liquid level sensor (Honeywell, Düsseldorf) and an eluent reservoir at the top of the resin. Two selenoid valves at the top and the end of the purification column are switched automatically depending on the signal of the liquid level sensor.

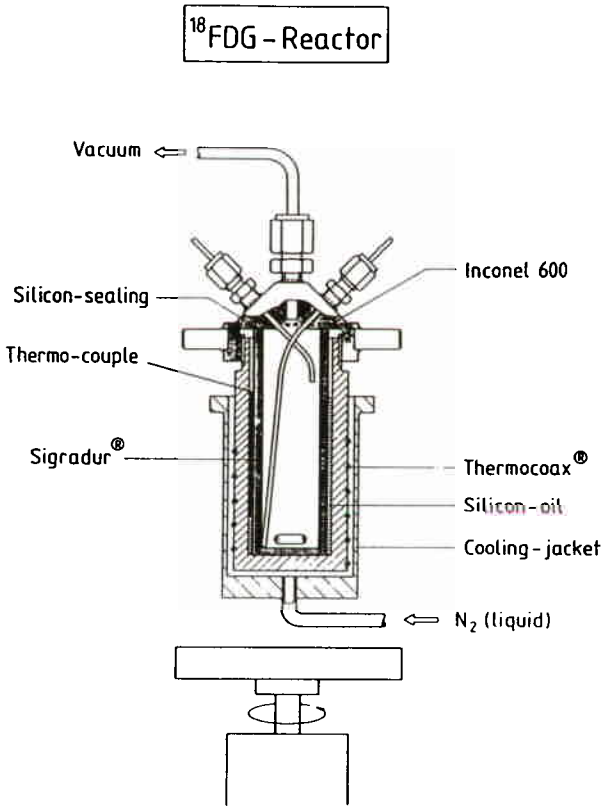

Figure 2 Sectional view of the single unit reactor with a cylindrical reaction vessel (17x70 mm) made of glassy carbon (Sigradur$^R$).

Filling Unit

The total amount of the 2-[$^{18}$F]FDG produced is collected in a glass vessel with a lead shielding and measured by the System 414 Monitor in order to get the radioactivity concentration in mCi per ml via the computer. After sterile filtration through a Millipore filter (0.22 $\mu$m) the isotonic FDG solution is portioned into evacuated ampoules via pneumatical piloting of a sterile tube cannula. The filling unit is installed below the synthesis device in a separate small lead shielded box (40x40 cm).

Computer Control Unit

All processes in the synthesis of 2-[$^{18}$F]FDG are electronically controlled by an IBM PC/AT clone equipped with standard peripherals. The microcomputer was allowed to communicate with an OPTOMUX$^R$ network via a directly plugged in RS 422/485 adapter card which provides up to 4000 V isolation between the PC and the communication link. For standard input/output signals, three OPTOMUX digital and one analog brain board with up to 16 I/O channels are used. We have programmed 6 lines to read status information from the system, such as temperature, pressure, the level of liquid in the purification unit, and the actual scale of the three radioactivity detectors. The temperature is controlled by a 12-bit digital-to-analog converter. A total number of 28 selenoid valves can be operated directly. In the OPTOMUX$^R$ unit an optical coupling is used to prevent any electrical feed back of the valves as they can induce high voltages when they are switched. As a programming language we have chosen TURBO-PASCAL$^R$. The run-down in a time-command sequence in the program and a feed back of intensity factors such as temperature and pressure control the procedure of the synthesis. The actual process carried out is described in a menu. Interruption and continuation of the automated process at any time is possible. A report, comprising the date, starting and ending time of the synthesis, and the calculation of the radiochemical yield of 2-[$^{18}$F]FDG, is automatically provided.

Solvents and Reagents

All organic solvents were of high purity grade and were obtained from Merck (Darmstadt, F.R.G.). Acetonitrile which was used as solvent for the fluorination was of very high purity which is suitable for DNA synthesis. This special solvent from Merck with a water content of max. 0.003 % was used without further purification. 1.3.4.6-Tetra-0-acetyl-2-0-trifluoromethane-sulfonyl-$\beta$-D-mannopyranose was either produced ourselves as described (Hamacher, 1984) or obtained from Aldrich. The aminopolyether Kryptofix$^R$ 222 and potassium carbonate (Suprapur) were purchased from Merck. Water used for the fluoride separation process was five times distilled in a quartz glass apparatus.

## PROCESS SEQUENCES

Separation of [$^{18}$F]Fluoride

At the end of bombardment, the fluorine-18 target water was remotely transferred via He-pressure from the target to the fluoride separation unit through a polypropylene tube of 0.8 mm diameter. In our case, the pipe length was about 40 m. The $H_2{}^{18}O$ transport into the degassing vessel required ~5 min and the loss of radioactivity ($^{18}F^-$) due to adsorption on the surface was < 5%.

According to Scheme 1, the $H_2{}^{18}O$ was passed through a column containing 10 mg of anion exchange resin AG 1x8, 100....200 mesh, in the carbonate form. The flow through the resin was 0.5...0.6

ml/min which was achieved by decreased pressure. $H_2{}^{18}O$ was collected, purified via distillation, and reused for $^{18}F$ production. The desorption of $^{18}F^-$ was performed by eluting the resin with a solution containing 3mg potassium carbonate in 0.3 ml of pentadistilled water. The desorption of fluoride took about 30 s. The eluted $^{18}F$/carbonate was mixed with a solution of 20 mg Kryptofix$^R$ 222 (MERCK) in 1.5 ml acetonitrile and transferred to the reaction vessel. The time necessary for the preparation of the fluoride cryptate solution was 5 min; it was 10 min if transport from the water target was included.

Reactive Cryptate Fluoride System

The acetonitrile-water solubilized cryptate of fluoride-18 and potassium carbonate coming from the fluoride separation unit was evaporated to dryness in a two step sequence. To prevent splashing of the solution during evaporation the reaction vessel was heated only to 50°C under a stream of He or Ar. The pressure inside the vessel was in the range of 600 mbar. After drying for two and a half minutes most of the solvent was removed. The system was heated to about 105°C during 1 min. and the gas stream interrupted. The reaction vessel was evacuated and the drying process completed in additional 4 min.

Nucleophilic Fluorination and Hydrolysis

A solution of 20 mg (0.04 mmol) 1.3.4.6-tetra-0-acetyl-2-0-trifluoromethane-sulfonyl-$\beta$-D-mannopyranose in 1 ml dry acetonitrile was added to the dry cryptate and the resulting solution kept at 85°C for 5 min. The solution was concentrated to dryness under diminished pressure and the residue extracted two times with 5 ml of 5 mmolar hydrochloric acid. The acid water phase in which the tetracetylated 2-$^{18}$FDG was suspended was passed through an activated (THF,$H_2O$) Sep-Pak C-18 cartridge. The water solution containing cryptate and residual [$^{18}$F]fluoride was discarded and the fluorinated product adsorbed on the reverse phase cartridge was eluted with 3 ml tetrahydrofurane and transferred back into the reaction vessel.

After evaporation to dryness, 2 ml 2 molar hydrochloric acid was added and the mixture heated at about 120°C for 15 min. under isometric conditions. The pressure inside the vessel was in the range of 1.9...2.0 bar during hydrolysis.

Purification of FDG-Solution

The hydrolysate was transferred via pressure on to an ion-retardation column (AG11-A8, 7x200 mm), the reaction vessel was washed with 12 ml of water and the solution transferred through a bypass to the ion retardation resin. It was necessary to wash the commercial resin with 100 to 200 ml of distilled water to prevent the contamination of the FDG solution with unwanted impurities attendant to the original retardation resin. The water phase passing through the column was subsequently led through a SEP-PAK C-18 and alumina SEP-PAK into a mixing vessel containing 1.2 ml sodium chloride solution (100 g NaCl/l). The radioactivity of the isotonic FDG solution was monitored and finally sterilized by passage through a Millipore filter (0,22 $\mu$m). The FDG solution was successively filled into different sterilized and evacuated ampoules. The filling unit can be operated by remote control, and the ampoules are filled with a well-defined amount of $^{18}$FDG as required by the customer.

## RESULTS

The uncorrected radiochemical yield of 2-$^{18}$FDG obtained with the automated device is 40...55 % which is comparable to the yields obtained by manual preparation. The $^{18}$F-labelled glucose can be produced routinely in amounts of more than 550 mCi, sufficient to supply five PET centres with adequate quantities of $^{18}$FDG. The radiochemical purity is 99 % (HPLC- and/or TLC analysis). The radiochemical impurities (< 1 %) are presumably four different partially acetylated FDG's of unknown structure.

According to our original design [$^{18}$F]fluoride containing $^{18}$O-enriched target water was evaporated

in the presence of the cryptate $(222/K)_2CO_3$. However, significantly lower radiochemical yields were found, perhaps due to traces of impurities such as metal ions or oxoanions. To overcome this problem of reduced reactivity of fluoride-18, the radioisotope was separated via anion exchange resin. The fluoride separation unit was similar to that described by Schlyer et al. (1987), but rather than the OH-form we used the less basic carbonate form which allows an effective fixation and desorption of [$^{18}$F]-fluoride using the appropriate amount of potassium carbonate solution. The recovery of [$^{18}$F]fluoride was > 95 %. We used pentadistilled water to prepare the potassium carbonate solution to prevent a recontamination with impurities which would reduce the [$^{18}$F]fluoride reactivity.

The routine production of 2-$^{18}$FDG has been accomplished more than 100 times with excellent reliability. The integrated automatic cleansing programme at the end of synthesis makes it possible to reuse the apparatus for the next synthesis within one hour after end of the first synthesis. Nevertheless, it is necessary after each third synthesis to open the reactor and to clean the glassy carbon vessel with a soft tissue. In the course of several syntheses, polymeric side products accumulate which are not soluble in the solvents used for the purification sequence such as boiling water, ethanol and acetone. Beside the manual purification step, the only manual procedures which have to be done before running a new synthesis are to fill up the reservoirs and to change the column and the SEP-PAK cartridges.

Although some minor problems exist with respect to the decreasing reliability of some membrane valves after a number of forty syntheses, the automated system is a convenient apparatus for the routine production of high amounts of the most widely used radiopharmaceutical [$^{18}$F]FDG. The same device without any apparative modification has been used for the routine production of 2-$^{18}$FDM using the epimeric form of the FDG precursor (Hamacher et al. in preparation).

## DISCUSSION

The routine production of 2-[$^{18}$F]FDG and some other radiopharmaceuticals has been a goal of numerous PET investigators (see Brodack et al. 1988b and references herein). Besides the development of remote or automated apparatus, some laboratory robotics are applied for the synthesis of carbon-11 and fluorine-18 labelled radiotracers. The main argument for users of robotic systems is that it would be possible to circumvent problems concerning a flexible synthesis of different radiotracers (Brodack et al. 1988). This argument is certainly true if the chemistry is basically different such as electrophilic or nucleophilic fluorinations. However, the automated system described here has been designed in a modular set-up to perform the routine synthesis of various $^{18}$F-containing radiopharmaceuticals via aminopolyether supported nucleophilic fluorination. This sophisticated single unit reactor system which can also be operated remotely is used for the routine production of different relevant PET-tracers e.g. [$^{18}$F]FDM, [$^{18}$F]FESP and [$^{18}$F]-N-Methylspiperone. In the case of $^{18}$F-labelled spiperone, the single unit reactor is easily connected on-line to a device for HPLC purification of the radiotracer (Hamacher et al. in preparation).

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