

NMR Sample Preparation

The Open Access NMR service in CAF frequently runs more than a hundred samples per day, and it is quite normal for your sample to be “waiting” to be run in a “queue” of NMR samples. For this reason, it is important that you pay proper care and attention to sample preparation so as not to waste NMR spectrometer time by submitting samples which give poor quality spectra, or even give spectra which are impossible to interpret. A little time and care spent in properly preparing your NMR samples will reward you many times over with consistently high quality NMR spectra.

NMR Sample Tubes

All the NMR instruments in CAF accept 5mm NMR tubes:

- For the Open Access NMR instruments (Nano400 and DPX400), please use the Norrell S 5-400-7 tubes, which are available from the Chemistry Food and Pharmacy Stores. NMR tubes should be at least 7' long and should not be broken at the top. Tubes which do not meet these requirements may malfunction when submitted to the BACS sample changers, potentially resulting in damage to the NMR instruments.
- For the Internal NMR service, please prepare your sample in a Wilmad 528-PP-7 tube for submission to the AV500 MHz instrument or in a higher quality Wilmad 535-PP-7 tube for submission to the AV700 MHz instrument.

Quantity of Sample Required for NMR

1-10 mg of sample is generally sufficient for acquiring a ^1H NMR spectrum of an organic compound in a short space of time (ca 5 mins) on either of the Open Access NMR instruments. Although, it is possible to obtain spectra from smaller quantities of material (see Sample Requirements for Experiments in ICONNMR), much greater care needs to be taken with sample preparation, so that peaks from common contaminants such as water, plasticizers and grease do not overwhelm peaks from your sample. Characteristic peaks for such contaminants in ^1H NMR spectroscopy are listed below in the section on Evidence of Poor Sample Preparation. Much more sample (5-50mg) is required in order to obtain an acceptable ^{13}C or DEPT NMR spectrum – and carbon samples also require a longer period of time (5-30 mins), owing to the very much reduced sensitivity of ^{13}C relative to ^1H . Note that a sample which has been prepared at a high concentration for ^{13}C NMR may exceptionally produce a ^1H NMR spectrum which has much broader lines than would be expected from a dilute solution, as a result of the increased viscosity of the solution.

NMR Solvents

The NMR sample must be dissolved in a solvent which contains deuterium in place of hydrogen (fortunately, almost all of the common organic solvents are commercially available in deuterated form). CDCl_3 is the most generally useful NMR solvent, as well as being the cheapest, and for this

reason CDCl_3 is always suggested as the “default” solvent by ICONNMR, when setting up experiments on the Open Access NMR instruments. Usually, a small amount (ca 0.03%) of tetramethylsilane (TMS) will already have been added by the manufacturer to the CDCl_3 which you are using. This TMS serves as a zero frequency reference for both ^1H and ^{13}C NMR spectra (if TMS is absent from the deuteriated solvent, then the residual protons in the deuteriated solvent can also be used as a secondary reference). d_6 -DMSO and D_2O are more expensive than CDCl_3 and are the second most common solvents for NMR. All three NMR solvents are kept in the stores in the Chemistry Department. Other deuteriated NMR solvents are considerably more expensive and should be purchased by research groups directly from the manufacturer on an individual basis (in general, the cost of the less common deuteriated solvents increases in proportion to the number of hydrogen atoms which have been replaced by deuterium).

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Samples should be dissolved in ca. 0.6-0.7 ml of a deuteriated solvent and then filtered through a Pasteur pipette, equipped with a glass wool plug, that discharges into an NMR tube. The purpose of filtration is to remove any undissolved sample, particulates, dust, hairs etc... from the solution, any of which may adversely affect the resolution and lineshape of your NMR spectrum. A simple set-up for effecting such a filtration is depicted in the diagram below:

NMR tubes need be filled to a depth of approximately 4 cm for optimum results (this sample depth represents 0.6-0.7 ml of deuteriated solvent). Using a smaller sample height/volume can make it very difficult for the NMR spectrometer to shim the sample. This wastes valuable instrument time, and will not only result in a long delay in recording your spectrum, but also add to the length of time that other users must wait for their samples to be run in the NMR "queue". Furthermore, spectra recorded from samples which have been made up in too small a volume of deuteriated solvent will almost always exhibit line broadening, relative to a sample which has been prepared properly. In extreme cases, ICONNMR will not be able to shim the sample at all, and the instrument may crash. Conversely, although the use of a much larger sample height/volume than is required does not adversely affect either the NMR instrument or the resolution of the NMR spectrum, it does entail an unnecessary "dilution" of your sample. This is a waste of costly deuteriated solvent. In addition, for samples which are available in only "limited" quantity, you should generally be aiming to prepare your sample at the highest possible concentration which is consistent with obtaining good NMR spectra (i.e. in the minimum permissible volume of 0.6-0.7 ml of deuteriated solvent), as this could well be the critical factor in determining the success or failure of your NMR experiment (see Sample Requirements for Experiments in ICONNMR).

Evidence of Poor Sample Preparation

The Open Access NMR instruments can quickly provide a very large amount of information from a few hundred micrograms of sample (for ^1H NMR). The corollary of the high sensitivity of a modern NMR spectrometer, however, is that any impurities which are present in comparably small quantities will also appear as "unexpected" peaks, possibly dominating the ^1H NMR spectrum and overwhelming the "real" peaks. Most procedures for organic synthesis involve a concentration step in which several hundred ml of solvent are reduced in volume down to 0.6-0.7 ml in an NMR tube. It is therefore quite possible that involatile impurities, which are present in only trace amounts in a solvent will come to dominate the NMR spectrum, following the work-up of a reaction, and its "concentration" in an NMR tube. The following "impurity" peaks appear most commonly in the ^1H NMR spectra of the products of a chemical reaction:

- Two characteristic double doublets at around δ_{H} 7.5 and 7.3 ppm. These two multiplets generally indicate contamination by phthalates. Phthalates are plasticizers which are readily leached by most organic solvents from any laboratory apparatus which contains plastic (plastic tubing is an especially common source of phthalates). Avoid using plastic apparatus wherever possible.
- A broadish peak at δ_{H} 1.56 ppm which is due to water. CDCl_3 can be saturated with up to approximately 1% of dissolved water - if there is more than 1%, then undissolved water will appear as a separate phase (droplets of liquid water suspended in CDCl_3 give a broad resonance at around 4.7 ppm). This contaminant can be removed by careful drying of solvent during the work-up of a reaction and by ensuring that the deuteriated NMR solvent is stored over molecular sieve.
- A broadish peak at δ_{H} 1.26 ppm and a much smaller associated triplet at δ_{H} 0.88 ppm. These peaks are due to higher molecular weight hydrocarbons, which have not been removed during sample concentration under reduced pressure. Generally these peaks are caused by grease which has been leached into the sample from dirty apparatus or as a consequence of using undistilled solvents. It is therefore a good idea to distil all solvents prior to use.
- A singlet at δ_{H} 0.1 ppm which is normally due to silicone grease. It is surprisingly easy for organic solvents to leach vacuum grease that has been applied to the quickfit joints of a reaction flask, even when liquid solvent has apparently not come into

contact with these joints. In general try to avoid using vacuum grease, where possible.

Cleaning of the NMR Sample Tube after Running an NMR Spectrum

After running an NMR spectrum, if the NMR sample is no longer required it is generally discarded into the appropriate halogenated or non-halogenated waste (otherwise the sample can be recovered by transferring to a sample vial and removing the solvent under a gentle stream of nitrogen gas). The NMR tubes should then be soaked in solution of detergent, rinsed several times with distilled water and then again several times with acetone. Although it is normally very easy to remove acetone from laboratory glassware, it can be surprisingly difficult to completely remove all acetone from NMR tubes (a consequence both of their length and comparatively narrow bore). The preferred method for removing residual wash acetone is to leave an NMR tube in a “cool” oven (say 50 °C) for several hours. It is not advisable to attempt to accelerate the process by using a hotter oven (100 °C and above) as the high temperature may result in “warping” of the NMR tubes. The cost of an NMR tube is largely determined by how straight and how parallel its walls are (which is quantified as the “tolerance” of the tube). Any degradation in the specified tolerance will degrade the quality of the spectral resolution which is theoretically possible from that NMR tube (this is the reason that higher quality tubes are generally specified for higher field instruments – see NMR Sample tubes). If time is at a premium, an alternative method for removal of residual acetone is to blow nitrogen or air through the tube, with a Pasteur pipette, while warming it **gently** for a few minutes with a heat gun. Some textbooks recommend that NMR tubes containing intractable substances, such as polymers, be cleaned by digesting these compounds with chromic acid. This should, however, be considered as a procedure of last resort as there is the potential for depositing residual paramagnetic chromium in the glass of the NMR tube, which will inevitably lead to line broadening in NMR spectra.