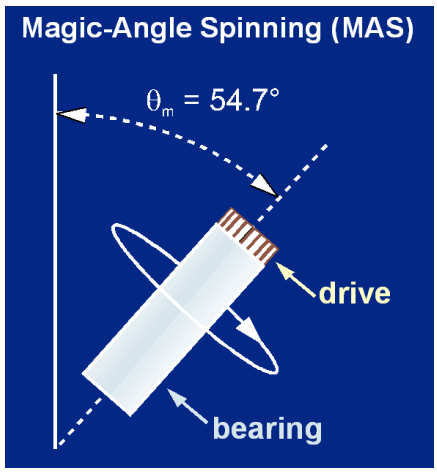
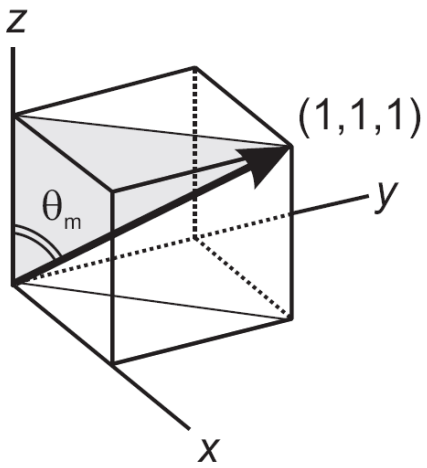
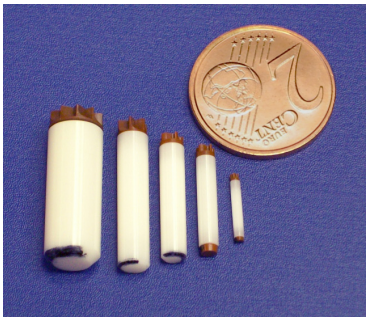
Practicum Solid-state NMR – Barth van Rossum

Introduction



Solid-state NMR has many similarities with liquid-state NMR. Differences between the two techniques find their origin entirely in the different nature of the samples that are being studied (which is, 'solid' versus 'liquid'). Since in the solid state the rapid isotropic tumbling (= in all directions) of molecules like in solution is absent, we have to help a bit. We employ a technique that is called 'magic-angle spinning', in short MAS. During MAS, the sample is rapidly rotated around the so-called magic angle, an axis inclined at an angle of 54.7° with respect to the magnetic field in the *z*-direction. Thus, in contrast to liquid-state NMR, where molecules tumble isotropically, under MAS the molecules 'tumble' anistropically, all around the same axis and all with the same speed (which is, the spinning frequency). This difference has important consequences for the design of NMR experiments in the two techniques. In MAS NMR, the most important interaction between nuclear spins is the dipolar interaction. In liquids, the dipolar interaction is basically averaged out by the rapid isotropic tumbling; in solids it is only partly averaged by the sample spinning and can be brought back by RF pulses (in so-called 'recoupling' experiments, that bring back a coupling that otherwise averaged out). Hence, in solids many pulse program make use of the dipolar coupling to obtain the required results, whereas in liquids mostly the *J*-coupling is used (although nowadays this borderline is not as strict anymore, there are many examples where *J*-couplings are exploited in solids and (residual) dipolar couplings in liquids). The typical range of the dipolar coupling (tens of kHz) is about a factor 1000 times larger than the range of *J*-coupling (tens of Hz), which is also reflected in the NMR set-up. To be able to





‘tame’ the much stronger dipolar couplings, high-power (1000 Watt) RF pulses are required, whereas in liquid-state NMR mostly low-power RF pulses will do the job.

Preparing a measurement

First we will take a look at the set up. We will look at the signal flow (from the electronics that create the RF pulses to the probe head) and at some of the ‘peripherals’: the pneumatic unit (for controlling the MAS) and the BCU (the cooling unit).

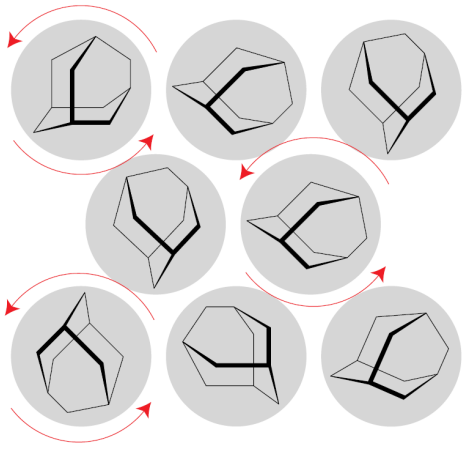
Next we will load a rotor filled with KBr into the probehead (using ‘eject’ and ‘insert’). On the computer, we will load a standard parameter set for KBr. Start the sample spinning by using the bearing and drive flow. Bearing gas lifts the sample, creating an ‘air film’ to minimize friction, drive gas is used for the actual spinning. Choose a spinning frequency (something around ~5 kHz will do) and let the pneumatic unit stabilize the MAS. Tune and match the probe head to get maximum absorbance at the ^{79}Br frequency (around 100 MHz). Record a simple one dimensional spectrum.

The many satellite signals are the so-called spinning side-bands.
What is the frequency spacing between the side bands?

Change the spinning speed, and look at the side bands again. *What happened*?

Now we will carefully change the magic angle. Observe how the spectrum changes. KBr is normally used to adjust the magic angle, since it is very sensitive to minor maladjustments.

Stop the sample spinning (press ‘stop’ on the pneumatic unit) and eject the sample. Next we will load a sample of adamantane. Adamantane is sometimes called a ‘liquid-plastic’; it consists of the smallest-possible Bucky ball. Adamantane is a solid, but at the same time extremely mobile and the molecules can rapidly spin around their position in the lattice. When



the sample is inserted, start the spinning (again, ~ 5 Kz will do). Tune and match the probe head. We will record a ^{13}C spectrum using cross polarization (CP) from protons to carbons. CP is used to enhance the ^{13}C signal. First we load a standard parameter set for adamantane, and record a 1D spectrum. Although there are several (10) carbon atoms in a molecule, there are only two chemically unique positions, and hence, the spectrum consists of two lines.

Change the length of the ^{1}H excitation pulse. *What happens*?

Optimize the length of the ^{1}H pulse to get a proper 90° excitation pulse. *Calculate the power of the RF pulse*.

Change the RF powers of the cross polarization. Only for certain power levels of the ^{1}H and ^{13}C spin lock pulses we get a large signal (an effect which is called 'Hartmann Hahn' matching). *Find some good matching conditions by sweeping the ^{1}H spin lock power*.

During acquisition we are decoupling the ^{1}H from the ^{13}C. We want a decoupling power of ~35 kHz. We will calculate this power using the RF power we found above as a reference. Now change the acquisition time (from 50 ms to 200 ms). *What happens*?

While recording 1D spectra, change the shim parameters of the magnet and see how the spectrum changes (or, how the shape of the free induction decay (FID) changes). A narrow line corresponds to a long FID. Play a bit around with the shim values and see how the spectrum changes. Adamantane gives very narrow lines due to its intrinsic mobility; it is often used in MAS NMR to shim the magnet.

<u>Running a 2D experiment</u>

After optimizing the shims, stop the spinning, eject the adamantane sample and load a protein sample (SH3 domain). This sample is ^{13}C and ^{15}N-enriched, to enhance the signal intensity (remember that the natural abundance of ^{13}C is only ~1 %, the rest is ^{12}C, which does not give an NMR signal). Spin the sample at ~10 kHz. Load a standard parameter set, and record a 1D spectrum. Assuming that all parameters are

more-or-less okay, we will now start a simple 2D experiment, a proton-driven spin-diffusion (PDSD) experiment. Take a look at the pulse program and try to get a rough idea about how it works. The experiment starts with a 90° pulse on ^{1}H; next, the magnetization is transferred from ^{1}H to ^{13}C with CP. Following CP, the ^{13}C spins are allowed to evolve under ^{1}H-decoupling. Next, a ^{13}C-^{13}C PDSD mixing period follows, to transfer magnetization between ^{13}C spins. Finally, the ^{13}C-FIDs are acquired under ^{1}H-decoupling. While the 2D experiment runs, we will Fourier-transform the data a few times to see how the 2D spectrum 'grows'. The more slices have been recorded, the better the resolution gets in the indirect dimension. While the spectrum is running (this will take several hours…), we change to a different data set with a finished PDSD experiment. Study the spectrum, and try to understand what you see. *What is the difference between the strong diagonal and the off-diagonal signals? What signals carry the important information? Can you identify the spinning-side band signals? Try to assign a few fingerprint patterns (e.g. for threonines)*.

threonine