Sample preparation for NMR measurements and points to keep in mind

NMR Sample preparation

In solution NMR, the sample for study is dissolved in an appropriate deuterated solvent ($CDCl_3$, Acetone-d_6, DMSO-d_6, C_6D_6, CD_3OD, D_2O, CD_3CN, DMF-d_7 etc). A standard substance is also added as the reference for chemical shifts. In order to achieve full performance of the instrument, it is necessary to be sufficiently careful not only in selection of the solvent and standard substance and also in handling of the sample itself.

Sample tubes

Use the best quality NMR tubes **(Wilmad 528, 535, and 541, New Era Enterprises MP5, HP5, and UP5; Aldrich Z569348, Z569364, and Z5569380)** and clean the outside of each tube with a solvent such as isopropyl alcohol. Cleaning should be followed by careful wiping with a wiper tissue before placing the tube in the probe. This helps keep the inside of the magnet bore (the "upper barrel") clean, to make the sample spinning more reliable (when needed).

Use of mixed solvents

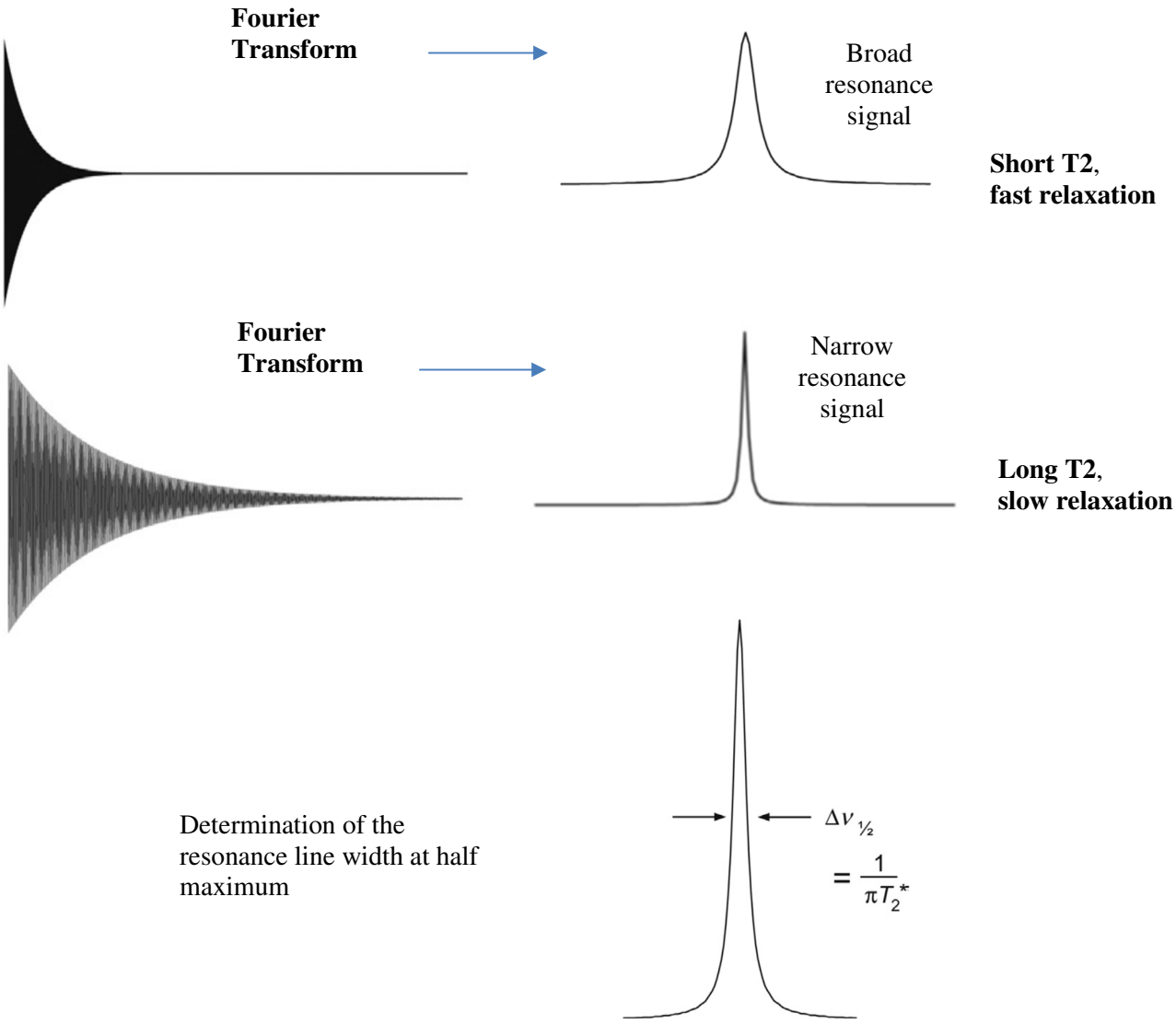
For some kinds of sample by using a mixed solvent, it is possible to enhance separation of peaks or to increase the solubility and to improve the S/N ratio or to reduce the cost of the solvent.

For example, in the cases of drugs or natural products, frequently the solubility is higher in a mixed solvent of deuterochloroform and deuteromethanol ($CDCl_3$:CD_3OD = 4:1). Also, for high polymers such as polyethylene and polypropylene a mixture of ortho-dichlorobenzene and deuterobenzene ($C_6H_4Cl_2$:C_6D_6 = 4:1) can be used *as* a relatively economical solvent. In other cases, also by using a mixed solvent suited to the target sample better spectra can frequently be obtained.

Concentration (Xmg/0.7mL) and Line width

A suitable sample amount should be selected according to the nucleus to be observed and the purpose of measurement. In ^{13}C observation to increase the sensitivity, as much sample as possible should be used. However, in ^{1}H observation too much of the sample dissolved in the solvent increases the viscosity of the sample solution (slows down molecular tumbling) and deteriorates separation of peaks. Slow molecular tumbling increases spin-spin (T_2) relaxation rates, thereby shortening spin-state lifetimes and increasing observed NMR resonance line widths. Fast molecular tumbling allows us to observe narrow resonances.

Fast-relaxing spins produce rapidly decaying FIDs and broad resonances, while spins that relax slowly produce longer FIDs and narrower resonances. ($\Delta\nu_{1/2} = 1/\pi T_2^*$):



In order to obtain the best spectrum, therefore, it is necessary to adjust the sample amount according to the observed nucleus. Usually, several milligrams (0.1mg-10 mg) of sample are used in ^{1}H observation and as more sample as possible in ^{13}C observation. When using the same sample for ^{1}H and ^{13}C observation, use 20 - 50 mg of the sample, considering the sensitivity to ^{13}C as the most important factor.

Filtration

Samples for solution- state NMR should be homogeneous liquids. Samples that contain heterogeneous material in the tube, solids, bubbles, or immiscible liquids exhibit broad NMR lineshapes, and this broadening cannot be shimmed out. Such samples should be filtered before use, if high- resolution spectra are desired.

Height of liquid

Powdered sample is put in an NMR sample tube as it is. Then an appropriate solvent is added.

Each probe has an optimum height for the solvent. The best S/N ratio and resolution can be obtained at the height of 40 mm in the case of the ROYAL probe (400/500MHz JEOL) and of 60 mm in the case of the VNMRS Probes (400/500 MHz VNMRS) and 600 MHz (Varian INOVA).

Immediately after the solvent is added, even if the sample seems to be well dissolved immediately it may not have been sufficiently dissolved. Caution is required, especially when handling high molecular weight polymers. If the sample has not sufficiently been dissolved, separation of peaks may not be good, or the target peak may not appear at all. In such cases performing sufficient heating and stirring the sample or heating and leaving the sample for some time to let it dissolve before measuring it can frequently improve the quality of the spectrum.

Shorter samples take longer to shim and require more re- shimming between each sample. For best results and minimum shimming time, samples should be prepared of similar heights. If you need to use less than **0.7 ml** of solvent for any reason, you can center the liquid volume in the cross- hatched area.

Internal reference compounds for ^{1}H NMR

Usually, TMS (Tetramethyl silane) is used as the standard substance. In high-temperature measurements, however, since TMS is volatile, HMDS (Hexamethyldisilane) or HMDSO (Hexamethyldisiloxane) is frequently used. In deuterated solvents, which do not dissolve TMS, TSP sodium Trimethylsilylpropionate) or DSS (sodium Dimethylsilapentane sulfonate) are used.

To samples using heavy water as the solvent TSP is suited.

One thing to be remembered is that the signal intensity of the standard substance should not exceed 30% of the signal intensity of the target sample. Also, if measurement is performed in the state in which the standard substance is not mixed sufficiently with the sample substance such as immediately after the standard substance has been added, the peak of the standard substance may split.

Degassing

In order to obtain good high-resolution spectra, it is sometimes necessary to remove oxygen dissolved in the sample solution. Usually, it is removed by repeating freezing and evacuation using a vacuum system. A simplified method of substituting an inactive gas (Ar, N2, or He) for oxygen is also frequently used. Dissolved oxygen influences measurements of relaxation times and NOE. To precisely perform these measurements, it is necessary to degas the sample well.