Fluorinated (^{19}F) Nuclear Magnetic Resonance (NMR) Spectroscopy Protocol

About

When performing a trifluoroacetic acid (TFA) salt exchange removal, it is important to determine if the removal of the TFA was successful. If it is desired to know how much TFA is removed, ^{19}F-Nuclear Magnetic Resonance (NMR) spectroscopy should be performed on the sample before the salt exchange. After lyophilization of the sample following the salt exchange, users can run a similar experiment to determine how much TFA is still present in the sample. The preparation of these samples often includes the incorporation of a standard. There are a variety of different standards that can be used. Within the PPMC, it has been found that potassium fluoride (KF) has acted as a good standard. Trifluoroethanol (TFE) is also a good choice as well.[1]

Glassware and Equipment

- 1 x Analytical Microbalance
- 1 x Spatula or Scoopula
- 2 x Microcentrifuge Tubes
- 1 x Vortex Mixer
- 1 x Adjustable 1000-µL Micropipette
- 1 x 5 mm Diameter, 7", NMR Tube with Cap
- 1 x Bruker Neo 400 MHz NMR Spectrometer

Materials

The materials needed for this protocol are provided below. The Fisher Scientific catalog numbers are provided in parentheses.

- Deuterium oxide (AC166300250)
- Potassium fluoride (AA1413018)

Safety Measures

When performing this protocol, users must wear safety glasses, laboratory gloves, pants, closed-toe shoes, and a fire-retardant laboratory coat. These chemicals have the following hazard identifications:

Potassium fluoride (KF): 

Procedures

1. Begin by weighing out a known amount of the lyophilized peptide sample into a microcentrifuge tube using the analytical microbalance. This should be on the order of ~0.5 mg.
2. Prepare a 1 mg/mL solution of KF in deuterium oxide (D_2O) in a microcentrifuge tube.
    a. This concentration may be too high for certain peptide samples. If this does not work, it is recommended users make a 30 mM KF solution and then dilute to 200 µM.[1]
3. Dissolve the weighed out lyophilized peptide in the deuterium oxide solution to a 0.1 mM peptide concentration.
4. Add at least 500 µL of this solution to an NMR tube.
5. The preparation of this sample is now completed. The ^{19}F NMR method can now be run on the Bruker Neo 400 MHz NMR spectrometer.
6. Once the sample has been run, there should be two major peaks. A peak at ~-123 ppm represents the KF standard. A peak at ~-76 ppm represents the TFA in the sample. These two peaks can be integrated after applying Auto Phase Correction and Auto Baseline Correction. The ratio of the absolute integration values can then be used as a molar ratio to convert between the known amount of KF in the sample and determine the amount of TFA in the sample.[2]

References

(1) Ayotte, Y.; Woo, S.; LaPlante, S. R. Practical Considerations and Guidelines for Spectral Referencing for Fluorine NMR Ligand Screening. *ACS Omega* **2022**, *7* (15), 13155–13163. https://doi.org/10.1021/acsomega.2c00613.

(2) Little, M. J.; Aubry, N.; Beaudoin, M.-E.; Goudreau, N.; LaPlante, S. R. Quantifying Trifluoroacetic Acid as a Counterion in Drug Discovery by 19F NMR and Capillary Electrophoresis. *J. Pharm. Biomed. Anal.* **2007**, *43* (4), 1324–1330. https://doi.org/10.1016/j.jpba.2006.10.039.