Sample Preparation and Positioning

Key Points for Sample Preparation

- ***Choose a high quality 5 mm NMR tube that is free from defects (e.g. flat bottom, cracked top):***

  The tube should be rated for the instrument you are using. If you are using the VXR-500, you should use tubes that are rated for 500 MHz or better. The minimum tube quality we recommend for the facility is a Wilmad 528. We strongly discourage the use of economy NMR tubes because poor lineshape and difficulty in shimming can be a result of poor tube quality.

- ***Decide on the amount of sample:***

  The sample amount depends on the experiment you are performing. For ^{1}H NMR, 1 mg to 40 mg is a good range (this assumes small molecules below 700 g/mol). The higher concentration will lead to difficult shimming and broadened lines. It is recommended to keep the amount of sample below 20 mg. Since, for ^{13}C NMR and most 2D experiments, signal-to-noise is the most important concern, the more you use, the better. I have found that 100 mg to 300 mg gives good signal to noise (S/N) with minimal scans (*nt*=128). If you use less sample, more scans may be required.

- ***Choose an appropriate deuterated solvent:***

  If you are not sure of which solvent to use, start with non-deuterated solvents until you find the appropriate solvent. The most used NMR solvent is deuterated chloroform ($CDCl_3$), but be careful, it can be acidic and acid sensitive compounds may decompose when dissolved in $CDCl_3$. Also, since it has only 1 deuterium, it can be more difficult to get a lock. Other popular solvents include D_2O, acetonitrile-d_3, acetone-d_6, benzene-d_6, DMSO-d_6, THF-d_8, and CD_2Cl_2. Many of these solvents are available from the Chemistry Department Stockroom.

- ***Use the appropriate amount of deuterated solvent:***

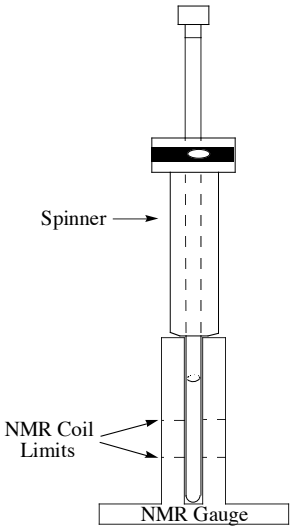
  Be sure that your sample is completely dissolved; undissolved particulate matter will lead to poor NMR lineshape. An appropriate volume is 0.7 mL or 50 mm in a 5 mm NMR tube. A sample that is 10 mm shorter or higher than 50 mm may be tolerable, but if you attempt to achieve higher sensitivity by concentrating your sample down to 30 mm or less, you are making a big mistake! The loss in sensitivity due to poor shimming on a short sample (which is inevitable and will result in broader line widths and poorer lineshapes) will always outweigh any gain from a higher concentration. Having more solvent than you need in the tube is less of a problem, except that substantial shimming may be required as the standard shim values are determined with the 50-mm sample height and may not accommodate unusually short or long samples. (Samples that are longer than 50 mm should be avoided in VT experiments to minimize temperature gradients over the length of the sample.)

- ***Remember that your solvent may contain dissolved water:***
  Many solvents will contain trace amounts of water when bought and as you use the solvent, the water content will increase. You can store your solvents over molecular sieves to minimize water, but be aware of the particulates from the sieves. The chemical shift of water in various solvents is shown below.

Solvent	H_2O chemical shift (ppm)
Acetone-d_6	2.8
CH_3CN	2.1
Benzene-d_6	0.4
$CDCl_3$	1.5
DMSO	3.3
Methanol-d_4	4.8
THF-d_8	2.5

- ***Cap and label your NMR tube:***
  Be aware that any tape, parafilm, Teflon tape, or improper sealing may prohibit proper positioning of the tube in the spinner and may also cause spinning problems.

Sample Positioning



A NMR Sample depth gauge is located on the console of every instrument. The depth gauge with correct sample position is pictured to the left. The sample is inserted in the spinner and placed in the gauge. The tube is positioned so that the spinner touches the gauge. If necessary, the tube can be raised to center it around the two white lines on the gauge (signifies the NMR coil edges as shown in the picture). When positioning the sample, make sure that your eyes are at the same level as the bottom of the tube in the gauge. Otherwise, the sample could be positioned improperly by as much as 5 mm. Ask the NMR staff or your trainer for a demonstration of this the first time you train on an instrument.

If you do not position the NMR tube to within 1 mm of the correct position, the standard shim libraries may not be very close and a substantial amount of shimming may be required. A few seconds spent here can save you many frustrating minutes in shimming.