Oxygen Present During Azeotropic Drying Drastically Reduces the Radiochemical Yield of 2-FDG.

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Summary
The effect of varying reaction conditions on the yield of 2-FDG via the Jülich synthesis (1) has been investigated. The yield is found to be relatively immune to changes in many reaction parameters. On the other hand, we have found that the type of sweep gas used to remove the vapor phase during the azeotropic drying step is critical. The incorporation of ^{18}F into the 2-FDG triflate precursor is reduced fourfold when the sweep gas contains oxygen. However, using either of the inert gases nitrogen or helium as the sweep gas insures a consistent 65% ^{18}F incorporation. This phenomena was investigated with proton and ^{19}F NMR spectroscopy at the University of Wisconsin. Oxygen in the sweep gas does not alter the proton environment of the phase-transfer catalyst (Kryptofix K$_{222}$) but it does change the environment of the ^{18}F atom, that is loosely bound to the crown ether, inducing less favorable conditions for the SN$_2$ reaction.

Keywords: FDG, PET, 19F-NMR.

Introduction

Our initial failure to duplicate the FDG production results of Hamacher *et. al.* (1) prompted us to examine the details of the synthesis. From the beginning, it was evident that the ^{18}F label was not being incorporated into the precursor effectively. Wide variation of the "standard" parameters in the incorporation reaction (reaction temperature, reaction time, etc.) had minimal affect on the outcome, suggesting that a critical parameter was as yet unknown to us. The goal of this work was to find the critical reaction parameters. In order to asses the criticality of different parameters, proton and ^{19}F NMR spectroscopy were used to examine the precursor reactants after fluorination.

Experimental procedure

Parameters that might affect the success of the 2-FDG synthesis have been varied in an effort to maximize the radiochemical yield. The varied parameters include: glassware and accelerator target cleaning techniques, target type, target water, irradiation current and time, the dry-down ratio of K_{222}/K^+ and precursor concentrations, the incorporation reaction time and temperature, hydrolysis time and temperature, and the type of gas (or gas mixture) used to remove the vapor phase during azeotropic drying. Investigating the complete space spanned by these parameters is prohibitive. For this reason, the overall synthesis was broken into the following three steps: 1) azeotropic dry-down, 2) incorporation and 3) hydrolysis. The effect of varying the parameters for each step was monitored via chromatographic analysis. Thin layer chromatography with $CH_2Cl_2 + 2\%$ MeOH as the solvent phase on 0.25 mm silica gel plates was used to separate the labeled peracetylated organic compounds from the unreacted fluoride. The reaction was carried out as described by Hamacher *et. al.* (1) except for the variations in the parameters mentioned above and more fully described in (2).

Results

The data was collected in a set of 135 consecutive experiments where all parameters were varied. In order to determine which parameters have the greatest effect on the reaction yield, the data was projected onto planes defined by the synthesis yield and the parameter under investigation. The yield is defined here as
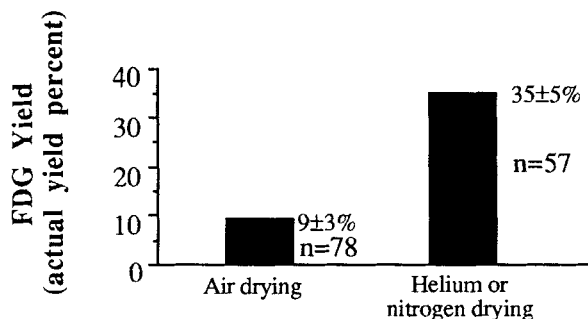

Figure 1. History of 2-FDG production during testing and optimization. The dramatic improvement after the first 78 experiments is due to performing the azeotropic drying using an inert gas.

the fraction of the ^{18}F activity removed from the target that is converted to 2-FDG (not decay corrected). This analysis demonstrates that the three most critical parameters are the incorporation reaction temperature and time, the ratio of K_{222}:K^+:triflate, and type of sweep gas used during the azeotropic drying. Variation of the first two parameters produced minor changes in the radiochemical yield while the sweep gas was found to be critical. For example, the yield varied less than 5% over the 75-95 degree temperature range employed during a ten minute incorporation. The lower temperatures (80-85 degrees) and smaller time intervals (5-10 minutes) are slightly preferred for increased yield, suggesting decomposition of the precursor at higher temperatures. The reagent ratio is similarly a minor factor

Azeotropic drying atmosphere: Helium
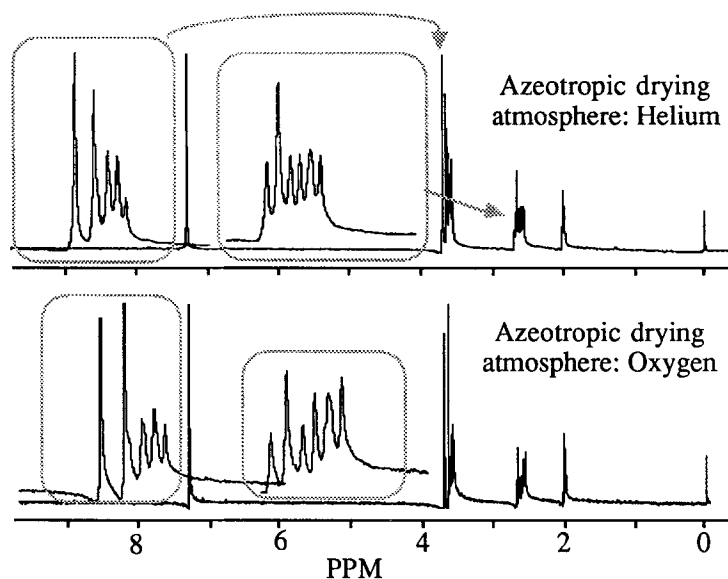
Azeotropic drying atmosphere: Oxygen

Figure 2 Proton NMR spectra of the potassium-Kriptofix® solution prepared under different drying conditions. The shaded regions are expansions of the indicated groups of peaks. The integrated areas of the corresponding peaks in the two graphs are identical.

but stoichiometric ratios (K_{222}:K^+:triflate 27:5:20 mg) result in slightly higher yields, and larger overall quantities appear to be less sensitive to impurities in the reaction mixture.

Figure 1 shows the dramatic increase in the radiochemical yield following a switch from utilizing a room air gas mixture to either helium or nitrogen in the azeotropic dry-down step. The first 78 syntheses, performed with room air as the

sweep gas, resulted in an average yield of 9%. The subsequent 57 syntheses with either helium or nitrogen as the sweep gas exhibited a fourfold increase in average yield. Separate experiments using either pure oxygen or helium were performed in order to investigate this phenomenon.

In order to determine whether the poor yields were caused by the kryptofix crown ether reacting with oxygen, identical solutions of K_{222} and KF in CH_3CN were dried under helium and oxygen atmospheres and examined with proton NMR spectroscopy (Bruker 200 MHz proton only spectrometer). The two spectra shown
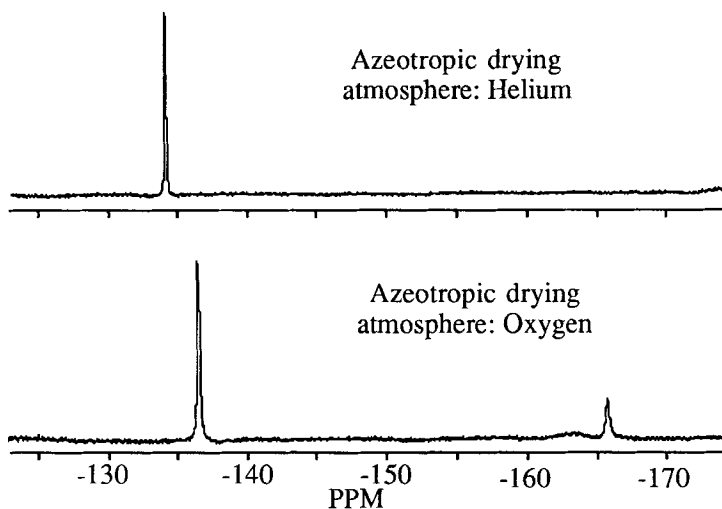

Figure 3 Fluorine-19 NMR spectra of the same solutions of figure 2. The contaminant peak in the oxygen sample represents 40% of the total signal.

in Figure 2 are identical, implying that the proton environment of the K_{222} molecule is unchanged as the result of oxygen drying. Therefore, the oxidative breakdown of K_{222} can be eliminated as the reason for the poor radiochemical yields.

The stability of the K_{222} molecule suggested that an investigation into the attachment of the fluorine atom to the K_{222} molecule might provide insight into the

cause of the low reaction yields when oxygen is used. A Bruker AM-400 MHz multinuclear spectrometer was used to obtain ^{19}F NMR spectra (Figure 3) with samples prepared as described above. The existence of a separate peak in the oxygen dried sample confirms that the environment of the Fluorine is drastically altered. The area under the contaminant peak is 40% of the total ^{19}F area, which corresponds directly to the reduction in the 2-FDG synthesis yield. Neither of the spectra, however, exhibit the peak splitting that one would expect for fluorine in the vicinity of hydrogen atoms. This implies that either the fluorine is held several bond lengths away from any protons, or that it is influenced by a magnetic field averaged over the surface of the K_{222} molecule. Some of the possible candidates for the contaminant fluorine-containing peak are: FO, F_2O, F_2O_2 or KF. The observed chemical shift (-165.7 ppm) of the unknown peak eliminates the above compounds as the contaminating species. These studies thus suggest that the fluorine remains bound to the K_{222} molecule but the oxygen has altered its position relative to the crown ether.

Conclusion

Our results show that any oxygen in the azeotropic drying gas will significantly reduce the overall reaction yield. We have ruled out oxidative breakdown of the phase-transfer catalyst as the cause of this effect. Also, the NMR spectra do not support the conjecture that oxygen combines with the fluorine to form a compound separate from the K_{222} molecule. We hypothesize that oxygen is affecting the reaction yield by altering the fluorinated phase-transfer catalyst. There are two mechanisms by which this might occur. The first is that extraneous oxygen is bound to the ^{18}F atom. In this case, the oxygen atom would physically shield the fluorine atom, preventing it from closely approaching the second position of the triflate precursor during the incorporation reaction. Since the probability that the SN_2 reaction will proceed depends upon the proximity of the reactants, the yield is reduced. In addition, the bond strength is increased which further reduces the probability of nucleophilic substitution based on thermodynamic grounds. If this mechanism is involved, then the observed change in the ^{19}F NMR spectra is due to a change in the bond length between the fluorine and the K_{222} molecule. The second possible mechanism is that an extraneous oxygen atom is bound to the K_{222} molecule in addition to but independent of the ^{18}F atom. There are many symmetric positions on the K_{222} molecule where the fluorine atom can bind. The very efficient fluorination possible with this phase transfer catalyst implies that (under ideal conditions), effective fluorination may occur with the ^{18}F atom bound at any of these

different positions. An additional oxygen atom would break this symmetry. The SN_2 reaction is hindered because the orientation of the fluorinating agent with respect to the 2-FDG precursor is now critical. In this case, the observed variation in the NMR spectra is due to a distortion of the K_{222} molecule caused by the oxygen atom.

Since this discovery, either nitrogen or helium gas has been used at the University of Wisconsin during azeotropic dry-down in the 2-FDG Jülich synthesis. A typical synthesis now produces in excess of 100 mCi of 2-FDG starting from 300 mCi of aqueous ^{18}F-fluorine.

Acknowledgement

References

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