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NMR Sample Preparation

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This handout covers a variety of considerations for good preparation of NMR samples. This covers the basic preparation for most small-molecule organic compounds. Additional preparation procedures for large molecules, polymers, biomolecules, inorganic molecules, etc. may be found in the literature.

1. **Use the proper amount of material.**
    a. For small molecules (less than 1000 g/mol), typical ^{1}H NMR spectra require 5-25 mg of material. Typical ^{13}C spectra require 50-100 mg of material. This amount of material will allow you to obtain a ^{1}H spectrum in a few minutes or a ^{13}C spectrum in 20-60 minutes. When the amount of material is doubled, the resultant signal will be doubled. Bear in mind that a very concentrated sample will produce a quick ^{13}C spectrum, but may result in a broadened ^{1}H lineshape. Overly concentrated samples can also be difficult to shim.
    b. For larger molecules and polymers, the amount of material may need to be significantly greater. Please consult literature affiliated with your molecule of interest for advice regarding sample concentration in these situations.
2. **Use the proper solvent and amount of solvent.**
    a. Deuterated solvents, in which ^{1}H atoms are replaced with ^{2}H atoms, are typically used in solution NMR for a variety of reasons. These reasons include deuterium lock, shimming, and providing an "invisible" background material that will not be observable in a ^{1}H or ^{13}C spectrum.
    b. Common deuterated solvents are available in small quantities from ISU Chemistry Stores. Common solvents include chloroform-D, acetone-D6, benzene-D6, deuterium oxide (D_2O), DMSO-D6, ethanol-D6, and methanol-D4. Additional less common deuterated solvents can be ordered through Chemistry Stores from Cambridge Isotope Laboratories or Sigma-Aldrich/Isotec.
    c. Typical NMR samples contain 0.6-0.7 mL of deuterated solvent. Do not fill the NMR tube full of solvent. This will dilute your sample, waste solvent, and make shimming more difficult.
3. **Prepare your sample in a secondary vial.**
    a. Use a small vial to dissolve the solid sample and transfer it to the NMR tube with a glass Pasteur pipette. Once the sample is in the NMR tube, effective mixing can be difficult. This also gives you the opportunity to treat the sample with heat or vortexing in order to get complete dissolution. If the sample contains significant solids, it is best to filter any particulate from the sample before transferring to the tube. Solid particles will not show up in a solution NMR spectrum, and may interfere with proper shimming.

4. **Use clean, unscratched NMR tubes and clean caps.**
    a. Particulate stuck to the inside of the NMR tube or scratches/defects in the tube can interfere with proper shimming. Use an NMR tube cleaner (either purchased or homemade) to clean tubes after using.
    b. Disposable NMR tubes are not appropriate for use on the high-field NMR instruments available in the CIF – the low glass quality will not allow for adequately good shims. High quality NMR tubes are available from Chemistry Stores or can be ordered from Wilmad Lab Glass or Norell NMR Tubes.
5. **Use an internal standard.**
    a. Residual ^{1}H in deuterated solvents can often be used for spectral calibration. However, in situations where an exact chemical shift is desired, or there is not solvent available for reference (such as for ^{13}C conducted in D_2O or ^{31}P), an additional internal standard must be used for chemical shift calibration.
    b. Internal standards such as TMS (for organic solvents) or DSS and TSP (for aqueous samples) will give you an exact NMR reference. For nuclei other than ^{13}C or ^{1}H, additional standards can be used such as phosphoric acid for ^{31}P.
    c. Internal standards can be added directly to the sample if desired. In this situation, just a drop of TMS is often too much for one NMR tube. Add a drop of TMS to 5-10 mL of deuterated solvent that can be used for several samples.
    d. Alternatively, if you are concerned about an internal standard reacting with the compound of interest, a capillary tube can be filled with an internal standard and placed in the NMR tube. This situation is not appropriate if you are using the internal standard for quantitation purposes. For quantitation, the internal standard must be added directly to the sample in order to achieve the same filling factor in the coil.
6. **Label your sample.**
    a. Permanent marker can be used to label the sample to ensure you do not mix up your NMR tube with another NMR user, or it can be retrieved at a later time. If you use a sticker or tape to label the tube, the sticker must be flush with the tube so it does not interfere with insert, eject or spinning inside the magnet.
7. **Air sensitive samples.**
    a. Some samples must be degassed to remove oxygen. Oxygen can be paramagnetic and broaden lineshape or interfere with T_1 relaxation measurements.
    b. For degassing air-sensitive samples, J-Young Tubes can be directly attached to a vacuum line.