Cryptand [2.2.2] Quantitation in the Synthesis of 2-[Fluorine-18]Fluoro-2-Deoxy-D-Glucose

P.J. Kothari, J. Ginos, R.D. Finn and **S.M. Larson**
Department of Medical Imaging
Memorial Sloan-Kettering Cancer Center
New York NY, U.S.A.

J.M. Link and **K.A. Krohn**
University of Washington Medical Center
Seattle WA, U.S.A.

K. Garmestani
The National Institutes of Health
Bethesda MD, U.S.A.

Abstract. Most automated synthetic devices for the preparation of [^{18}F]2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) employ cryptand [2.2.2] (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo(8.8.8)-hexacosane) to facilitate the ^{18}F displacement reaction [1]. Lack of simple spectroscopic and/or chromatographic determinations for cryptands prompted our investigation with tritiated [2.2.2] cryptand reagent [2] to determine the absolute concentration of this reagent throughout the synthetic procedure [1,3] leading to the final formulation. The concentration of cryptand [2.2.2] in the final formulation has been determined to range from 0.39 µg/ml to 0.60 µg/ml which is well below the detection limit by a method recently reported [3].

Introduction

Several methods to prepare ^{18}F labelled 2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) have been reported in literature [4]. Many of these methods employ cryptand [2.2.2] to facilitate a nucleophilic displacement reaction. However, the presence of cryptand [2.2.2] in the final formulation of [^{18}F]FDG has been of concern based upon toxicity [5]. The lack of simple sensitive spectroscopic and/or chromatographic methods to accurately determine the cryptand [2.2.2] concentration in the radiopharmaceutical formulation prompted this investigation. This report represents preliminary results from our investigation using ^{3}H labelled cryptand [2.2.2].

Materials and Methods

The1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose (TAM), cryptand [2.2.2], tetrahydrofuran (THF), anhydrous acetonitrile, Dowex 50-X-8 resin, Dowex ion retardation resin and anhydrous potassium carbonate were purchased from ALDRICH Chemicals Company, Milwaukee WI and used without additional purification. The C-18 and alumina Sep-pak cartridges and 0.22 micron Micropore filters were purchased from MILLIPORE Corporation, Bedford MA. ^{18}O enriched water was purchased from MOUND Laboratory, Miamisburg OH. Concentrated hydrochloric acid was purchased from FISHER Scientific Company, Fairlawn, NJ and was diluted with deionized water to achieve appropriate concentration. ^{3}H labelled cryptand [2.2.2] was prepared according to a procedure we have published [2]. The scintillant DUPONT Formula-963 was purchased from NEN RESEARCH Products, Inc., Boston MA.

Production of [^{18}F]Fluoride. The no-carrier-added [^{18}F]HF was produced via ^{18}O(p,n)^{18}F nuclear reaction from an ^{18}O enriched water target. The [^{18}F]HF was concentrated by distillation to approximately 200 µl with the [^{18}O]H_2O being recovered for future use.

^{18}F Labelled 2-Fluoro-2-deoxy-D-glucose Synthesis (Fig. 1). To the vessel containing [^{18}F]HF, a solution containing 4.6 mg potassium carbonate, 7 mg [^{1}H]cryptand [2.2.2] and 7 mg [^{3}H]cryptand [2.2.2] in 88% acetonitrile : 12% water was added. The solution was concentrated *in vacuo* to dryness (water bath temp. 55-60°C). The process was repeated twice with 1-2 ml anhydrous acetonitrile. A solution of 20 mg TAM in 1.5-2.0 ml anhyd. acetonitrile was added to the reaction vessel and the solution was heated under reflux for 5 minutes (oil bath temp. 125-130°C). The solution was concentrated to approx. 400 µl *in vacuo*, followed by the addition of 5 ml water. The aqueous solution was passed through C-18 Sep-pak (pre-conditioned with 2 ml THF and 5 ml water). The Sep-pak cartridge was eluted with 6-7 ml 0.1 M hydrochloric acid. The ^{18}F labelled 2-fluoro-1,3,4,6-tetra-O-acetyl-D-glucose was eluted from the Sep-pak cartridge with 3 ml THF. The THF solution was evaporated to dryness under reduced pressure and 1 ml 2 M hydrochloric acid was added. The solution was heated under reflux (oil bath temp. 125-130°C) for 20 minutes. The hydrolysate containing ^{18}F labelled FDG was loaded onto a column containing Dowex-50X-8 and Dowex ion-retardation

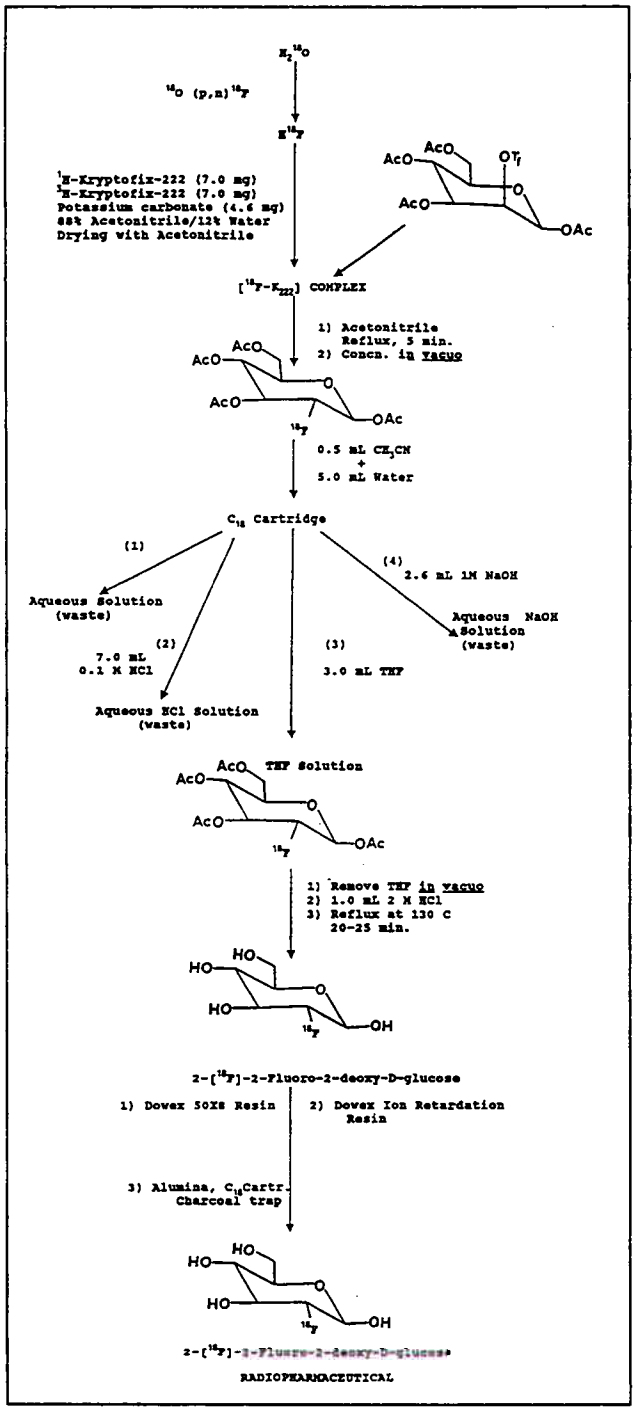

Figure 1: Scheme of the Synthesis of [^{18}F]2-FDG.

resin. The charcoal cartridge (prepared by loading a charcoal suspension onto a 0.22 micron vented micropore filter), C-18 and alumina Sep-pak cartridges were connected to the resin column in series. [^{18}F]FDG was eluted from the column with 20 ml water. The product was rendered isotonic with NaCl.

Determination of ^{3}H Labelled Cryptand [2.2.2]. In separate experiments, to verify its stability, ^{1}H labelled cryptand [2.2.2] was subjected to extended reaction conditions (*i.e.* separately refluxed in acetonitrile for 10 minutes and 2 M HCl for 30 minutes). For these preliminary results with labelled cryptand [2.2.2], the solution containing 2-[^{18}F]-2-fluoro-2-deoxy-D-glucose was evaporated to near dryness *in vacuo*. The residue was redissolved in 1 ml deionized water and 5ml scintillation cocktail (DUPONT Formula-963) was added. The aliquots were removed from aqueous solution and 0.1 M HCl solution and mixed with the scintillant. The charcoal, C-18 and alumina cartridges were separately eluted with 1 M NaOH and appropriately mixed with the scintillant. The Dowex 50-X-8 and ion-retardation resins were separately eluted with 1 M NaOH and mixed with the scintillant. Also, a finite amount of ^{3}H labelled cryptand [2.2.2] was dissolved in 1 M NaOH and mixed with 5 ml scintillant. The solutions were assayed in a BECKMAN Liquid Scintillation System model 3801. This data was used to determine the distribution of cryptand [2.2.2] during the synthesis. The experimental sequence and results are shown in Fig. 1 and Table 1, respectively.

Table 1: Distribution of Cryptand [2.2.2] at Various Steps of ^{18}F Labelled 2-Fluoro-2-Deoxy-D-Glucose Synthesis

SYNTHETIC STEP	% of Tritium Labelled Cryptand [2.2.2]
(1) Aqueous Solution	1.24
(4) C-18 Cartridge	0.09
(2) Aqueous HCl Solution	98.51
DOWEX 50-X-8 Resin Column	0.07
Ion Retardation Resin Column	0.008
Charcoal, Alumina & C-18 Cartridge	0.003
[^{18}F] Labelled 2-Fluoro-2-deoxy-D-glucose	0.069

Conclusion

The use of ^{3}H labelled cryptand [2.2.2] enabled us to accurately determine the presence of this chemical impurity throughout the various steps of the synthetic procedure leading to ^{18}F labelled 2-Fluoro-2-deoxy-D-glucose. The radiopharmaceutical formulation contained an average of 0.49 µg/ml of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo(8.8.8)hexacosane.

References

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