

Epimerization study on [^{18}F]FDG produced by an alkaline hydrolysis on solid support under stringent conditions.

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Abstract

Since 1998, routine [^{18}F]FDG syntheses are carried out by alkaline hydrolysis on solid support i.e. the labeled intermediate is trapped on a tC18 SPE cartridge, purified and finally hydrolyzed within the cartridge, at room temperature, using sodium hydroxide. The present study demonstrated that no epimerization of [^{18}F]FDG to [^{18}F]FDM occurs even when 12 N NaOH is used and when the hydrolysis is extended up to one hour. The alkaline hydrolysis on solid support appears as a simple method leading to [^{18}F]FDG with high purity.

Key Words: Fluoride, [^{18}F]FDG, [^{18}F]FDM, alkaline, hydrolysis, SPE, epimerization

Introduction

The increasing demand of 2-deoxy-2- ^{18}F fluoro-D-glucose ([^{18}F]FDG), for clinical PET investigations requires this compound to be producible in large amounts, with a high purity and with reliable results. The most frequently applied method to produce [^{18}F]FDG is based on a nucleophilic substitution of the triflate leaving group from the precursor, 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulphonyl- β -D-mannopyranose (mannose triflate) in presence of 4,7,13,16,21,24-hexaoxa-10-diazabicyclo(8.8.8)-hexacosane (Kryptofix 2.2.2) as phase-transfer agent (Hamacher, Coenen et al., 1986). After labeling, the removal of the acetyl protecting groups from the resulting 2- ^{18}F fluoro-1,3,4,6-tetra-O-acetyl-D-glucose ([^{18}F]FTAG) can be performed by an acid hydrolysis (Hamacher, Coenen et al., 1986. Mulholland, 1995) or an alkaline hydrolysis (Füchtner et al., 1996). During the last few years, we have improved the alkaline method by performing the hydrolysis on a commercially available low polarity solid phase extraction (SPE) cartridge (Lemaire et al., 1997. Mosdzianowski et al., 1999. Lemaire et al., submitted for publication). The alkaline hydrolysis on solid support presents several advantages: preliminary purification and hydrolysis are performed on the same solid support, no second evaporation is required for solvent removal, the hydrolysis is performed in a few minutes at room temperature and thus the synthesis time is reduced. The simplicity of the method facilitates the automation of the process and allows to carry out the synthesis within a disposable kit assembly made of standard commercially available single-use components.

For the alkaline hydrolysis in liquid phase at room temperature, it has been reported that the yield is maximum with a 0.3 M NaOH solution. Beyond this concentration [^{18}F]F⁻ is formed by defluorination of acetylated [^{18}F]FDG (Füchtner et al., 1996). When the hydrolysis is achieved on a low polarity cartridge at room temperature, the yield is optimal with 2 M NaOH (>98 %) and no significant defluorination occurs (Lemaire et al., submitted for publication).

Concern in a possible epimerization of [^{18}F]FDG to 2- ^{18}F fluoro-2 deoxy-D-mannose ([^{18}F]FDM) at the C-2 position according to the "Lobry de Bruyn-van Eckenstein"-rearrangement under alkaline conditions has risen since a method has been published, showing how to produce [^{18}F]FDM by epimerization of [^{18}F]FDG under basic conditions at high temperature (Varelis et al., 1996). A multi-center study has been performed recently to check the safety of the alkaline hydrolysis for routine productions of [^{18}F]FDG in view of the stability of [^{18}F]FDG towards epimerization (Meyer et al., 1999). This study revealed that the epimerization of [^{18}F]FDG to the corresponding mannose derivative [^{18}F]FDM is reliably limited to 0.5%, when the alkaline hydrolysis in liquid phase is carried out under mild conditions, at room temperature. According to this publication, the main reaction parameters, which may induce an epimerization of [^{18}F]FDG, are the base strength, the reaction time and the temperature.

The aim of the present study was to quantify [^{18}F]FDM in [^{18}F]FDG solutions produced by alkaline hydrolysis on a low polarity SPE cartridge under stringent hydrolysis conditions such as highly concentrated sodium hydroxide (up to 12 N), extended hydrolysis duration (up to one hour) and also to evaluate the effect of temperature.

Methods

[^{18}F] F^- was produced by proton bombardment of 94% H_2^{18}O (from Cortec) in a 2 mL silver target fitted with a 50 μm Havar window, using an IB A 18/9 Cyclotron.

The [^{18}F]FDG-syntheses with alkaline hydrolysis on a low polarity cartridge were carried using the FDG-Synthesizer from Coincidence Technologies. The labeling of the mannose triflate precursor (from ABX) was performed according to Hamacher and Coenen (1986) using the aminopolyether potassium complex ($[\text{K}/2.2.2]^+^{18}\text{F}^-$) as phase transfer catalyst (Kryptofix K222 and potassium carbonate purchased from Merck). The resulting [^{18}F]FTAG diluted in 3 mL of acetonitrile (J.T. Baker) was mixed with 26 mL of water and passed through a Sep-Pak Plus tC18 cartridges (Waters), preconditioned with 3 mL of ethanol (Riedel Dehaën) and 22 mL of water. Thus the [^{18}F]FTAG was trapped on the cartridge. Afterwards, the cartridge was flushed with water (43 mL) to eliminate the acetonitrile, Kryptofix 2.2.2., unreacted [^{18}F] ions and other byproducts. After this preliminary purification, 800 μL of a 2N NaOH solution (Merck) were injected into the cartridge. The [^{18}F]FTAG was hydrolyzed within the cartridge in 2 min at room temperature. The resulting [^{18}F]FDG was collected in 10 mL of sterile water, 5 mL of sterile citrate buffer solution and 1 mL of 2N hydrochloric acid (Merck). The isotonic neutralized [^{18}F]FDG solution was purified by passing through a Sep-Pak Plus tC18 (previously conditioned with ethanol and water) and a Sep-Pak Plus Alumina N cartridge (Waters). Finally the solution was filtered (Cathivex GS 0.22 μm , Millipore). The synthesis was carried out within a disposable kit made of standard components. The synthesis duration was about 25 minutes. The hydrolysis conditions were modified in order to study a possible epimerization of [^{18}F]FDG under various conditions.

The HPLC analysis was performed using a Dionex Carbo Pac PA10 Guard 4-mm (10-32) P/N 46115 precolumn and a Dionex Carbo Pac PA10 4-mm (10-32) P/N 46110 column (from Dionex). The system is equipped with a Waters 626 pump, its 600S Controller, a PAD (Pulsed Amperometric Detector) detector ED40 (from Dionex) and a NaI(Tl) scintillation detector with associated electronic (Eberline). Before analysis, the columns were equilibrated with 200 mM NaOH at a flow rate of 1 mL/min during 15 minutes until a stable base line was obtained. The mobile was 55 mM NaOH protected against contamination by carbon dioxide and the flow rate was 1 mL/min. The columns were maintained at a temperature between 20 and 30°C. Following parameters were used for the PAD detector: $E_1 = +0.1\text{ V}$ ($t_1 = 400\text{ms}$); $E_2 = -2\text{ V}$ ($t_2 = 20\text{ms}$); $E_3 = +0.6\text{ V}$ ($t_3 = 10\text{ms}$); $E_4 = -0.1\text{ V}$ ($t_4 = 70\text{ms}$). In order to identify the product, non-radioactive FDG and FDM were added to the [^{18}F]FDG solution. Standards for FDG and FDM were obtained respectively from Aldrich Chemicals and Toronto Research Chemicals. It has been reported that no [^{18}F]FDM is produced during the HPLC analysis on the column using 75 mM or 220 mM sodium hydroxide as mobile phase (Lemaire et al., submitted for publication).

Results

The FDG-Synthesizer performing the alkaline hydrolysis on a Sep-Pak Plus tC18 cartridges was used for its routine productions at the Cyclotron Research Center from the University of Liège since September 1998. The yields were found very reliable and reproducible (average yield 61% uncorrected for decay and 72% corrected for decay) (Mosdzianowski et al., 1999). Until now, more than 600 routine [^{18}F]FDG productions have been carried out including an HPLC analysis to determine the radiochemical purity. No [^{18}F]FDM was detected in any of these [^{18}F]FDG solutions. The only radioactive peak in the radio-HPLC chromatogram was identified as being [^{18}F]FDG, by adding cold FDM and FDG standards. The identity was also confirmed by adding some radioactive [^{18}F]FDM, synthesized as described in another paper (Lemaire et al., submitted for publication). In this case, two separate radioactive peaks appeared in the radio-HPLC chromatogram, one peak corresponding to the added [^{18}F]FDM and one peak corresponding to the [^{18}F]FDG.

The routine synthesis conditions were modified in respect of sodium hydroxide concentration, hydrolysis duration and reaction temperature in order to study a possible epimerization under more stringent conditions.

In the first set of experiments the hydrolyses were carried out using 2N, 8N and 12N sodium hydroxide. The amount of hydrochloric acid, citrate buffer and water for dilution were modified in order to ensure a proper neutralization of the resulting alkaline [^{18}F]FDG solution in such a way that the dilution factor of the analyzed sample was kept the same. No [^{18}F]FDM was produced even when the hydrolysis was performed in presence of the most concentrated sodium hydroxide solution. These results are summarized in table 1.

The figure 1 shows the radio-HPLC chromatogram and the corresponding PAD chromatogram obtained when the [^{18}F]FDG was produced by a hydrolysis on solid support using a 12 N NaOH solution. In order to identify the product, the [^{18}F]FDG-solution was diluted twenty times with water, 5 μL of this diluted solution was mixed up with 25 μL of a solution containing 1mg/mL of cold FDG and 25 μL of a solution containing 1mg/mL of cold FDM. 20 μL of the resulting solution were injected into the UPLC system. The two major peaks in the PAD chromatogram were identified to be the peaks corresponding to the cold FDM standard (retention time = 12.2 minutes) and the cold FDG standard (retention time = 13.8 minutes). The comparison between the

electrochemical and the radio-HPLC chromatogram shows that the only peak in the radio-HPLC chromatogram (retention time = 14 minutes) was the [^{18}F]FDG peak and that the solution was radiochemically pure. The shift of the retention times between the electrochemical chromatogram and the radio-HPLC chromatogram were due to the dead volume between both detectors (40 cm of tubing with an inner diameter of 0.03").

In the second set of experiments the duration of the hydrolysis was extended to 10 minutes and to one hour, instead of 2 minutes (using 2N sodium hydroxide). Finally, the hydrolysis was carried out using an extended duration of one hour and using a 12 N sodium hydroxide solution. No [^{18}F]FDM was produced under these conditions. These results are summarized in table 1.

In the third set of experiments, the 2N sodium hydroxide solution as well as the tC18 cartridge were heated up to 60 °C using a hot air gun. When the hydrolysis was carried out in 2 minutes, 0.74 % of [^{18}F]FDM were detected and with a 15 minutes hydrolysis the amount of [^{18}F]FDM was 7.22 %. These amounts of [^{18}F]FDM are below the limit of 10 % recommended by the European Pharmacopoeia. It has to be mentioned that clinical investigation revealed no significant diagnostic outcome with [^{18}F]FDM versus a [^{18}F]FDG (Wienhard et al., 1991).

Finally, a [^{18}F]FDG synthesis was carried out under routine hydrolysis conditions but without final neutralization: the 800 μL of [^{18}F]FDG in NaOH 2N were collected in 16 mL of water. The resulting pH was above 14. This solution was analyzed for [^{18}F]FDM 3 minutes, 60 minutes and 240 minutes after end of synthesis. No [^{18}F]FDM was detected, showing that [^{18}F]FDG remains stable in respect to [^{18}F]FDM epimerization in a 95 mM NaOH solution for at least 4 hours.

Table 1: Summary of hydrolysis conditions and detected amount of [^{18}F]FDM (RT = Room Temperature, ND = Not Detected)

Duration (min.)	NaOH (normality)	Temperature (°C)	[^{18}F]FDM (%)
2	2	RT	ND
2	8	RT	ND
2	12	RT	ND
10	2	RT	ND
60	2	RT	ND
60	12	RT	ND
2	2	60	0.74
15	2	60	7.22

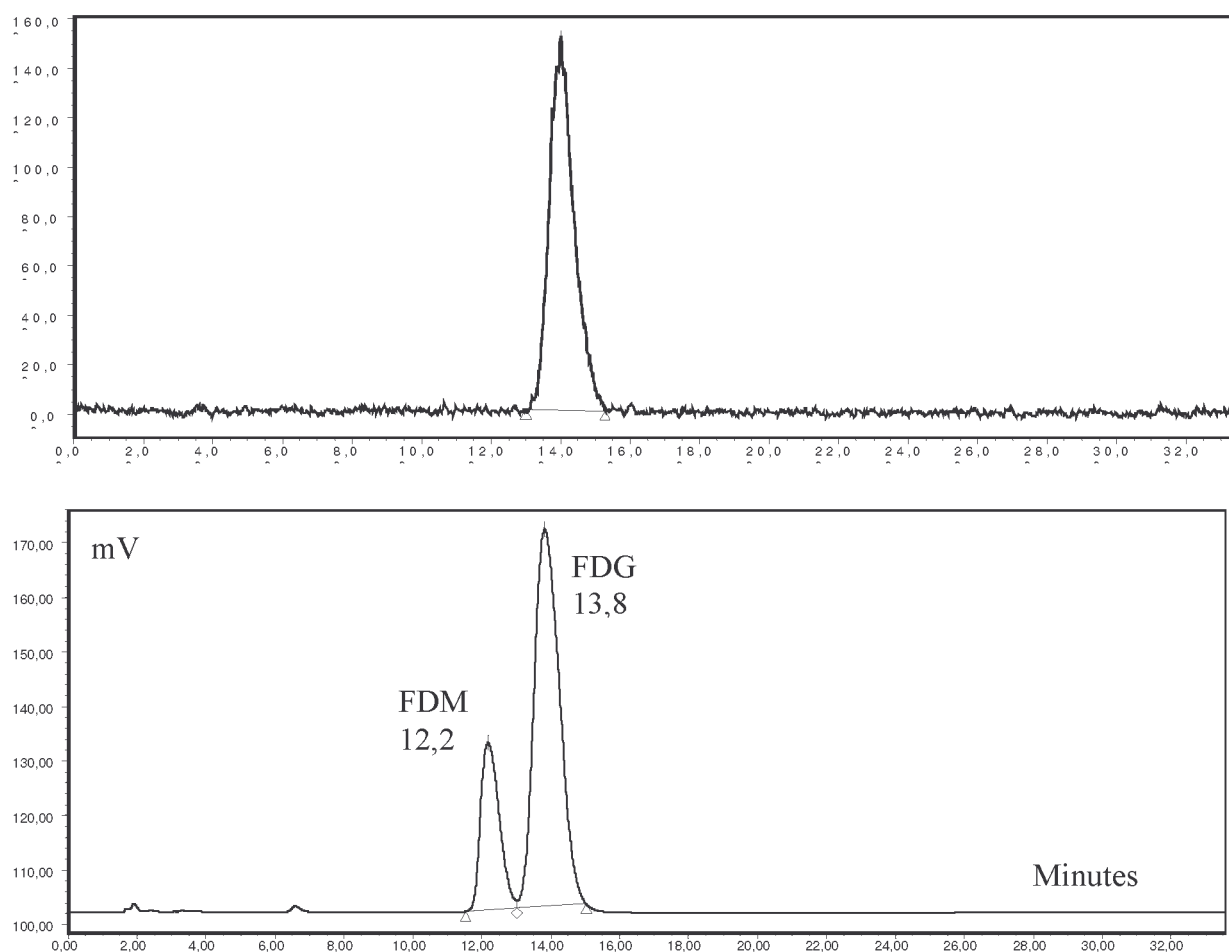
Conclusion

Since it has been showed that the hydrolysis of the labeled [^{18}F]FTAG intermediate can be achieved on a low polarity SPE cartridge under alkaline conditions, the [^{18}F]FDG synthesis was considerably simplified. This technique presents several advantages over classical hydrolysis methods: after trapping of the [^{18}F]FTAG onto the SPE cartridge, the solvents are removed without need for heating, the intermediate product is pre-purified and then hydrolyzed in 2 minutes at room temperature within the cartridge. This method was implemented in the FDG-Synthesizer from Coincidence Technologies.

The present study demonstrated that no epimerization of [^{18}F]FDG to the corresponding mannose derivative occurs when the hydrolysis on solid support is carried out under standard routine conditions (i.e. 2 minutes, room temperature and 2N sodium hydroxide). When the [^{18}F]FDG solution was not neutralized, no epimerization occurred for at least 4 hours after end of synthesis. Even when 12 N sodium hydroxide was used for the hydrolysis and when the reaction duration was extended up to one hour, no [^{18}F]FDM was detected in the final product. It was necessary to heat-up intentionally the SPE cartridge during hydrolysis using an external heating device to produce some [^{18}F]FDM. Among the 3 parameters that we investigated, namely sodium hydroxide concentration, duration and temperature, only temperature was likely to induce epimerization. In a conventional [^{18}F]FDG production process using alkaline hydrolysis in liquid phase, the nucleophilic substitution reaction is followed by an evaporation of the organic solvent in a heated vessel. Sodium hydroxide is then added to the dry residue and hydrolysis occurs. A risk of epimerization persists in case the heater does not cool down to sufficiently low temperature before hydrolysis. This risk is totally avoided by the alkaline hydrolysis on solid support since no heating device is used and the solvent is removed by rinsing the SPE cartridge with water. The

alkaline hydrolysis on solid support at room temperature appears to be an efficient method to reliably produce [^{18}F]FDG with high yield and high purity.

Fig 1: Radio-chromatogram and PAD-chromatogram corresponding to a [^{18}F]FDG solution produced with 12 N NaOH



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