

Lab Course 1

The aim of this course is to get familiar with the acquisition parameters of some simple NMR spectra and to study strongly coupled spin systems at 9.4 Tesla.

Coil Selection

We will first study a mixture of citric acid and taurine in a 10 mm NMR tube. Several ^1H RF resonators are available.

- Have a brief look at the hardware design and the electrical connectors of the probe setup.
- Choose an appropriate RF resonator. Important factor is the geometry compared to the object to be studied. Which diameter is to choose for optimum registration of the sample's RF signals?
 - 5 mm
 - 10 mm
 - 20 mm
 - 25 mm
 - 30 mm
- Connect the resonator with the probe tube.
- Insert the NMR tube all the way and immobilize it if necessary.
- Insert the NMR probe into the gradient system. **WARNING: avoid breaking the NMR tube!**

Tuning and Matching

The next step is to adjust the resonance frequency and impedance of the RF coil, i.e. the tuning to $400 \pm \text{MHz}$ and matching to 50 Ohm.

- Select an acquisition protocol: ADJ_SHIM_1st_2nd_Z3 and activate the instruction name by double clicking on it
- Open the adjustment platform (top left icon above the instruction list) and double click on the protocol Wobble Adj. instruction. Start it with "setup" and open the Acq/Reco real time window
- Use the T- and M-knob at the lower end of the probe head to adjust the resonator.
- Observe the changes for the reflected RF components on the monitor and try with an iterative approach to achieve a narrow absorption line at the center frequency (see Fig. 1).
- Leave the menu by choosing "Stop" on the bottom left of the protocol list.

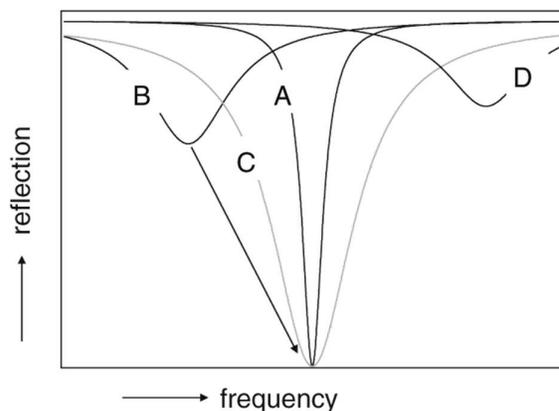


Fig. 1:

A) Tuned and matched resonator without load.
 B) Inserting the sample changes the load and hence the resonance frequency and the impedance. C) Broadened profile after re-tuning with sample.

Shim

The quality of NMR data depends on the homogeneity of the static field B_0 . The spectrometer has additional coils generating static field that in sum compensate for inhomogeneities by inserting the sample and the probe into the magnet bore. Hence, the sample needs to be 'shimmed' after positioning.

We will first work with a sample that contains 750 μL of a citric acid stock solution, 750 μL of taurine stock solution (D_2O as the solvent in both cases) and 30 μL H_2O to obtain a strong signal for shimming.

The parameter to monitor the shim process is the line width (usually of the water resonance) because the frequency components in the NMR spectrum are directly related to the experienced field through $\omega = \gamma B_0$. We therefore want to optimize the water signal.

- Improvements in the field homogeneity is related to
 - a broader resonance line because this is equivalent to a stronger signal.
 - a more narrow signal because more spins experience the same field.
- Start the shim routine Adj_Shim_1st_2nd_z3 in the directory \Scan Programs & Protocols\Micro2.5\Any Object\Any Region\Training
- Open the adjustment tool and activate "FID shim" (double click) to adjust automatically the shim currents in the coils. Start the adjustment with "start"
- Write down start and end values for the most intense peak

Line width FWHM _____

Signal intensity (GS normalized area) _____

- Once the routine has finished, save the current values with the „Apply“ button
- After you optimized the shim currents, the static net field is slightly changed. Therefore, you must
 - measure the basic resonance frequency again (protocol: Basic Frequency).
 - re-position the sample within the coil.
- Select "Apply" to save use all current settings for the next measurements

Pulse Calibration

The next step is the accurate pulse calibration, i.e. measuring the correlation between pulse amplitude for the B_1 field and the achieved flip angle for the spins. To do this, we switch to the spectroscopy software Topspin.

- Open the directory /Praktikum and choose the first experiment; create a copy of this data set under a new experiment no.
- Acquire a simple spectrum and perform the FFT using the "ft" command
- Apply a phase correction if necessary.
- Set the transmitter frequency directly onto the water resonance frequency.

- Zoom into the area of the water peak and save the display with a right mouse click.
- Make sure PH_mod is set to "pk" in ProcPars; this ensures the phase correction is now applied to all following acquisitions in this data set
- Adjust the relaxation delay (D1) to 1 s make sure the pulse power level is set to 1 W.
- Open the command popt for parameter optimization and vary p1 from 0 to 150 μ s in steps of 10 μ s.
- What do you notice for the displayed nutation curve?

- We therefore have to reperform the calibration with
 - increased repetition time.
 - decreased repetition time.
- After performing the popt procedure in a new experiment no. with proper adjustments, write down the time for a 90° and a 180° pulse:

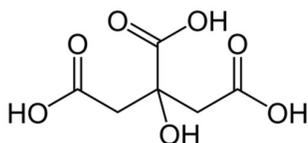
Acquiring simple FID Spectra

Now that we know the pulse calibration, take 8 acquisitions of the sample with the following parameters:

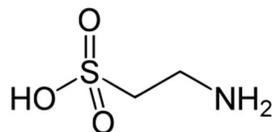
- Bandwidth 5000 Hz, 1000 points acquired in the time domain, acquisition time 0.1 s.
- Judge the spectral quality:

- Which improvements might be useful?
 - increase bandwidth
 - increase number of data points
 - decrease bandwidth
 - decrease number of data points
- Take a new spectrum. What is still wrong?
 - acquisition time too short
 - acquisition time too long
- Now that we have a decent spectrum, have a closer look at the signals right from the water ("upfield"). You will see four resonance groups. Use the roof effect to assign the coupling partners:

- The structures of the substances are as follows:
citric acid



taurine



- Assign the resonances:

Tau:

Cit:

- Measure the coupling constant for the multiplets using the mouse cursor, pretending to zoom into the spectrum:

Tau:

Cit:

Spin Echo Experiment

Next, we want to study the sample with a spin-echo experiment and see what happens to the different multiplets.

- Select a spin-echo sequence, enter 0.1 W for the pulse power level and the appropriate pulse durations you determined in the calibration experiment.
- Perform one experiment with an echo time of 1 ms and one with 20 ms (saved as separate data sets).
- Phase the spectra on the water resonance and compare the multiplet structures. What do you see?

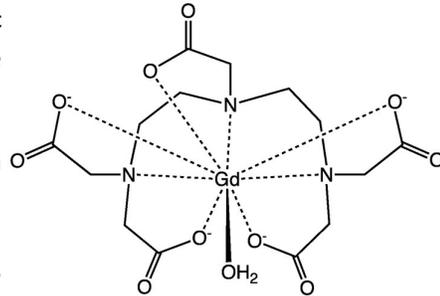
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- Now let us observe the multiplet evolution in more detail. Zoom into the multiplet region and save the display. Use the popt command again and change the parameter for the echo delay from 1 to 35 ms in steps of 5 ms.
 - Why does the citrate multiplet appear in anti-phase at ca. 30 ms echo time?

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- Now select the spectral region including the water signal and save the display. Let the popt command run with echo times from 0 to 4000 ms in steps of 400 ms. Make sure D1 is 30 s.
 - Why does the signal intensity change?
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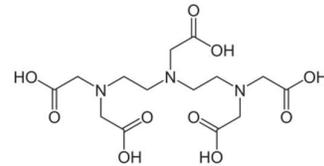
Relaxation Enhancement

Now we want to study the influence of a paramagnetic substance onto the NMR spectra. For this purpose, we use the commercial contrast agent Magnevist. It has this structure:

- Take the sample out of the magnet and add 30 μL of a Magnevist stock solution (0.005 mM).
- Take again a simple FID spectrum with 8 acquisitions.
- What do you observe for the signals from Cit and Tau?



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- Repeat the procedure by adding stepwise more Magnevist so that you have a series with 30, 60, 90, 150, 240 μL of the contrast agent.
 - Why are the two molecules affected differently by the paramagnetic substance? Consider the molecular structure compared to the DTPA of Magnevist.



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- Have a look at the FID signal of the last spectrum. What do you notice?

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- Beside the (site-selective) signal distortion, relaxation enhancement can also have positive effect. Perform the pulse calibration again, but now with a repetition time of 2 s. Did this calibration work?