NMR SAMPLE PREPARATION

JB Stothers NMR Facility
Materials Science Addition 0216
Department *of* Chemistry
Western University

1. INTRODUCTION

1.1. About these Notes

These notes describe how to prepare samples for analysis using NMR spectroscopy. No additional notes need to be consulted in order to understand the material presented here.

1.2. About NMR Samples

NMR experiments are generally performed on liquids, more specifically, on solids dissolved in a small amount of solvent. In order to get the best possible NMR spectra in the shortest amount of time, it is important that your solution used for NMR is prepared properly. An ideal NMR sample must be made with a deuterated solvent, be of the correct concentration, be free of particulate matter, have the correct volume and be in a good quality NMR tube.

1.2.1. Deuterated Solvent

Solutions used for NMR must be prepared using a deuterated solvent (i.e. a solvent in which the ^{1}H isotopes have been replaced by ^{2}H isotopes). Usually the deuteration is about 99.5 %, meaning that 0.5 % of the H atoms within the solvent will still be the ^{1}H isotope that give rise to peaks in a ^{1}H NMR spectrum. Deuterated solvents are described by formula, such as $CDCl_3$, D_2O, or name followed by a small *d* and the number of deuterons, such as DMSO-d_6, methanol-d_4. The deuterated solvent serves three essential purposes:

- The ^{2}H signal from the solvent is used to "shim" the NMR sample (shimming being a procedure in which the homogeneity of the magnetic field around the sample is optimized)
- The ^{2}H signal from the solvent is used to "reference" the NMR spectrum
- For ^{1}H NMR, the deuteration reduces the residual ^{1}H signal from the solvent enough that ^{1}H signals from your sample are observable

Although NMR experiments, except for ^{1}H NMR, can be performed for solutions prepared with standard (i.e. not deuterated) solvents, the samples must be referenced to an external solution, the resolution of the NMR spectrum will be poor and the peaks will likely suffer from false splittings or asymmetry. Deuterated solvents are available from several companies, the largest being Sigma-Aldrich and CIL.

1.2.2. Solution Concentration

The correct concentration is necessary to acquire an NMR spectrum of sufficient signal-to-noise and resolution in a reasonable amount of time. Since the NMR signal is directly proportional to the concentration, concentrated samples are obviously desirable. However, samples that are too concentrated can lead to problems with sample shimming and thus spectra with poor resolution, plus the sample can actually produce too much signal for the spectrometer to handle properly. Complicating matters further is that some experiments, such as a ^{1}H or COSY, are sensitive and good spectra can be obtained with only a millimolar solution, whereas experiments such as ^{13}C or ^{1}H–^{15}N HSQC are insensitive and require solutions that are at least 100 mM.

Table 1.1. Approximate Minimum Concentration and Masses for Select NMR Experiments[ab]

Experiment	Type	Concentration (mM)	Mass (mg, MW=100)[c]	Mass (mg, MW=250)[c]	Mass (mg, MW=500)[c]
^{1}H or ^{19}F	1D	10	0.7	1.8	3.5
^{13}C	1D	100	7.0	17.5	35.0
^{31}P	1D	25	1.8	4.4	8.8
^{1}H–^{1}H NOESY/ROESY	1D	20	1.4	3.5	7.0
^{1}H-^{1}H COSY	2D	10	0.7	1.8	3.5
^{1}H–^{1}H TOCSY	2D	10	0.7	1.8	3.5
^{1}H–^{1}H NOESY/ROESY	2D	40	2.8	7.0	14.0
^{1}H–^{13}C HSQC	2D	40	2.8	7.0	14.0
^{1}H–^{13}C HMBC	2D	40	2.8	7.0	14.0

[a]These values are guidelines, not hard and fast rules. The actual sensitivity of the NMR experiment depends on the spectrometer, nature of the sample, temperature and other factors.
[b]The maximum concentration is usually limited by the solubility of the sample and the amount of the sample that is available. If neither of these is a limitation, a maximum concentration of 100 mM should be used for ^{1}H or ^{19}F experiments and 1000 mM for all other experiments.
[c]MW = molecular weight. Assumes a 0.7 mL solution.

NMR SAMPLE PREPARATION

1.2.3. Solution Composition

To obtain the best possible resolution it is important to not have any particulate in the solution. Any particles in the solution should be filtered out and the clean solution transferred to the NMR tube. A Pasteur pipette stuffed with about a centimeter of cotton wool (not glass wool) makes an ideal filter.

1.2.4. Solution Volume

The NMR tube should be filled to a height of 50 mm from the bottom of the NMR tube. This corresponds to roughly 0.7 mL of deuterated solvent, depending on the density of the solvent. Too little solvent will cause problems locking the sample and with sample shimming. Too much solvent will result in minor problems, but is mostly a waste of money.

1.2.5. NMR Tube

Prepared solutions must be transferred to NMR tubes, which are essentially small glass test tubes without the upper lip, and sealed with a small plastic cap. NMR tubes come is various types of glass, widths, lengths, and quality, with the quality being determined by the camber (the degree of warping of the tube), the variation in the outer and inner diameters of the glass wall, and the concentricity (the consistency of the thickness of the glass wall). Essentially the smaller the camber, variation, and concentricity, the better and more expensive the NMR tube is. Using too low a grade NMR tube will result in poor shimming and can affect the resolution of the NMR spectra. It is also important to ensure that the NMR tube is not warped, and is free of cracks. NMR tubes are manufactured by Wilmad-Labglass, Norell and New Era and are available from ChemBioStores, directly from the manufacturers or through numerous distributors. In our facility, a New Era mid-grade borosilicate ASTM Type 1 Class B glass NMR tube of 7" to 8" in length and a 5.0mm outer diameter is generally used.

1.2.6. Sample Guidelines

An ideal sample should have the following:

- Deuterated solvent: *Solutions for NMR must be prepared using deuterated solvents*
- Concentration: *Between 10 to 100 mM for ^{1}H or ^{19}F, and 100 to 500 mM for ^{13}C*
- Solution Composition: *NMR sample must be free of particles and dust*
- Solution Volume: *NMR tube should be filled to a height of 50 mm from the bottom of the tube (about 0.7 mL of solvent)*
- NMR tube: *Relatively new NMR tube free of cracks, not warped and at least 7" in length*

2. PREPARING AN NMR SAMPLE

The procedure described below is the most thorough way in which to prepare an NMR sample. Once you obtain a feel for how much solid you require, the weighing step (step 5) can be omitted.

1) To prepare an NMR sample, you will need:
   a) NMR tube with cap
   b) small (~250 mL) Erlenmeyer flask or beaker
   c) balance with a minimum precision of 1 mg
   d) 1-dram vial with cap
   e) spatula
   f) minimum of 0.7 mL of the appropriate deuterated solvent
   g) 1.0 mL syringe
   h) metric ruler
   i) fine-tipped permanent marker
   j) 2 Pasteur pipettes, Pasteur pipette bulb and cotton wool (if filtering is necessary)
2) Place the NMR tube into the Erlenmeyer flask or beaker.
3) Use Table 1.1 to determine the approximate concentration you will need for your NMR experiment(s).
4) Based on the molecular weight of your sample, determine the mass needed to make a 0.7 mL solution of the correct concentration.
5) Place the uncapped 1-dram vial cap on the balance; tare the balance.
6) Add the appropriate amount of sample into the vial until the desired mass is reached. Note: you do not need to be too exact with the mass.
7) Using the syringe, draw 0.7 mL of the appropriate deuterated solvent and add the solvent to the vial.
8) Cap and shake the vial, or stir the contents with a stir-bar and magnetic stirrer, until all solid has dissolved.
9) If no particles are visible in the solution, transfer the solution into the NMR tube using a Pasteur pipette.

NMR Sample Preparation

10) If particles remain suspended, do the following:
    a) add a small amount of cotton wool to a Pasteur pipette
    b) use another Pasteur pipette to push the cotton wool down to the neck of the Pasteur pipette
    c) place the Pasteur pipette with the cotton wool into the NMR tube
    d) place the Pasteur pipette bulb on the empty Pasteur pipette and use the pipette to remove the solution from the vial and insert the solution into the Pasteur pipette containing the cotton wool
    e) place the Pasteur pipette bulb on the pipette with the cotton wool and push any remaining solution into the NMR tube
11) Use the ruler and ensure that the height of the solution is at least 50 mm from the bottom of the NMR tube. If it is not, add additional deuterated solvent until the height is 50 mm.
12) Place the cap firmly onto the NMR tube.
13) Using a marker, label the sample as desired. It is best to write on the cap, as opposed to the NMR tube itself as marker tends to smear easily on glass. If the sample is being submitted to the Mercury 400, you must write your supervisor's initials on the top of the cap. For the Inovas, any label is fine as long as you can identify the sample.