Things to know before you begin operating an NMR

NMR Sample Preparation

These are some guidelines for preparing a good NMR sample:

The NMR tube

Make sure that the NMR tube is clean inside AND outside. Tubes that are dirty on the outside cannot be put into the NMR instrument and will also result in bad spectra. The tube should be in good condition with no cracks or chips. If the top of the NMR tube breaks take it to the glass blower to grind it down – however the tube must not be shorter than 16 cm.

Always label your NMR tube with the name of your sample. DO NOT write all over the tube but rather write the label on the NMR tube cap.

Choosing a solvent for NMR

NMR samples need to be dissolved in deuterated solvents. Deuterated solvents tend to be expensive so to find out what solvent you should use. Take a small amount of your material and try dissolving it in a protonated solvent first. Evaporate properly before testing the next solvent.

Do not put any of the protonated solvent in your NMR tube! The cheapest and most common deuterated solvent is $CDCl_3$ but there are many others available which can be found in a chemical supplier's catalogue.

NMR Solvents

Solvent	Formula	^{1}H shifts	^{13}C shifts	mp °C	bp °C
Acetic Acid-d_4	CD_3COOD	2.0, 11.7	20.0, 180.0	16.6	117.9
Acetone-d_6	CD_3COCD_3	2.09	29.9, 206.7	-94.7	56.1
Acetonitrile-d_3	CD_3CN	1.94	1.4, 118.7	-43.8	81.6
Benzene-d_6	C_6D_6	7.16	128.4	5.5	80.1
Carbon Disulphide	CS_2	none	192.8	-11.6	46.3
Carbon Tetrachloride	CCl_4	none	96.7	-23.0	76.8
Chloroform-d	$CDCl_3$	7.24	77.23	-63.5	61.3
Dichloromethane-d_2	CD_2Cl_2	5.32	54.0	-95.1	40.8
Diethyl Ether (-100 °C)	$(CH_3CH_2)_2O$		14.5, 65.3	-116.3	34.6
Dimethyl Ether (-100 °C)	CH_3OCH_3	3.2	60.08	-138.5	-23
N,N-Dimethyl formamide-d_7	Me_2NCHO	2.95, 2.75, 8.03	29.8, 34.9, 163.2	-60	153
Dimethyl Sulfoxide-d_6	CD_3SOCD_3	2.50	39.5	18.6	189.0
1,4-Dioxan	$(OCH_2CH_2)_2$	3.53	66.7	11.8	101.4
Ethanol-d_6	CD_3CD_2OD	1.11, 3.56, 5.19	17.3, 57.0	-117	78.3
Methanol-d_4	CD_3OD	3.31, 4.78	49.2	-97.8	64.6
Nitrobenzene	$C_6D_5NO_2$	7.5, 7.7, 8.1	123, 129, 135	5.7	210.8
Nitromethane-d_3	CD_3NO_2	4.3	57.3	-28.6	101.2
Pyridine-d_5	C_5D_5N	7.22, 7.58, 8.74	123.9, 135.9, 150.2	-41.6	115.3
1,1,2,2-Tetrachloroethane	$CHCl_2CHCl_2$	5.91	74.2	-43.8	146.3
Tetrahydrofuran-d_8	C_4D_8O	1.73, 3.58	25.4, 67.6	-108.5	65.4
Toluene-d_8	$C_6D_5CD_3$	2.09, 6.98, 7.00, 7.09	20.4, 125.5, 128.3, 129.4, 137.9	-94.9	110.6
Trichlorofluoromethane	$CFCl_3$	none	117.6	-111	23.7
Trifluoroacetic Acid-d_4	CF_3COOD	11.5	116.6, 164.2	-15.3	72.4
Trifluoroethanol-d_3	CF_3CD_2OD	3.88, 5.02	61.5, 126.3	-15.3	72.4
Water	D_2O	4.8	none	0.0	100.0
Solvent	**Formula**	**^{1}H shifts**	**^{13}C shifts**	**mp °C**	**bp °C**

Most solvents used for NMR analysis are toxic – please read the warnings on the data sheets for the solvent and take care when making up samples!

Quantity of Material

This will vary from sample to sample.

For a small molecule: Use 20-50 mg of your compound if you would like to get a ^{13}C spectrum in a reasonable time. (^{1}H experiments are much more sensitive and so fewer samples can be used if you only have a small amount of material and don't need a ^{13}C spectrum.) Overnight or longer experiments can be run on small quantities of material if you do not have a lot of your compound. If you find that the compound is not very soluble and some is not dissolved then filter off the suspended material before placing the sample in the NMR tube as solid materials in the tube will result in a bad spectrum.

For larger molecules (eg. polymers): For larger molecules try adding more compound. 50 mg and more will probably be required. This will obviously also depend on the solubility of your sample.

As NMR is not a very sensitive technique (compared to other spectroscopic methods) it is desirable to have a reasonable amount of material to get quick results, however having too much sample can also be a problem. Extremely viscous samples will have broad lines which is one of the reasons not to add too much sample. Try to avoid preparing very viscous samples if possible.

Getting to know how much sample you should add for your types of compounds will be trial and error in the beginning.

Solid Particles and Paramagnetic Materials

Remove all solid particles from your NMR sample by filtering it before placing it in the NMR tube. Solid particles will result in bad spectra and if your entire sample does not dissolve you will not get a true representation of what you have in your vial. The instrument only 'sees' what is dissolved, and that can be your target compound or impurities. Either way, you will not get a true representation of your sample. Solid particles result in a reduction in the transverse relaxation times (T_2's) of the nuclei causing broad peaks. In addition it is impossible to homogenise the magnetic field resulting in bad peak shapes and loss of resolution.

Paramagnetic materials will also result in unsatisfactory spectra as the lines become extremely broad and resolution is lost. Line width at half height is calculated as $1/T_2$ and paramagnetic nuclei or suspended solids result in very fast T_2 relaxation times. Thus a very small T_2 value results in broad line widths.

Adding the solvent

For a good NMR spectrum the height of the sample in the NMR tube should be around 5 cm. It is better to have a slightly lower concentration and a sample of reasonable height than a short sample with higher concentration. If you have very little material there are different NMR tubes that can be used and you should ask your NMR operator for assistance.

DO NOT fill up the NMR tube with solvent. Samples should not be higher than 5 to 5.5 cm. Using too much solvent is not only wasteful but can cause technical problems for the operator of the NMR instrument due to convection in the sample being increased. If you make a mark on the NMR tube for measuring the correct solvent height, clean it off before you submit the sample for analysis.

High/Low Temperature Samples

Please ensure that you know the boiling/freezing point of your solvent when submitting a sample for high/low temperature NMR. For high temperature samples please test your sample for one hour at 20 ˚C above the temperature you intend to have the NMR experiment run at.

Note that users do not need to provide UHP nitrogen gas for high temperature and low temperature samples. Liquid nitrogen will however be required for low temperature samples. These will not be provided by the NMR laboratory.

REMEMBER:

CLEAN TUBE (INSIDE AND OUT)

TUBE LONGER THAN 16cm

NO SOLID PARTICLES OR PARAMAGNETIC MATERIALS

DEUTERATED SOLVENT HEIGHT 5cm

ENOUGH SAMPLE

REASONABLE VISCOSITY

Operating the NMR instrument (after you had training from the NMR staff)

NMRs are expensive and delicate. Misusing or abusing them can cause hundreds of thousands of Rands worth of damage. Most damage is avoidable, some is not. If you understand how the instrument works, you will also develop some feeling for why things should be done a specific way. If, on the other hand, you do things your own way and disregard normal procedure, you run the risk of damaging the instrument so that you and everyone else will not be able to use it for an extended period of time. Because of the danger posed by improper use of the instrument, those taking care of the NMRs often will revoke or severely curtail the access certain individuals have to NMR instrumentation. This is simply the reality of NMR operation in a multi-user environment. Please keep this in mind if and when a staff member has a word with you about proper operating procedure. The Staff can and will limit your access if they feel it is needed. You can request access to the NMR unit entrance once you got your training.

Basic Safety:

NMR magnets are always live and always at field. They cannot be turned off like a light switch. Therefore, nothing ferromagnetic is allowed near them. This includes tools (hammers, wrenches, and screwdrivers), paper clips, staples, bobby pins, metal barrettes, costume jewellery, wallet chains, metal buckets, metal chairs, and floor buffers. Items such as iPods, cell phones, and media storage devices can also be damaged by the magnetic field. ATM and credit cards will become unusable. Please leave all of these items in your lab or next to the instrument's computer when you approach the magnet.

You will also receive a safety agreement to read and to sign off on.

Liquid-state NMRs normally operate in the following manner:

Your sample is dissolved in the selected deuterated solvent and transferred to a NMR tube. The solvent is usually deuterated, meaning that is has deuterated protons (^2H) in place of its protons (^1H). A proper amount (0.6 to 0.7 mL (5cm height) when using a 5mm diameter NMR tube) of the sample solution (solute and solvent) is placed in an NMR tube. Normal NMR tubes range in price from about a R10 per tube to a few hundred rand per tube. It is important to keep in mind that you get what you pay for. A cheap (economy) tube will often have a widely varying wall thickness, a varying outside diameter, or a pronounced bend or bow that will make it difficult to spin and shim. The more expensive the tube, the less likely it is that the tube can be blamed for poor results. Using an expensive NMR tube does not guarantee good results, but using a cheap NMR tube will often give problems with spinning, shimming, and hence with the line shapes you obtain, not to mention the possibility that you may break your tube (if it is badly warped) and contaminate the NMR probe with your sample.

Improper cleaning of NMR tubes can also render an expensive tube useless. Do NOT leave NMR tubes in a drying oven for extended periods of time – this causes the tubes to bend and flow under the force of gravity, thus making them non-cylindrical. The best way to clean a tube is to rinse it with appropriate solvent and finish with a couple of rinses of high grade acetone.

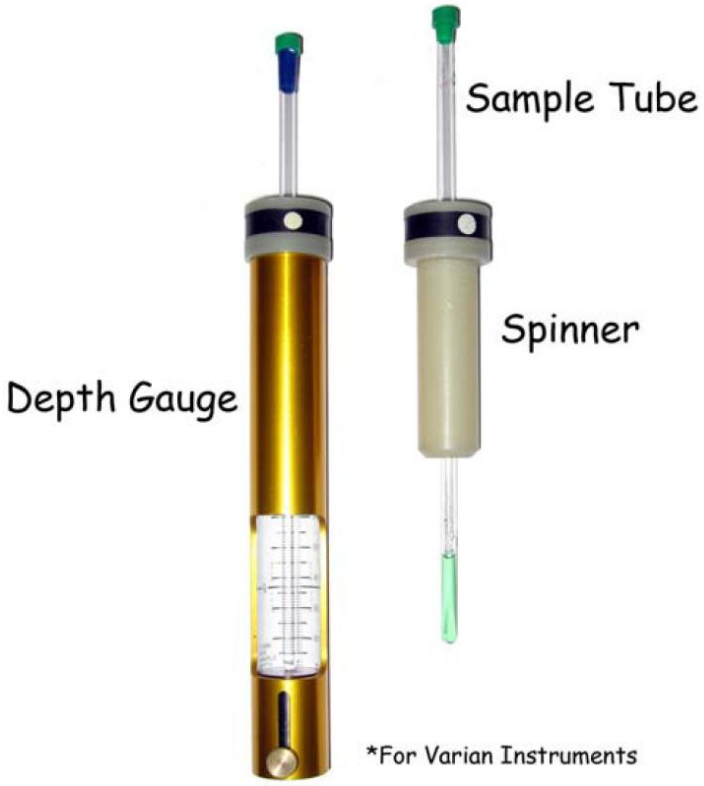
This final solvent rinse can be quickly expelled from the tube with dry air, nitrogen, or argon blown through a drawn-out Pasteur pipet. If you want, you can also place the nearly dry tube (don't start a fire!) flat in a glassware oven for ten minutes. Placing the correct amount of solvent in the NMR tube is important for a number of reasons. The most important reason is that the standard shims you use as a starting point for shimming on your particular sample assume that the solution meniscus is located at a certain position above the detected region of the sample. This means that with a normal amount of shimming, you will obtain better signal-to-noise if you use 0.6 to 0.7 mL of solvent, even if this means diluting your sample.

In cases when the amount of the solute is limited and gradient shimming fails, an extensive amount of manual shimming of the higher-order shims (z3, z4, z5, etc.) might be necessary to compensate for the use of a smaller sample volume. That is, you have a choice: you can either spend more time manually shimming, after gradient shimming, on a smaller volume of solution or you can dilute your sample down to 0.7 mL and spend all the time that you would have had to spend on shimming just collecting data instead. Informed individuals often choose the latter, but in some cases – e.g., those working with small amounts of natural products – people actually do find it necessary to resort to nonstandard shimming methods, special NMR tubes (e.g., Shigemi tubes), or even special NMR probes (e.g., Varian nano probes or cold probes).

The NMR tube must be positioned properly in a spinner (See Figure 1) before it can be introduced into the NMR instrument. A spinner is usually a piece of kel-F or some other polymer with some gripping mechanism (e.g., a rubber band or a rubber o-ring) to hold the tube securely in place. Before the tube is inserted into the spinner, it should be thoroughly wiped off with a clean paper towel or equivalent. Failure to wipe off grease and other chemicals off of the outside of the NMR tube will contaminate the spinner and may even cause the spinner to fail to grip the tube properly.

WIPING OFF THE TUBE IS IMPORTANT.

Figure 1

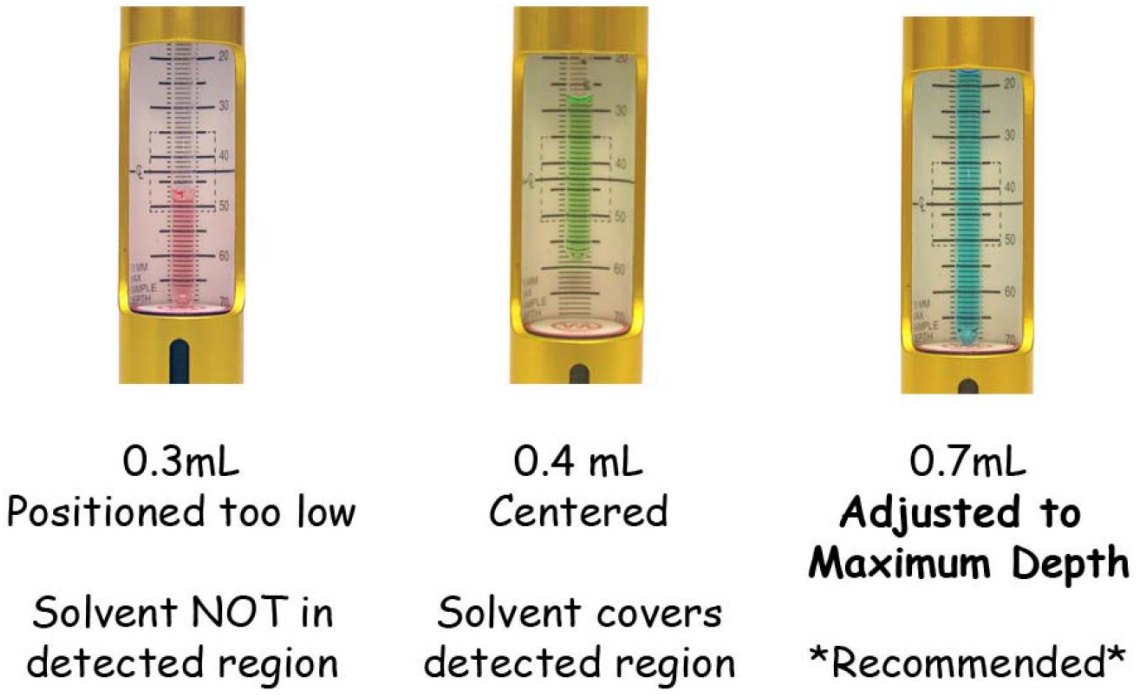


If the NMR tube slides up and down too easily in the spinner, then somebody has already contaminated the spinner (or it is old). If you notice that the tube slides up and down too easily, then you should ask the NMR staff for the spinner to be cleaned or swapped. If the tube slides down too far upon insertion into the magnet, bad things can happen (broken probe, broken sample, impossible to shim by hand).

The NMR tube needs to be positioned in the spinner so that your solution is in the detected region of the NMR probe once the spinner is in place. The tube should never exceed the maximum allowable sample depth for the NMR probe that is inside the magnet. Exceeding the maximum allowed sample depth can break sample or probe or both. That is, the tube should not stick down so far that it hits a

part of the probe that it is not supposed to touch. The sample depth gauge should contain information on what the maximum allowable sample depth is for a specific type of NMR probe (the maximum depth is usually only a function of the diameter of the NMR tube that the probe is designed to accommodate). Another important consideration to keep in mind when positioning the sample tube in the spinner is that the bottom of the NMR tube and the solution meniscus at the top should be equidistant from the center of the detected region (see Figure 2). The only time this condition should be violated is when the tube cannot go any lower because one has slid the tube down to the maximum allowable sample depth. Failure to properly position your sample (especially if you are using a smaller-than recommended volume of solution) will make your sample difficult to shim. The sample depth gauge is already set to the correct setting so please do not change the setting.

Figure 2



After the NMR tube has been positioned, it is important to again wipe off the tube (taking care not to disturb its alignment with respect to the spinner). This is necessary because others may not have been as conscientious in wiping off their NMR tubes prior to inserting them into the spinner. That is, your tube may have picked up contaminants as you slide it into the spinner. Inserting a dirty tube into the NMR will cause two problems: one, it will deposit chemicals and/or dirt in the probe that will generate an unwanted background signal; and two, it will make sample spinning difficult. Introducing the spinner with the NMR tube into the NMR magnet is normally done on a stream of air. Prior to placing the sample/tube at the top of the upper barrel (the tube that sticks out of the top of the NMR magnet), the lift or eject air must be turned on. All modern NMRs turn on the lift/eject air

with the computer software (manual switches are only there as an emergency backup in the event that a sample needs to be removed and the computer is down).

NEVER DROP YOUR SAMPLE INTO THE MAGNET WITH NO LIFT/EJECT AIR FLOWING!

This causes a great deal of damage and happens more than you might think. Once the sample has been gently lowered into the magnet on a cushion of air, you should be able to spin, lock, shim, tune, and acquire NMR data.

Spinning:

Sample spinning is done to improve the observed NMR line shapes. This occurs because any solute molecule not on the sample spinning axis will travel in a circular path twenty times a second if the sample spin rate is 20 Hz. Since the acquisition time for each scan of the sample typically takes one or more seconds, the solute molecule will feel only the average of the magnetic field strengths as it travels in a circular path since each rotation occurs in 50 ms (1/20th of a second). Sample spinning basically eliminates the need to adjust any shim with an **x** or a **y** in the name, unless the spinning sidebands are very large. Spinning sidebands will occur at 20 Hz on either side of the center peak in the frequency domain spectrum (after the Fourier transform has converted the signal from the time domain to the frequency domain) if the xy shims need significant optimization and if the spinning rate is set at 20 rotations per second (20 Hz).

Locking:

Locking is a means of compensating for transient variations of the magnetic field strength.

All superconducting magnets run down slowly over time – this phenomenon is called magnet drift and is caused by slight imperfections in the main superconducting coil of the magnet – usually at the weld where the two ends of an incredibly long piece of wire (many kilometers in length) are fused back together. A tiny amount of resistance at the weld will cause a slight dissipation of power which will slowly draw down the many tens of amperes flowing in the main coil over time. Typical drift rates are in less than ten Hz per hour (for proton observation), with many magnets having drift rates of less than one Hz per hour. Even at 10 Hz/hr, a 300 MHz magnet will take more than a year to lose a tenth of a MHz, and will take more than 3400 years to drift down to zero field. Superconducting magnets are the closest thing we have to a perpetual motion machine, as long as they are kept cold with liquid helium and liquid nitrogen. While 10 Hz/hr of drift may seem small, it is quite large if one considers that we can often shim our solute lines down to less than 1 Hz and we often need to collect data for more than an hour at a time. Without the lock, a sample with a line width of 1 Hz being run in a magnet with a 10 Hz/hr drift rate would, after one hour acquisition, show an apparent line width

of 11 Hz. The field lock works by using the proportionality of the resonant frequency of the deuteron to the resonant frequency of the NMR active nucleus of interest, e.g., ^{1}H, ^{13}C, ^{31}P, etc. (Those that observe ^{2}H and want to lock often resort to a 19F lock channel, but this is very unusual). That is, the lock circuitry in the NMR monitors the frequency of the NMR signal of the deuterium in the NMR solvent and adjusts the frequency being used to collect data on the nucleus of interest in proportion to the change in the ^{2}H NMR frequency. The reliance of the lock circuitry of the NMR on observing the NMR signal from deuterons requires the use of isotopically enriched solvents. This is why deuterated solvents are often referred to as NMR solvents. Deuteration of the solvent also serves another important purpose in that it allows one to observe the signals of the solute without a large signal from the solvent. The solute proton NMR signal would otherwise be overwhelmed by what would be a much more intense proton NMR signal from the solvent were it not for the replacement of the protons on the solvent molecules with deuterons. Momentary changes in the magnetic field strength are also compensated for by the deuterium lock, as long as the changes do not occur too rapidly. This means that the momentary turning on or off of high amperage electrical devices, e.g., laser power supplies, arc welders, etc., will normally not render an NMR completely unusable – although the effect of external perturbations are often apparent to the discerning eye. In general, the best NMR results are obtained when the rest of the world is asleep and not using electrical appliances and other devices. The lock also serves another important function – it allows one to improve the homogeneity (evenness) of the magnetic field in conjunction with the shims.

Shimming:

Shims are coils of wire (see Figure 3) wrapped in such a way that changing the current being passed through these coils will affect the strength of the magnetic field in the detected region of the sample in a specific way. Many modern NMR instruments have many different shim gradients that can be optimized to obtain the narrowest possible line shape for a given sample. Since each sample is different, there is no one ideal set of shim currents that will give the best results every sample. Shimming has a firm basis in geometry, but one often lacks sufficient information to make informed decisions as to what shim (e.g., z1, z2, z3) should be adjusted in which direction (higher or lower current). Shimming is often done by adjusting a given shim and observing its effect on the lock level. The lock level essentially corresponds to the height of the deuterium line shape. Under normal circumstances the area of the deuterium NMR signal will be constant with respect to time. Making the field more homogeneous by improving the shimming will cause all deuterons with a particular chemical identity – e.g., all of the deuterons of the $CDCl_3$ molecules in the sample – to resonate more nearly at the same frequency. Since the area of the deuterium NMR signal is constant and the difference in the frequencies (the line width) of the deuterons goes down, the height of the line must increase to keep the product (area) constant. This means that empirical adjustment of the shims to

maximize the lock level is often a reasonable way of optimizing shims. If a good starting shim set (rts ('CDCl3')) is used, a normal sample should only require adjustment of z1 and z2 to obtain decent shims.

The most common reasons for poor shims are:

1. Bad starting shims (did not load a good starting shim set)

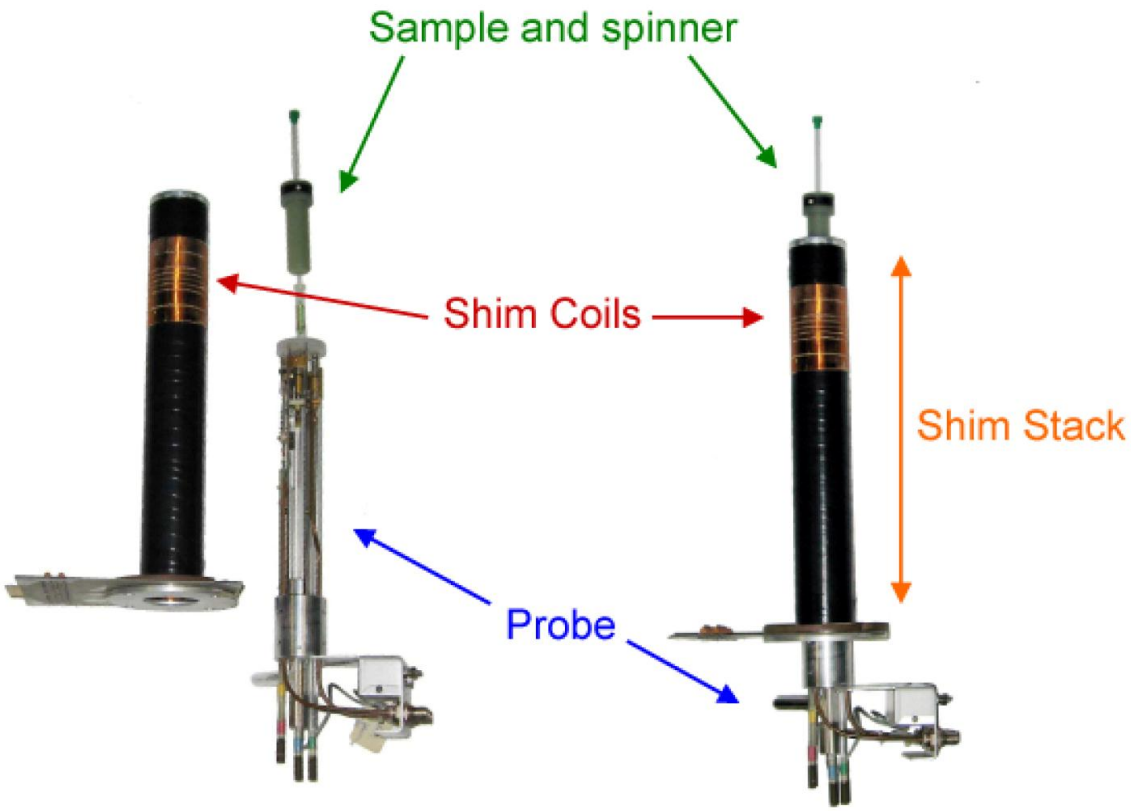
2. Low quality and or flawed NMR tube

3. Insufficient volume of solution

4. Improperly positioned NMR tube

5. Unusual nature of sample, e.g., highly concentrated, viscous

Figure 3



Starting with terrible shims means that you will have to do a lot of shim adjustments to get to that ideal point where your sample is perfectly shimmed. In practice, you never obtain perfect shims. The reason why optimal shims for each sample vary so much can be explained very simply: everything has a unique magnetic susceptibility. Magnetic susceptibility is essentially a measure of how well a

particular material can accommodate magnetic field lines. Whenever there is a transition from one material to another, the density of field lines will change at the interface and this will introduce magnetic field heterogeneity. Shims are how we compensate for these heterogeneities to make the field as homogeneous as possible. Keeping the bottom of the NMR tube and the field heterogeneity associated with this interface away from the detected region of the sample is just common sense. The problem is that moving the bottom of the tube too far down may bring the meniscus (and the much lower density vapour with a different magnetic susceptibility) at the top close to the detected region. This is why the sample liquid volume needs to be centred (without putting the sample too far down into the probe). Low quality NMR tubes have walls with a varying thickness, and the glass may itself have a heterogeneous composition. A perfect NMR tube will have an unvarying chemical composition, will be perfectly cylindrical, and will have a perfectly rounded bottom. Cheap tubes deviate farther from this ideal than do expensive ones, and hence, cheap tubes introduce more variations in the magnetic field strength than do good ones. After a sample has been shimmed, you must load a set of experimental parameters from computer memory before you can collect any NMR data. Usually there is a specific command (*su* on a Varian, *ii* on a Bruker) that will let the NMR hardware know what nucleus (frequency) you are about to observe.

Tuning:

Once you have told the hardware what frequency you are going to be using, you will often want to tune the NMR probe for that exact frequency. Failure to tune the probe in tuneable systems can have very grave consequences for the instrument (and possibly the operator). Probe tuning serves two purposes. One, probe tuning maximizes the forward power and minimizes the reflected power; and two; probe tuning maximizes the transmission of the NMR signal from your sample to the receiver. In some cases, a poorly tuned NMR probe will cause a great deal of damage to the instrument because the power intended for the sample will instead be reflected back into some other part of the instrument not designed to dissipate large amounts of power. The other problem with having the power not go into the sample in the probe is that the power will not be able to excite the sample as intended. In order to observe an NMR signal, the NMR-active spins must first be perturbed from equilibrium with the application of radio frequency (rf) electromagnetic radiation. If the rf never gets to the sample, it cannot excite the sample and hence there will be no signal to detect. Although many NMR experiments do not require that the amount of power being used to excite the spins be well-controlled, some do. If there is any doubt as to whether or not one should tune the probe, it is always better to tune the probe (as long as one knows how to move the cables back to their proper configuration when one is done tuning). Another reason to tune is to maximize the transmission of the NMR signal from sample to receiver. Fortunately, tuning to maximize forward power and to maximize receiver sensitivity does not involve compromise – that is, both are

optimized when the probe is properly tuned for a particular sample. A poorly tuned probe will often give no signal and just show noise – even if the sample is concentrated. Probe tuning is done by either minimizing reflected power at the frequency of interest (the tune box meter reading will be minimized) or by locating the dip in a line using a sweep generator (complex impedance as a function of frequency). Probe tuning is done by adjusting variable capacitors located inside the NMR probe that are near the detected region of the sample. These capacitor adjustments are done by turning or sliding rods that stick out of the bottom of the probe at the base of the magnet. Often it will be necessary to play the 'tune' capacitor off of the 'match' capacitor to arrive at the optimal probe tuning. With practice, one can become quite adept at probe tuning. Mastering probe tuning will, in the long run, save you time. This is because the improvement in the signal-to-noise per amount of experiment time will more than offset the time initially investing in tuning the probe. Optimal probe tuning will vary from one sample to the next.

Factors that influence tuning frequencies:

1. Variations from one NMR tube to the next (expensive tubes exhibit less variance)

2. Different NMR solvents have different magnetic susceptibilities

3. Solutes present in appreciable (non-dilute) amounts will give the solutions different magnetic susceptibilities

4. Slight differences in sample positioning

5. Different volumes of solution

6. Different temperatures

For routine ^{1}H and ^{13}C spectra the tuning should be fairly close to optimal and don't need to be re-tuned. In general, using low concentrations of solutes (or similar solutes with similar concentrations), the same NMR solvent, and the same type of high quality NMR tubes will often render probe tuning for every sample unnecessary. *Only the NMR staff and a few trained senior students are allowed to tune the probes.*

Different NMR instruments and their probes will tolerate different amounts of abuse before they fail. The only way to know how much abuse is too much is to break them. This is not information you want to obtain. At this point, the acquisition of the NMR data set is relatively easy, although some of the more sophisticated NMR experiments will require the calibration of the rf pulse or pulses in order to work properly. If one understands the material and heeds the advice given in this document, then it is very unlikely that one will cause a great deal of avoidable damage to an NMR (with the exception

of bringing a large ferromagnetic object near the magnet or when conducting a variable temperature experiment). To summarize, one should understand the importance of the following:

1. A good quality NMR tube

2. Using a deuterated NMR solvent

3. Using the proper amount of solution

4. Cleaning and positioning the NMR tube in the spinner

5. Introducing the sample and spinner into the NMR

6. Spinning the sample

7. Locking

8. Shimming

9. Tuning the probe