Development of MicroMAS NMR Probehead for Mass-limited Solid-state Samples

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The probehead (microMAS) is developed for high resolution solid-state NMR observation of mass-limited samples. The probehead gives extremely high sensitivity of the spectra, and largely improves the limit of detection for solid-state NMR. As the results, the NMR spectra of μg order samples could be successfully observed using conventional 400 MHz NMR within a few hours.

Solid-state nuclear magnetic resonance (NMR) is one of the most powerful analytical methods for insoluble organic or inorganic compounds including bio-macromolecules and thus, it has been used in several research fields widely. However, NMR itself has a basic disadvantage in sensitivity compared with other spectroscopic methods because the NMR phenomena come from the extremely small energy difference of nuclear spins in Boltzmann distribution. Therefore, conventional solid-state NMR experiments require more than "mg" order samples.

The signal to noise (S/N) ratio is usually used to describe the sensitivity of the NMR spectra quantitatively. The S/N ratio is generally written as;[1,2]

$$(\mathrm{S/N}) \propto (B_1/i)N\omega_0^2, \tag{1}$$

where B_1 is magnetic field induced in the RF-coil, i is current to the coil, N is number of spins in observed coil, and ω_0 is the strength of external magnetic field. In the case of solenoid coil, B_1/i can be expressed as;

$$(B_1/i) \propto n/(r^2 + l^2), \tag{2}$$

where n is number of turns of the coil, and r and l are radius and length of the coil, respectively. The eq 1 shows that higher magnetic field is very effective for improving the S/N ratio. Actually, recent development of NMR magnet with higher field is very active.[3] The other improvement is development of a microcoil NMR. Namely, equation 2 indicates that the S/N ratio increases for the solenoid coil with smaller radius and shorter length through the increase in the parameter B_1/i.

Some microcoil NMR performance has been reported for liquid-state NMR,[4] demonstrating sensitivity enhancement for mass-limited samples. Using single channel microcoil probehead, Yamauchi et al.[5] and van Bentum et al.[6] have reported remarkable improvement of S/N ratio in the proton (^{1}H) and aluminum (^{27}Al) solid-state NMR spectra of μg order samples. For carbon (^{13}C) measurement, the development of cross polarization (CP) technique could be attained and an application to fibrous polymers has been reported.[7] However, all of these solid-state NMR experiments are performed for the samples in the static state. Modern solid-state NMR is usually performed under magic angle spinning (MAS) for getting "high resolution" NMR spectra. Actually, increase in the use of solid-state NMR is largely due to the development of CP/MAS NMR technique.[8] There is a few approaches to use small sample rotor for MAS system. Samoson et al.[9] are making a high speed MAS system and they are using around 2 mm rotor system but they are not focusing high sensitivity.

In this paper, we will describe our work on the development of microcoil NMR with MAS. For example, when suitable crystals of peptide or protein samples cannot be obtained for X-ray diffraction studies, only solid-state NMR can be used for the structural determination under "high resolution" NMR condition. However, only limited amounts of such microcrystalline samples are usually available. Thus, the potential of the microcoil NMR with MAS is promising. In order to perform the microcoil with magic angle experiment (microMAS), such a microcoil must be fitted in the spinning rotor with spin at a rate of over several kilohertz to remove both the dipolar coupling and chemical shift anisotropy effects.

The rotor and the rotation system developed by us are shown in Figures 1 and 2, respectively. The microrotor is attached to the top end of the conventional rotor for high-speed spinning. This system is targeting for a small mount of sample and stable rotation as conventional MAS is realized with this approach. The material of the microrotor is zirconia (ZrO_2) to minimize unwanted background in the spectrum. The outer diameter is 1 mm, inner diameter is 500 μm and the length is 1 cm. The conventional rotor used here is the Varian–Chemagnetics 5 mm pencil-type rotor and the rotation is to 10 kHz with the microrotor. The microcoil is made with nine turns of 200 μm diameter copper wire and the diameter of the coil is 1.4 mm. Therefore, the gap from the coil to sample rotor will be 200 μm.

The microMAS system is built in a Varian–Chemagnetics APEX 400 type triple channel probehead. The microcoil is tuned for 400.1 MHz for H channel and 95 to 105 MHz for X channel of the original probehead for doing CP experiment, and the Y channel is tuned for 100.2 MHz to detect the ^{79}Br of KBr which is packed in original rotor to check the magic angle. All experiments are recorded with a BrukerBiospin Avance 400 spectrometer.

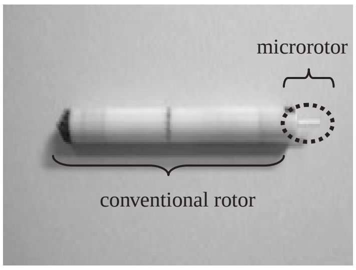

Figure 1. The photo of microMAS rotor (in the dashed circle) developed for mass-limited sample. The microMAS rotor is attached to be conventional rotor and rotates to 10 kHz.

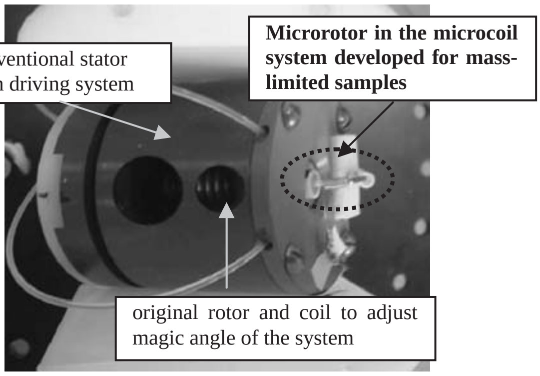

Figure 2. Top view of microMAS system. The driving unit for rotation from the conventional system is used. The sample rotor and coil of the micro system are in the dashed circle. The microrotor is outside of the conventional driving system, and it rotates together with the conventional rotor. The microcoil is fitted to only the microrotor part and feeds the RF and observes the NMR signal only from the sample in the microrotor.

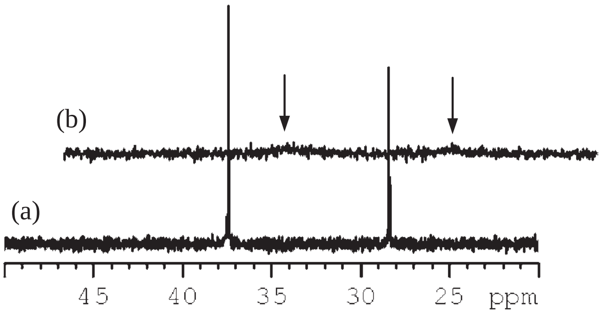

Figure 3. ^{13}C CP/MAS spectra of adamantine with 4.2 μg under 3 kHz spinning speed (a) and nonspinning (b). The CP/MAS spectrum is greatly improved compare to static case. The peaks of nonspinning "↓" are broad and of low intensity so they are hard to observe.

Figure 3 shows the ^{13}C CP/MAS spectra of the plastic crystal adamantine (natural abundance ^{13}C), 4.2 μg under 3 kHz spinning speed (a) and nonspinning (b). The spectra are accumulated with 512 scans. The S/N ratio was improved about 17 times and the resolution was improved about 40 times by spinning. The S/N is improved about 30 times compare to conventional probehead (4 mm MAS probehead), and the signal could not detect with the same amount of the sample because it is lower than the LOD (limit of detection).

Figure 4 shows ^{13}C CP/MAS and radio frequency dipolar recoupling (RFDR)[10] spectra of [U-^{13}C] Alanine with 6.5 kHz spinning speed. The CP/MAS spectrum with high resolution and high S/N ratio was obtained for a small amount of the sample (885 μg). Even with low RF power (about 10 W), and the decoupling field is strong enough (83 kHz) to decouple the dipolar interaction of protons. This means that the microMAS also has an advantage in the RF performance. The CP/MAS NMR spectra was obtained with 128 scans and RFDR spectra was obtained with 16 scans (6 h for obtaining one 2D spectrum). RFDR is one of the broadband recoupling methods to determine the internuclear distance and, therefore, it can be used for the structure analysis of biomolecules such as proteins.

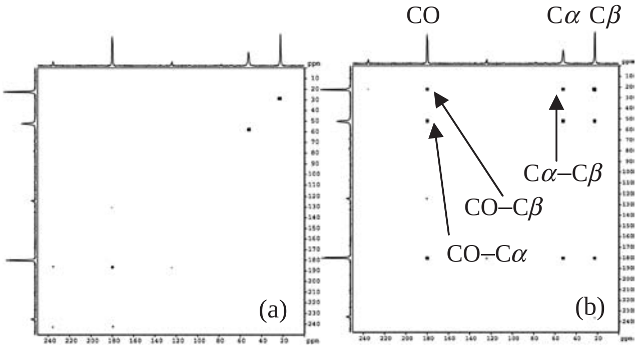

Figure 4. ^{13}C RFDR of [U-^{13}C] Alanine (885 μg). Top and side spectra in the 2D spectra are ^{13}C CP/MAS NMR spectra. The short mixing time (150 μs) gives only diagonal peak (a), and the longer mixing time (4 ms) gives cross peaks between carbons.

It can be concluded that the microMAS probehead developed here has the great potentiality for mass-limited samples. Now, samples under the "μg" amount is open for measurement with this probehead. We showed only some spectra here, but this probehead can be expected to be used in many applications of solid-state NMR.

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