NMR (Nuclear Magnetic Resonance) spectroscopy

Introduction

NMR is a technique that is used to analyze the structure of many chemical substances, primarily organic and inorganic compounds.

NMR experiment consists of three steps, in its simplest form:

1. Place the sample in a static magnetic field.
2. Excite nuclei in the sample with a radio frequency pulse.
3. Measure the frequency of the signals emitted by the sample.

Using NMR spectroscopy, information about the bonding and arrangement of the atoms in the sample can be deduced from the emitted frequencies. The frequencies also yield qualitative information regarding the local atomic environment. The integrated intensity of a signal (determined by integrating the area under the signal peak) is a measure of signal strength. The integral will be directly proportional to the number of nuclei contributing to a signal at a particular frequency (if all nuclei are equally exited) and hence will provide quantitative information regarding chemical structure.

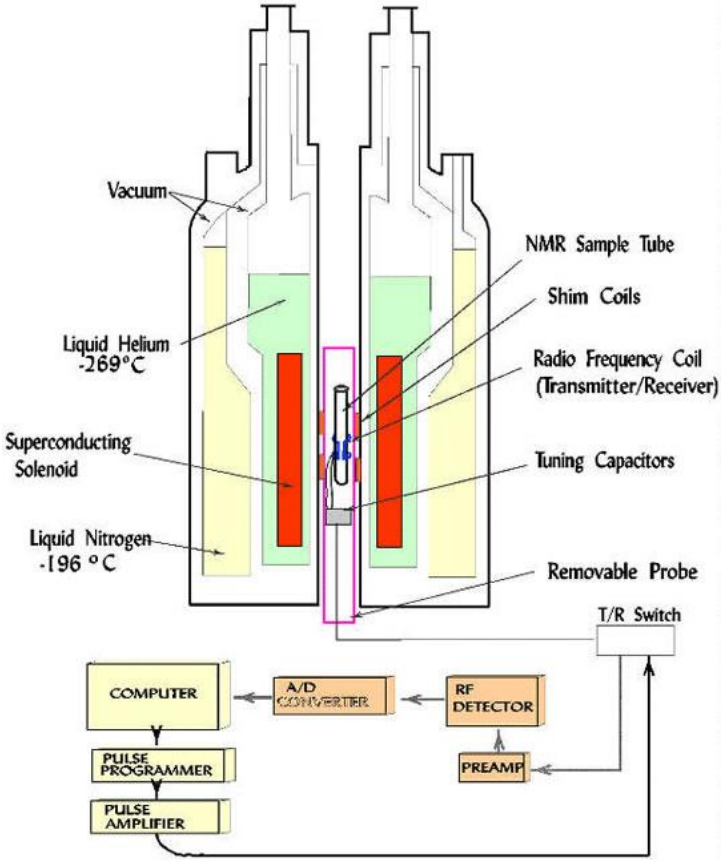

Figure 1: Schematic diagram of a cross section of NMR spectrometer

Pay attention before operating an NMR

NMRs are expensive and are delicate. If misused or abused, the damages caused could be worth several thousands of dollars. After understanding how the instrument works, you will be able to make the maximum use of it and use it properly for each specific task. If, however, you start using it without following required procedures, you risk causing serious damage, so that no one will be able to use it for a considerable period of time.

What to pay attention during sample preparation and usage of the NMR spectrometer:

A good quality NMR tube
Using a deuterated NMR solvent
Using the proper amount of solution
Cleaning and positioning the NMR tube in the spinner
Introducing the sample and spinner into the NMR
Spinning the sample
Locking
Shimming
Tuning the probe

In order to obtain the best possible data, you should pay attention not only to the sample preparation but also the vessel in which it is contained. By following only a few simple guidelines, you will be able to acquire quality NMR data in an efficient manner.

How to choose a suitable NMR tube and proper sample preparation for NMR analysis

NMR tube

- Standard NMR tubes range in price from a dollar to a two-digit number per tube. A better-quality tube usually will be more expensive, but you will avoid variations in wall thickness and/or outside diameter as well as some bends or bows that might result in poor spinning and shimming. A good quality tube can be seen as a predisposition to good NMR results. It does not guarantee them, but using a cheap tube will often result in problems with the line shapes (due to poor spinning and shimming) and even breaking the tube (if it is significantly bent) and contaminating the NMR probe with your sample. **Do not** use chipped, cracked or deformed NMR tubes!

- Improper cleaning of the NMR tube can render a good quality tube useless. **Do not** leave tubes in a drying oven for a long time. This can distort the tube, due to the high temperature and flow under the force of gravity, and cause them to lose their cylindrical shape. It is best to clean the tubes with an appropriate solvent and finish with a few rinses, using HPLC-grade acetone. The acetone can be easily expelled from the tube with dry air, nitrogen or argon. The nearly-dried tube can also be placed flat in a glassware oven for approximately 10 minutes. For more information on proper cleaning procedures follow the link: http://www.wilmad-labglass.com/Support/NMR-and-EPR-Technical-Reports/Proper-Cleaning-Procedures-for-NMR-Sample-Tubes/

Using a deuterated NMR solvent

- Using a deuterated solvent has two advantages: deuterium is used for field frequency lock, thus making spectral resolution easier to acquire in less time; the proton signal from the solute is more pronounced compared to the solvent one (if a deuterated solvent is not used, the proton NMR signal from the solvent will overpower the signal from the solute). Therefore, it is highly recommended to use deuterated solvents as often as you can.

Using proper amount of solution

- In order to have an NMR spectrum, the sample should be sufficiently soluble. The greater the solubility of the sample in the deuterated solvent the better the spectrum is. **Do not** leave any suspended particles in the sample. If your solution is turbid, first filter it and then introduce in the NMR instrument.
- Introducing the correct amount of solvent is also an important step in sample preparation. The amount of solvent in your NMR tube should be sufficient to ensure that the meniscus level is located at a certain position above the shimming detection region of the sample. It is recommended to dissolve 2 to 10 mg of your compound in between 0.6 to 1 mL of solvent so that the sample depth is about 4 to 5 cm in the tube. Thus, a better signal-to-noise ratio with a normal amount of shimming is obtained. (A small amount of solvent will give a bad spectrum, and large amount of solvent a good spectrum, but it's a waste of expensive deuterated solvent).

- If the amount of solute is very low, a long shimming of higher order shims (z3, z4, z5, etc.) can compensate for the smaller amount of solvent in the NMR tube. In this case you should adjust the depth of the NMR tube, using the sample depth gauge (Figure 2). The other solution to the problem is to dilute your sample to increase the volume to 0.7 ml and increase the scanning time, keeping the sample depth as for a normal sample.
- It is sometimes preferable to use higher concentrations in the case of compounds with high molecular weights. Too concentrated a solution, however, results in lower resolution due to increased viscosity. For ^{13}C NMR between 10 and 50 mg of sample is recommended. In case the sample amount is limited or the solubility is low, it is still possible to acquire a spectrum at the expense of more time.
- In certain cases, non-conventional shimming methods (e.g., pulsed field gradient shimming), special NMR tubes (e.g., Shigemi tubes), or special NMR probes (e.g., Varian nanoprobes) can be used, especially when working with very small amounts of natural products.

Cleaning and positioning the NMR tube in the spinner

- The NMR tube must be placed properly in the spinner, before introducing it in the NMR machine (Figure 2). The spinner is a cylindrical tube, made out of Kel-F or other polymer with a rubber band or rubber O-ring at one end, which holds the tube well in place. Before introducing the tube in the spinner, it should be cleaned with Kimwipe or equivalent wipes. If not some grease and other chemicals on the outside of the tube may contaminate the spinner and even cause not to keep it properly in place. **Therefore, wiping off the tube is very important.**
- It is important to introduce the tube into the spinner carefully, by gently gliding it inside, since otherwise it might break. On the other hand, if the tube slides too loosely inside the spinner, it might be that the latter is contaminated or too old. In this case clean the spinner, use another one or ask for help to clean or replace it.
- The NMR tube must be positioned in the spinner so that the sample depth does not exceed the maximum allowable depth inside the NMR probe (Figure 2). If the maximum allowable depth is exceeded, the tube or probe or both might break (the tube should not stick out of the spinner too much or otherwise hit part of the probe). In order to avoid this mistake,

always use a sample depth gauge that indicates the proper sample depth for a specific NMR probe (the maximum sample depth is usually only a function of the diameter of the NMR tube that the probe is designed to accommodate).

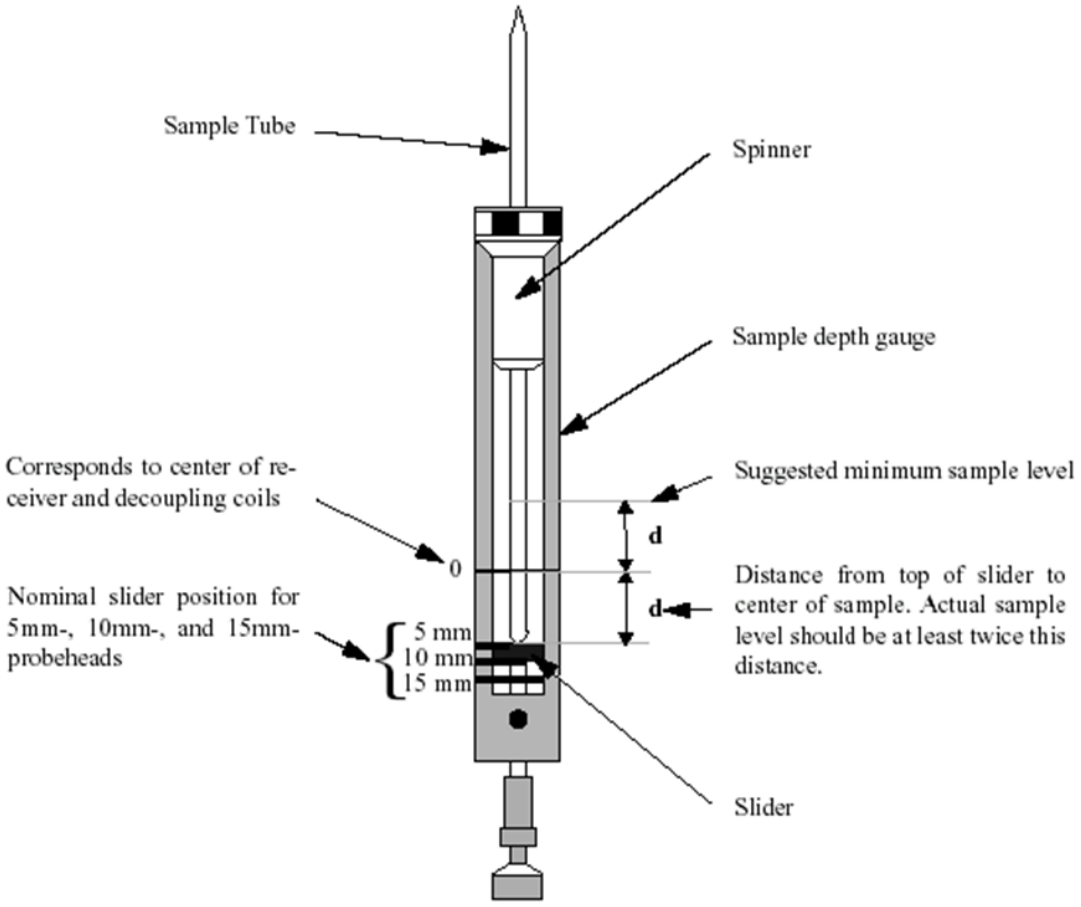

Figure 2: Sample positioning using sample depth gauge.

- The NMR tube must be placed inside the spinner so that the detection region is in the middle of the distance determined by the meniscus at the top and the bottom of the tube (Figure 2). Once again use the sample detection gauge provided to see where the detection region is. The above directions for placing the tube can be disregarded only when the tube cannot go any lower, because it is down to the maximum allowable sample depth. If the sample is not positioned properly (particularly in the case of insufficient solution volume), shimming will be very difficult.
- Wiping off the tube one more time, after inserting inside the spinner is also an important step (do this carefully so that the adjustment with respect to the spinner is not disturbed).

This is necessary, since others might have skipped wiping off the tube and thus introducing unwanted grease and chemicals on the inside of the spinner wall. As sliding the tube inside the spinner, it might collect some of them and contaminate the probe, thus making sample spinning difficult.

Introducing the sample and the spinner into the NMR

- An air flow coming from the NMR machine is normally present when introducing the NMR tube with the spinner inside. Before placing them at the top of the bore tube (this is the black tube that is situated on top of the NMR instrument), the LIFT or EJECT air must be turned on. The air flow is usually turned on automatically with the computer software for most of the modern NMR instruments (manual switches can be used in case of emergency, when a sample has to be removed while the computer is down). Do not place your sample inside the NMR machine with NO LIFT or EJECT air flowing. This can cause serious damage to the instrument.

With the sample inside, you can continue with the standard procedure for obtaining NMR spectrum - spin, lock, shim, tune, and acquire NMR data.

Student Training: Acquiring and Processing NMR Spectra

Part I – Running a sample (^{1}H NMR)

1. Login in Windows – You will see that Topspin will launch immediately after you logged in. The Topspin program allows you to 1) work with spectra and 2) launch Icon-NMR.
2. Opened Topspin window mainly consists of 4 parts:
   a). Toolbar panel on the top of the window
   b). Left half of the window is a browser window. Here you can see all the spectra you run.
   c). Right half of the window is the spectrum viewer. Here you can display a spectrum. To do so you need (in the browser) either double click on the spectrum or drag the spectrum in the spectrum viewer window by holding left button of the mouse pressed.
   d). Under the browser window and the spectrum viewer there is a command line (highlighted in pink), here you can type commands manually.
3. In the toolbar panel go to "Spectrometer", then Icon-NMR, then main screen. You will see the new program allows you to start the experiment and provides your communication with the spectrometer.
4. In the Icon-NMR window you can select the type of the run: "Routine" (usual) or "Automatic". "Routine mode" allows you to run 1 sample in 1 spectrum sequence. In "Automatic mode" (if the sample changer accessory is installed) you can set up as many as 6 samples, and on each sample you can set the sequence of the experiments to run. For basic skills, the knowledge of "Routine mode" is enough. Click "Routine mode", the window with users list will appear. Select the user and hit OK. Then the password window will pop up. Write your password and hit Enter. After that the second Icon-NMR window will appear. In this second window, you can set up the desired experiment.
5. Prepare sample for the run. **This part is extremely important. The improper sample preparation might cause damage to the spectrometer.**
6. Insert sample in the spinner (the blue piece).
7. In the second "Icon-NMR" window press "Insert new sample". In couple of seconds you will hear the air steam. This is the time to insert the sample in the machine (**Do not place your sample inside the NMR machine with no LIFT or EJECT air flowing**. This can cause serious damage to the instrument.) The magnet in the NMR instrument creates a strong magnetic field. Therefore, nothing ferromagnetic is allowed near the NMR

magnets. Among the objects not allowed are tools (hammers, wrenches, and screwdrivers), paper clips, staples, bobby pins, metal barrettes, costume jewelry, wallet chains, metal buckets, metal chairs, and floor buffers. Keep in mind that items such as phones, media storage devices, ATM and credit cards can become damaged by the magnetic field. Leave all ferromagnetic items on the computer desk before approaching the drawn perimeter (black line) on the floor around the NMR instrument. Only after you are free from such items can you cross the black line on the floor and place the sample on top of the bore tube (with the air flow).

8. Return to the computer and hit OK. You have to hear a click indicating that sample went in. On the BSMS control pad, the red light "missing" must change to green light "down".
9. Select the name of the experiment, you can write for example the current date. Write for the "No." any appropriate number or your experiment number. If the current number is occupied (meaning the spectrum already exits) the program will ask you if you want to use "next available No.". You can have several spectra with the same name but under different numbers. Hit "Continue".
10. In the same Icon-NMR window now specify the solvent, the type of the experiment and title. Then hit "OK" or "Continue". Then press "Start".
11. In the first Icon-NMR you can see the sequence of your experiment proceeding. Also on the BSMS control there is a reflection of your experiment. First the sample will spin. (*The spinning is done in order to improve the line shapes*.) You will see the numbers increasing from 1 to 20. If the spinning is not high enough (less than 15) the message will pop up saying that spinning is not ok. You have to remove the sample and change the spinner. After spinning is done the button "Spin on" on the BSMS control will turn solid green. Next the locking process will be started. You can see the lock signal either in the bottom right corner of the "Topspin" window or you can click lock button in the second Icon-NMR window. If the lock signal fluctuates a lot it might be because of the low amount of the deuterated solvent in the sample or the low quality of the NMR tube. After the lock is done, the button "Lock on" on BSMS control will turn solid green. Then the shimming process will be started. (*Shims are coiled wires that can affect the strength of the magnetic field at the detection region, by passing electric current through them. Often one shim is adjusted and then the effect on the lock level is observed. If poor shimming is*

observed, the most common reasons could be: bad starting shims (a bad starting shim set is loaded); bad quality NMR tube; low volume of solution in the tube; improperly positioned NMR tube; highly concentrated sample. While shimming the magnetic environment is adjusted to suit the current sample the best way. You can observe this process either in the lock window or in the command line of the Topspin window (the change Z^1, Z^2 and Z^3 values). After shimming is done (it might take as long as 15 minutes depending upon the sample) the collection of scans will be started, and you can see that happening on the bottom of the Topspin window near the small image of the lock signal. After the scanning is done, the spectrum will appear in the Topspin window (Spectrum viewer).

Part II – Work with the spectrum.

All operations with the spectrum are performed in the "Topspin" window. The use of one or another part of that window will be emphasized.

1. When the spectrum is done it will appear in "Spectrum viewer" as an FID. On the top of the spectrum there will be a command line. In this command line you can find the button (appear as a little spectrum) by clicking it you can display your spectrum as FT. If you would like to zoom in on the part of the spectrum then go to the "Spectrum viewer" window. Press and hold the left button of the mouse and drag it over the region you would like to be displayed in the expansion, after that release the button. In order to view the spectrum in its original size you need to press a button on the toolbar of the Topspin window, which looks like double side arrow) surrounded with a blue square.
2. Peak picking: To perform peak picking on the currently opened spectrum go to "Analysis" menu in the Topspin toolbar. Select "Analysis" and then click "Peak picking". In the window that appears, select the option "Define peak picking manually"' and hit OK on the bottom of the window. You will see that on top of your spectrum in the "Spectrum viewer" the command line will appear. To delete all peaks, select appropriate button (image of peaks with a red cross over them). To select particular peak, select the one of the three buttons corresponding for peak picking. The only difference between them is the way you select peaks. Select for an example one which has an image of an arrow pointing at the peak from top (). The selected button will turn green if it is activated. To deactivate it,

click on it again. When the button is selected you can click on the peak of interest (left click) and the value will appear on the top of the peak. To exit mode and save changes you made, click the button in the end of the command which looks like a floppy disk with the 90^o broken arrow.

3. Integration: To perform integrating on the currently opened spectrum go to "Analysis" menu in the Topspin tool bar. Select "Analysis" and then click "Integration". In the window that appears, select the option "define integrals manually" and hit OK on the bottom of the window. You will see that on the top of the spectrum in the "Spectrum viewer" the command line will appear. To delete all integrals (the machine automatically integrates all the signals) you have to select all of them. To do so you need to select the button with an image of horizontal green strips (). Then click the button next to it to the left. The window will appear asking you if you are sure to delete all integrals. Click OK. All integrals must disappear. Next select the button 2^{nd} from left, looks like the flopped bracket ( ). Once the button is green, the mode is activated. Now press the left button of the mouse then drag mouse through the peak you want to integrate and in the end release the button. The integral value will appear on the bottom of the peak. To calibrate this value right click on the value and in the pop up window select calibrate. If you want to change to the different extension of the spectrum you have to unclick the button and then you can expand spectrum to the desired region without integrating it. To exit mode and save the changes you may click the button in the end of the command, which looks like a floppy disk with the 90^o broken arrow.
4. Printing: To print the spectrum, go to menu "File" and then select "Print" option. In the window that appears, you have three options. To print just the picture of the spectrum select option #1, to "print the active window". To print the picture of the spectrum with the parameters, select the option #2, to print with starting the Plot editor. In the scrolled down line select the appropriate standard form *only if you need to* (in case of printing with the extensions or multiple spectra display). Then select the limits to be used. Usually the "limits from the screen" is working option. Then hit OK. Another program will start; the name is Topspin plot editor. This program is just for printing, you cannot work in it with the spectrum. Once spectrum is opened in the plot editor you cannot modify peak picking or integrations, but you can adjust X and Y scales and create expansions if you want. To

do so right click on the selected spectrum and select “Edit” in the appearing menu and then enter the values you would like to be displayed.

Part III – Shutting down the session.

In the second Icon-NMR window click button “Continue” (it will be highlighted in red) the select ‘Eject/Terminate’ button. You will hear the air stream and then your sample will appear in the sample holder on the top of the spectrometer. Make sure that you are not carrying any magnetically susceptible materials, then go to pick up your sample. After the removal of the sample place the black cup on the place where your sample was to prevent the inside of the spectrometer from dust. Then return to the computer and hit OK in the pop up window. This will automatically close all the windows. Then log off and sign in the book. You need to write down the date, your name, type of the spectrum you run, the amount and your comments. If everything was fine write OK. If you have experienced any problems please write exactly what was the problem/irregularity happened and if you find any major issues with the NMR machine immediately inform Shane Johnson (7-3989, shane.johnson2@und.edu) and Dr. Guodong Du (7-2241, Guodong.du@und.edu) and

Departmental Rules Regarding NMR Access and Reservation

Access:

Each potential user needs to:

1. be recommended by his or her research advisor to the faculty member in charge of the equipment,
2. contact the faculty member in charge of the instrument,
3. be trained by the faculty in charge of the instrument or by a person designated by that faculty (not by his or her research advisor), and
4. receive approval from the faculty responsible for the equipment.

If an uncertified student or other personnel is found operating the instrument without proper training and approval, he or she along with the person who provided the access will not be allowed to use the instrument. **This ban will be on 1st infraction for a week, on 2nd infraction for a month, on 3rd infraction the loss of access will be permanent.**

Reservation:

Weekday (Monday-Friday) Restrictions:

Day (8:00 AM - 6:00 PM):

Advance sign up: Each block is 20 min in length; consecutive blocks allowed, up to 60 min maximum (routine proton NMR spectra should take no more than one block).

Walk-on usage: No time limit, unless another user requires time. No long term experiments.

Evening (6:00 PM - 10:00 PM):

Advance sign up: Each block is 20 min in length; consecutive blocks allowed, up to 2 hour maximum.

Overnight (10:00 PM - 8:00 AM):

Can use entire block. Please be prepared to release the instrument at 8:00 AM

Weekend (Saturday-Sunday) Restrictions:

Day (9:00 AM - 5:00 PM): Advance sign up: 2 hour maximum.

Evening and Overnight: Can use entire block.

Note: Long-term and special experiments can be scheduled for an entire weekend.

Please contact Dr. Du to schedule days and times.

A Few Final Notes:

- ✓ Take good care of the NMR machine, since NMR is one of the most versatile laboratory instruments, which plays an important role in our research/academic carrier.
- ✓ Follow the NMR reservation principles and be thoughtful when you reserve a time (https://sites.und.edu/chemistry/). If you need to perform a special analysis (such as high temperature, low temperature, and kinetic studies using NMR, other nuclei, etc.) please contact Dr. Guodong Du. (For more information on such experiments follow the link: http://www.columbia.edu/cu/chemistry/groups/nmr/index.html)
- ✓ When you reserve the time, be aware that people from the same groups are not supposed to have more than two (2) consecutive hours in the day time.
- ✓ When you run experiments overnight, make sure you come back in time (by 8:00am) to take care of your sample. It is also a good idea to always leave a note about what to do with your sample before you go home. At the same time, it is certainly a huge waste of NMR machine time if you terminate others' overnight runs prematurely. Be considerate.
- ✓ Make sure you cancel your reservation(s) ASAP, if you decided not to use them.
- ✓ Clean the NMR tube and the spinner with Kimwipes before inserting to the machine.
- ✓ Keep the place clean by removing used Kimwipes, unwanted printed spectra, etc., from the table. Recycle any unwanted paper in the box by the door.
- ✓ **If you feel someone is not following these guidelines, you can file a complaint to Dr. Guodong Du with a short description and/or screenshot of the incident. He will investigate and keep a record of the incidents. Repeated violations may lead to further actions, including suspension of access.**

WELCOME TO THE GROUP OF NMR USERS ☺

References:

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6. The Hebrew University, Institute of Chemistry, NMR facility - http://chem.ch.huji.ac.il/nmr/techniques/expts.html
7. Wilmad-lab glasses, Proper Cleaning Procedures for NMR Sample Tubes: http://www.wilmad-labglass.com/Support/NMR-and-EPR-Technical-Reports/Proper-Cleaning-Procedures-for-NMR-Sample-Tubes/