7 The NMR Sample

When a **solid** is investigated using the NMR technique, signals tend to be broad and the fine structure, which is of most interest to scientists, cannot be resolved. Consequently, solid samples are usually dissolved in a suitable solvent prior to acquisition. The same goes for **liquid samples**. In organic solvents, small amount of a reference compound may be added. However, to achieve best results the sample should be as pure as possible. Signals from **impurities** will at best make the spectrum unnecessarily complicated and at worst mask genuine signals. Particular care should be taken to ensure that the sample is free of **magnetic impurities** as these can distort the magnetic field and hence degrade the spectrum resolution. Solid impurities can be most easily removed by filtering. For samples in **organic solvents**, dissolved water can be removed as far as possible by thoroughly drying the sample prior to dissolution.

7.1 Solvent Selection

Once the sample has been sufficiently purified and dried, the next step is to choose a suitable solvent. Since deuterium is by far the most popular lock nucleus the sample is usually dissolved in a deuterated solvent (a deuterated solvent is one in which a large proportion, typically more than 99%, of the hydrogen atoms have been replaced by deuterium). Commonly used **deuterated solvents** are benzene-d6, acetone-d6, and chloroform-d though many other solvents are available. Factors to be considered when choosing a solvent are:

1. **Solubility**: Clearly the more soluble the sample is in the solvent the better. This maximizes the amount of sample within the sensitive volume which increases the sensitivity of the experiment. High solubility is particularly important if only small quantities of the sample are available.
2. **Interference of solvent signals with the sample spectrum**: The solvent itself will inevitably produce NMR signals which will obscure regions of the spectrum. These ‘residual solvent peaks’ should not overlap with signals from the sample.
3. **Temperature dependence**: For experiments above or below room temperature the solvent‘s melting and boiling points are also important factors. Furthermore the solubility of the sample is likely to vary with temperature.
4. **Viscosity**: The lower the solvent viscosity, the better the resolution of the experiment.
5. **Cost**: Clearly for routine NMR where many samples need to be measured, the solvent‘s cost is an important consideration. As a rule of thumb, the price increases with the number of deuterated atoms.
6. **Water content**: Almost all NMR solvents contain water traces. Also many are hygroscopic (they absorb water from the atmosphere) and hence the longer they are stored the more water they contain. The presence of a water (HDO) peak will only serve to degrade the quality of NMR spectra. The water level in the solvent can be greatly reduced by filtration through a drying agent or by storing the solvent by adding molecular sieves.

The choice of solvent for a particular sample will be the best compromise between the various advantages and disadvantages of each. Surge the web for precise details of specific solvents.

7.2 Sample Tube

When the sample is being analyzed it may be rotated, depending on the type of probe or experiment. **Spinning** the sample has the effect of cancelling out field inhomogeneities in the X and Y direction and consequently improves the spectral resolution. A disadvantage of spinning is that it may lead to the presence of **spinning sidebands**. These are spurious signals (i.e. peaks) that result from the modulation of the magnetic field at the spinning frequency. The peaks always appear on either side of any large genuine peak at a separation equal to the spinning rate. The intensity of these sidebands will be proportional to the intensity of the genuine peak. Thus if the spin rate is 20 revolutions/second (= 20 Hz), you would look for spinning side bands at frequencies 20 Hz above and below the resonance frequencies of genuine signals.

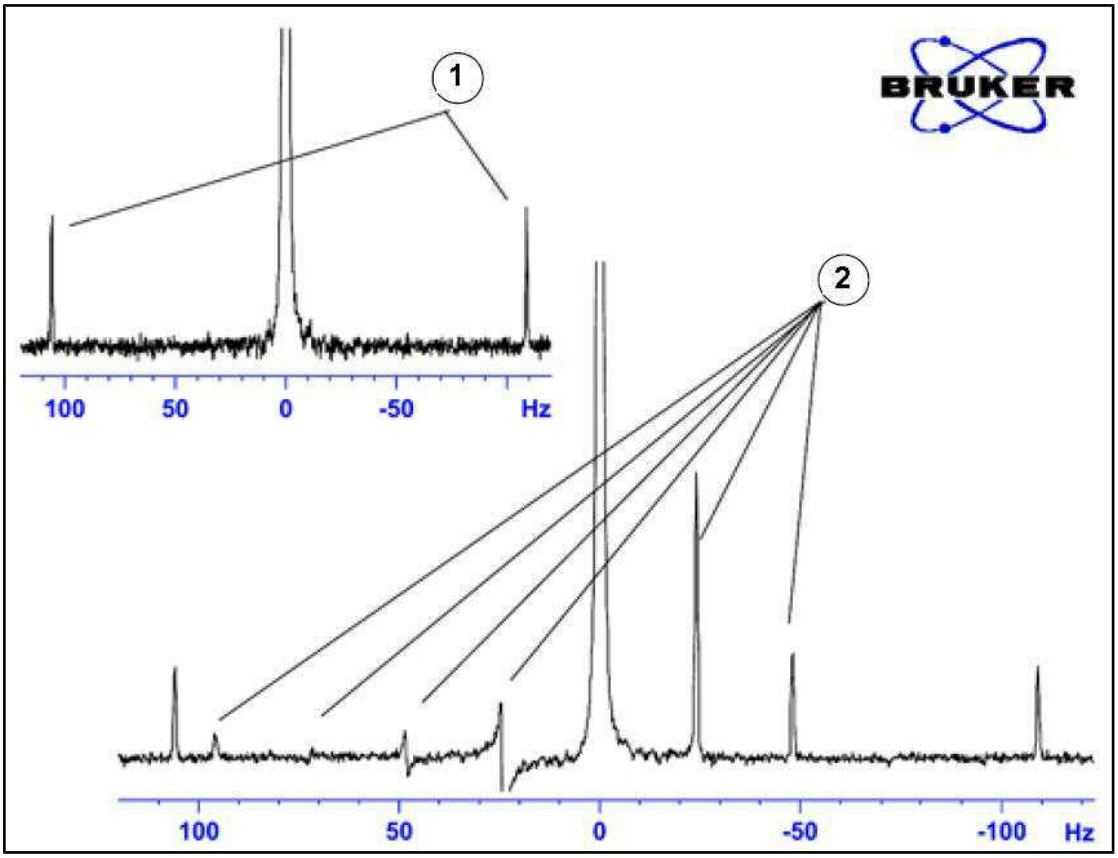

Figure 7.1: Spectrum Showing Spinning Sidebands

1.	13C Satellites	2.	Spinning Sidebands

While the presence of spinning sidebands may be inevitable, their size often depends on the sample tube quality. Ideally, the sample tube should have perfect cylindrical symmetry. Unusually large sidebands might suggest that the **tube symmetry** is inadequate and might warrant using tubes with higher specifications (and of course greater cost).

Sample tubes must always be kept clean and free from dust and scratches. Do not scrub the tubes with test tube brushes. Be aware that new NMR tubes cannot be assumed to be clean. The tubes may be cleaned by washing in Acetone or distilled water. Liquid detergent may be used as long as it is rinsed out within a few minutes to prevent etching of the tube. The tubes may also be cleaned ultrasonically in an appropriate solution. If all the above measures fail, the tubes should be soaked in AQUA REGIA for up to two days and then

rinsed thoroughly before being dried. NMR tubes can be oven dried but should not be heated above 100°C as they can become distorted and subsequently fail to spin as required. Drying is best achieved by passing filtered nitrogen through the tube.

7.3 Sample Handling

It is good practice to filter NMR solutions directly into the sample tube to keep the solution free from dust and other contamination.

Note: The sample tube should always be held by the top!

Typical procedures to prepare a sample might be as follows:

1. For a solid sample using a 5 mm tube dissolve up to 20 mg of the sample in about 0.6 cm3 of the chosen solvent (for 10 mm tubes dissolve 80 mg in 2.5 cm^3). Typically for a liquid sample, and when observing protons, dissolve 20% sample in 80% deuterated solvent.
2. Add a small amount (~0.1%) of reference compound Tetramethylsilane (TMS). Make sure the TMS signal is smaller than the most intense sample or solvent signal (otherwise the signal-to-noise ratio is wasted because of low receiver gain).
3. Filter the solution into the sample tube through a Pasteur pipette containing a small plug of Kimwipe.
4. Filter 0.2 cm^3 of solvent through the filter into the tube. The resulting solution should have a depth of three to four centimeters.
5. Close the tube with a cap, seal the top with parafilm to reduce evaporation and label the tube near the top. Be careful to ensure that the cap, parafilm and label are concentric or otherwise they will adversely affect sample spinning.

Note: Some problems may occur when using glass fiber for filtering the sample, especially when you want to measure T_1.