NMR Service

How to Prepare Samples for NMR

In NMR, unlike other types of spectroscopy, the quality of the sample has a profound effect on the quality of the resulting spectrum. If you follow a few simple rules, the sample you prepare will give a spectrum in which useful information is not lost or obscured. Your sample must be made up in a 5mm NMR tube, unless you have arranged to provide it free of solvent.

1) Use the Correct Quantity of Material. For ^{1}H spectra of organic compounds (except polymers) the quantity of material required is about 5 to 25mg. It is possible to obtain spectra from smaller quantities, but at very low concentrations, the peaks from common contaminants such as water and grease tend to dominate the spectrum. ^{13}C is ***six thousand times*** less sensitive than ^{1}H, and a good rule of thumb is to provide a saturated solution. If you can dissolve about 0.2 to 0.3 millimoles in 0.7ml, the spectrum will take no more than about half an hour to record. If the quantity of material is *halved*, the necessary data accumulation time will be *quadrupled*. You should also be aware that if you make up a sample at high concentration for ^{13}C, and then record a ^{1}H spectrum from it, the increased solution viscosity may result in a spectrum that has broader lines than you would get from a more dilute solution. If you wish to observe polymers or other nuclei, please contact me to discuss quantities.

2) Remove All Solid Particles. Solid particles distort the magnetic field homogeneity because the magnetic susceptibility of a particle is different from that of the solution. Thus, a sample containing suspended particles has a field homogeneity distortion around every single particle, causing broad lines and indistinct spectra. To remove solid particles from your samples, you must filter ALL solutions into the NMR tube. You should filter through a small plug of glass wool tightly packed into a Pasteur pipette. If the plug is not tight enough, the filtration will be ineffective; if it is too big, some of your sample will remain trapped in it. Do not use cotton wool, since most NMR solvents will dissolve material from it which can easily be seen in ^{1}H spectra. After filtration the sample should be clear though, of course, not necessarily colourless.

3) Make Samples to the Correct Depth. In the magnet, the main field direction is vertical, along the length of the sample. Each end of the sample causes a major distortion of the field homogeneity which is corrected using the spectrometer's shim controls. A partial correction is done for every sample, and takes a few minutes. A complete correction takes many hours using a high quality test sample. So that this lengthy task need be done as seldom as possible, your samples must be prepared so that they physically resemble the test sample so, after filtration, they must be made up to a similar depth. This must be between 5cm and 5.5cm, and requires about 0.6 to 0.7ml solvent. Shorter samples are very difficult to shim, and cause considerable delay in recording the spectrum. Samples that are too long are also difficult to shim and are a waste of costly solvent. Check your

sample depth using a ruler. After preparation, you should ensure that the cap is pushed fully onto the tube to minimise solvent loss through evaporation.

4) Use Deuterated Solvents. Samples must be prepared using solvents that contain deuterium in place of hydrogen. The NMR signal from the deuterium nuclei is used by the spectrometer for stabilisation and is called the NMR lock. Deuterochloroform is available from the stores, and a small selection of other deuterated solvents is kept for trial purposes in Room G.20. If you have a regular need for deuterated solvents other than $CDCl_3$, you should order your own supply. Please see me for up-to-date information about prices and suppliers. Because they are extremely costly materials, they must be used with great care to minimise waste. You must not use deuterated solvents to do solubility trials. If you keep a 1ml calibrated all-glass syringe solely for use with deuterated solvents, you will simplify handling and reduce the risk of contamination. Do **NOT** use mixtures of deuterated solvents, or mixtures of deuterated with non-deuterated solvents, without taking advice.

5) Use Clean Tubes and Caps. 5mm NMR tubes are available from the Stores. After use, they should be rinsed with acetone or some other suitable solvent, preferably using a tube washing device. If your tube is badly contaminated you should use solvent with a doubled-over pipecleaner. Tubes should then be dried with a blast of dry air or nitrogen. You must **NOT** dry tubes in a hot oven, firstly because it does not effectively remove solvent vapour, so solvent peaks will appear in your spectrum, and secondly because tubes distort significantly at the temperatures used in many ovens, and become dangerous to use. Tubes must always be capped, and caps should be cleaned the same way as tubes. You must not use NMR tubes with a chipped or broken top because they are dangerous, and very likely to splinter lengthwise. It is very easy to trim off the damaged part yourself, see me if you don't know how to do this.

6) Label Your Samples. Samples must be labelled, preferably with just your User Code and a Sample Code. Do not clutter up a necessarily small space with structures or anything else. You should write directly on the NMR tube with an solvent-based felt pen. If it does not write legibly then the tube is dirty. You should be aware that new tubes are usually covered with a film of grease from the manufacturing process, and should be cleaned.

7) Do Not Add TMS. The amount of TMS or any other reference material that is required for a ^1H spectrum is far less than can be added after the sample has been prepared. Even one drop of TMS in a sample causes serious problems due to distorted baseline and exceeded dynamic range. Suppliers can provide, on request, solvents with a small amount of TMS (about 0.03%) already added and, if you need it, you should specify this when you order solvents. Usually, the residual protons in the deuterated solvent are used as a secondary reference.

8) Degassing Samples. Some samples need to be degassed or have oxygen removed. The only effective way of doing this is by using the Freeze-Pump-Thaw technique, at least three cycles. It is sometimes sufficient to flush the space above the sample surface with nitrogen. This should be done with great care to avoid blowing the solution out of the tube. Do ***not*** bubble nitrogen through the solution in an NMR tube. This wastes costly solvent through evaporation, and is not an effective method of removing oxygen.

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NMR Data Naming Conventions

So that you may find your NMR data in the archive, all files from the AC200 are named in accordance with this pattern:

a a a b c c c c . d d d

a a a	User Code, generated by the registration program.
b	Observed nucleus, e.g. H or C, inserted automatically.
c c c c	User's Sample Code, provided by you.
d d d	Experiment serial number, generated automatically.

Because of limitations imposed by the computer on the AC200, you must use **only** upper-case letters and numbers. You **must not** use more than four characters. You **must not** use lower case letters, or punctuation characters such as / : ( ] '. You **should not** include your initials in a Sample Code. There are two reasons for this. Firstly, because the computer puts your User Code at the beginning of the data file name, so your personal identity is automatically attached to the file. Secondly, because you are limited to only four characters to identify each sample, and later on you may need all of these to provide enough distinct Sample Codes. If you use Sample Codes more than once, you should remember that any earlier data file is destroyed by any later one with the same name. You may use a Sample Code again if you are observing a different nucleus, since the nucleus is made part of the file name. However, the solvent you use is not made part of the filename, so if you record spectra of a sample in different solvents, each one should have a different Sample Code. Here are some Sample Code examples:

47/2	incorrect, because it contains '/'.
35b	incorrect, because it uses lower case 'b'.
***	incorrect, because it uses '*'.
34A57	incorrect, because it uses more than four characters.
5	correct
B6UD	correct
SKG	correct

You are strongly recommended to use a simple numbering system, e.g. 0 to 999, with resubmission of the 'same' sample designated by a letter suffix, A to Z. Sample Codes don't need any leading zeroes, but you may find them useful. It's generally unnecessary to use more than one or two. You should be careful to avoid confusion between letter 'I' and figure '1', letter 'O' and figure '0', letter 'S' and figure '5', and letter 'Z' and figure '2'. The AC200 automation system will detect some mistakes in a Sample Code entry, but not all.

For DPX400 data sets there are a few differences. Sample codes may be up to eight characters long and are case-insensitive. All Sample Codes will be changed to lower case for the data set name, and will be printed in upper case in the spectrum title. The experiment number is no longer an integral

part of the name, but is the name of an individual directory inside the data set. All the other restrictions and advice given above still apply.

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