



Chemistry Department

NMR/Instrumentation Facility

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NMR sample preparation

The sample preparation has a substantial effect on the quality of the spectrum. No shimming or change in spectrometer parameters can fix bad samples. For high-resolution experiments follow the guidelines below:

1) Tube quality

Only good quality NMR tubes should be used. A bad NMR tube will lead to large spinning side bands and bad lineshapes that cannot be fixed, no matter how long you shim or how many scans you acquire. This is especially important at higher fields, such as with the 400MHz instrument.

Here are a few suggestions from different companies:

Wilmad	Norell	Kimble-Kontes	Ace Glass
528-PP-8	508-UP-8	897240-0008	2528-08
527-PP-8	507-HP-8	897235-0008	
526-PP-8			

2) Tube care

The NMR tubes are made from thin glass and therefore are quite fragile. Do not use tubes with chipped or cracked tops, they can easily splinter lengthwise. Make sure to have the tube cap correctly placed on the tube. Always clean the tube before inserting the sample into the probe. Do not touch the bottom of the NMR tube.

Do not store NMR tubes in beakers or flasks; instead store them horizontally in a flat surface. Bent tubes may cause severe probe damage and spinning problems.

3) Tube cleaning

Tubes left with samples for a longer period of time may be more difficult to clean. Sample precipitation can cause material to adhere to the inner walls of the tube. Rinsing doesn't always remove this material. In severe cases, soaking the tubes in fuming HNO₃ for 1-3 days may be used. Carefully rinse tubes with a considerable amount of water.

Caps should also be cleaned; they can contaminate your samples.

Avoid heating NMR tubes in ovens. Tubes may warp, bent and flatten, destroying the tube's quality. It is best to dry the tubes with a blast of dry air or nitrogen. If you decide to dry tubes in the oven, Wilmad recommends placing tubes on a flat surface in the oven at 125°C for 30-45minutes. Again: store them in a flat surface.

4) Sample labeling and preparation

Label your tube, if you need to label the tube directly, use a permanent marker on the top of the tube, or on the cap. Do not use sticker or tape which will leave a flap. Remember that your sample will be spinning in the magnet, preferably at a stable speed!!

The presence of solids in your tube will distort the field homogeneity around every particle in solution and cause broadening of the NMR signals. Filter samples to remove any solid particles.

Use about 0.7mL of deuterated solvent for a 5mm tube. You can use less solvent, as long as you are within the sample gage depth requirements.

For ^1H spectra of organic compounds, the quantity of material required is about 5-25mg. At very low concentration, the peaks from the solvent and from contaminants (water and grease) tend to dominate the spectrum.

Grease will show large peaks at 0.5-1ppm (hydrocarbons) or around 0.1ppm (silicon grease)

^{13}C is much less sensitive than ^1H . If 0.2-0.3millimoles are dissolved in 0.7mL the spectrum should take about 30min to record. Generally a saturated solution will provide best results. However, be aware that a ^1H spectrum of a sample at high concentration may result in broader lines due to increase viscosity.

5) Degassing

Some experiments, such as NOE may require removing oxygen. The most effective way of doing this is by using the Freeze-Pump-Thaw method, at least 3 times.

An alternative method is to bubble nitrogen or argon through the solvent. Some solvent can be lost through evaporation.

Freeze-Pump-Thaw method:

- 1) freeze sample in liquid nitrogen or CO_2 /acetone
- 2) turn vacuum on to evacuate the space above the solution
- 3) turn vacuum off and allow sample to thaw, bubbling should be noticed
- 4) repeat steps 1-3 (at least 3 times)
- 5) fill with nitrogen and tightly close the tube

NOTE ON BROKEN TUBES

Be careful when placing a tube in a spinner. Adjust the depth with the depth gage and carefully place it on the magnet. Make sure it stays floating on the upper barrel until you insert the sample using the computer commands.

If a sample breaks inside the probe:

- 1) EJECT spinner and broken tube out of the magnet
- 2) place spinner next to the computer
- 3) discard the broken tube
- 4) leave a note on the computer keyboard informing others that a sample was broken
- 5) inform the manager immediately, at CB 126 or by email (leila76@ksu.edu) - information on solvent used and on the sample is helpful

DON'T DO ANYTHING ELSE!! Leave the magnet/probe without sample or spinner.
Never inject anything back into the magnet.