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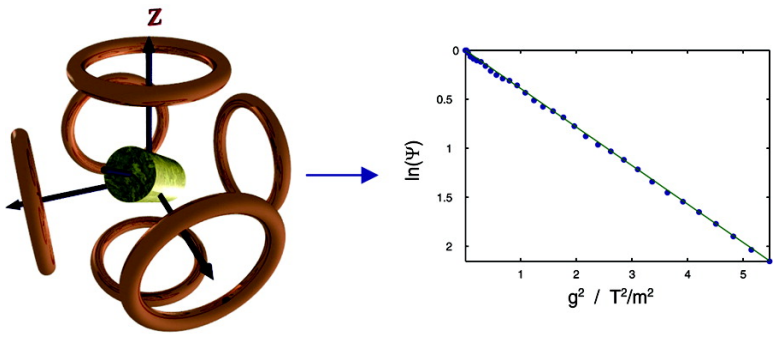

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Studying Lateral Diffusion in Lipid Bilayers by Combining a Magic Angle Spinning NMR Probe with a Microimaging Gradient System

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Pulsed field gradient (PFG) NMR is a versatile tool for studying diffusion processes and has been used for many years.[1,2] In PFG NMR, pulsed field gradients of duration δ are used to dephase magnetization, and, after a subsequent delay, the observation time Δ is used to rephase magnetization. After the rephasing pulse, an echo occurs, which is finally detected. Any motion of the spin carrying molecules prevents complete rephasing, which is, in the case of Brownian motion, reflected in a change of the intensity of the spin echo. The ratio of the spin echo measured without applied gradients and those measured with applied gradient, the so-called echo attenuation Ψ is a direct measure of the molecular mean square displacement and, thus, the self-diffusion coefficient. In high-resolution NMR applications, the echo is Fourier transformed into a high-resolution spectrum, which allows observation of the diffusion of many molecules in one sample at the same time.

In lipid membranes, or other semisolid systems, signals are broadened by several anisotropic interactions in the NMR Hamiltonian, such as magnetic susceptibility, chemical shift anisotropy, or even dipolar coupling.[3,4] Those interactions have to be suppressed by spectroscopic techniques[5] or by using solid-supported membranes oriented at the magic angle[6] in order to observe resolved spectra and to apply echo sequences that use pulsed field gradients. The most effective technique to get rid of the unwanted broadening mechanisms and to produce highly resolved spectra with high sensitivity is the application of magic angle sample spinning (MAS).[3,4]

Modern MAS probes for high-resolution MAS (HR-MAS) NMR are usually equipped with coils to produce pulsed field gradients.[7] Recently, it has been shown that lateral diffusion can be nicely studied by using a combination of PFG and MAS. The technique has been successfully applied to study the diffusion cell compartments[8] of molecules embedded in cubic liquid-crystalline phase[9] and even in lipid bilayers.[10,11] Although the spectroscopic resolution provided by MAS is superior compared to spectra of oriented samples, the gradient strengths available with MAS probes are usually limited compared to probes designed for PFG NMR studies.

In general, Ψ is a function of the gradient strength, the duration of the gradient, the observation time, and the mean square displacement, whereby the echo attenuation has to be large enough to be distinguishable from noise and other error sources.[1] The lack of strong gradients can be compensated using longer observation times or a longer δ. Both possibilities have serious drawbacks. During a long δ, the transverse magnetization may decay rapidly, especially if membranes are being studied. If longer observation times are used, effects of exchange[12] and NOE[13] can influence the echo attenuation and mask the true diffusion process. Furthermore, for studying membranes, diffusion along curved bilayers results in a time-dependent diffusion coefficient, whose interpretation might be difficult.[10,11,14] Therefore, it is highly desirable to develop MAS probes with PFG capabilities that allow the application of strong gradient pulses. However, there are disadvantages if MAS stators are equipped with gradient coils:[7,15] the Q factor of the resonance circuits may decrease, which negatively affects the performance with respect to efficiency (short pulses and high sensitivity), and due to the forces caused by the strong currents in the magnetic field, the mechanical stability might become an issue.

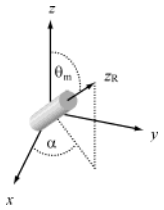

Figure 1. Geometrical arrangement of the MAS rotor with respect to the gradient direction. The z-direction of the gradient coincides with the magnetic field B_0. The z_R-direction in the rotor frame is along the rotation axes, which is oriented at magic angle θ_m.

In this report, we explore another setup, namely, the use of a conventional narrow-bore MAS probe that is inserted into a microimaging system. This technique has some important advantages compared to the construction of a MAS probe whose stator is wrapped by a gradient coil: (i) The gradient coils are separated from the radio frequency parts of the probe, thus minimizing any impact of the gradient coils on the radio frequency properties of the probes circuits. (ii) An external cooling of the gradient coils can be used, thus enabling high currents, high gradient strengths, and high linearity. (iii) There is no torque acting on the stator caused by high currents in the gradient coils, which otherwise could compromise the mechanical stability and could disorient the sample, thus leading to distorted NMR results.

The microimaging device we used can generate a field gradient of three independent components given by $\mathbf{G} = (\partial B_z/\partial x, \partial B_z/\partial y, \partial B_z/\partial z)$, where the z-direction is the direction of the magnetic field B_0. The additional field $\mathbf{B}$ generated by the gradients for a spin at position $\mathbf{r} = (x,y,z)$ is $\mathbf{B} = \mathbf{G}\cdot\mathbf{r}$. The position $\mathbf{r}$ in the laboratory frame (LAB) of a spin located in the rotor at position $\mathbf{r}_R = (x_R, y_R, z_R)$ in the rotor frame (R) is modulated by the sample spinning. The field seen by a spin located at the rotor frame can be calculated using an unitary transformation described by a set of Euler angles (α, $\beta = \theta_m$, $\gamma = \omega_r t$) (Figure 1) that transforms $\mathbf{r}_R$ into the laboratory frame.[15] If we adjust our gradient such that $\mathbf{G} = (G,G,G)$ and $\alpha = 45°$, it follows that $\mathbf{B} = (0,0,\sqrt{3}\,Gz_R)$, which means that the dephasing and rephasing depends only on the z_r-coordinate in the rotor frame, and thus, as one would have intuitively guessed, that the microimaging device produces a true magic angle gradient of the strength $\sqrt{3}\,G$. The angle α has to be adjusted manually by thoroughly orienting the azimuthal orientation of the MAS stator by turning the probe. It would be possible to use another α; however, this would require adjusting the gradients in x- and

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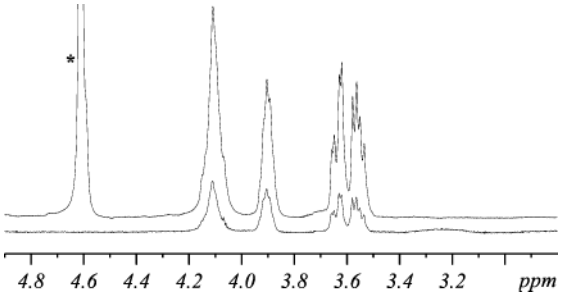

Figure 2. ^{1}H MAS NMR spectra (400.13 MHz) of a cubic phase composed of monoolein and water (D_2O) at 307 K and a 4 kHz rotation frequency. The spectra show the region of the glycerol backbone of the lipids (4.2−3.5 ppm).[18] The spectrum on top was measured using single-pulse excitation. The water signal (HDO) is marked by an asterisk. The spectra at the bottom was measured using a PFG sequence at maximum gradient strength. The water signal has now disappeared. Note the identical line shape for both spectra.

y-directions, which would prevent the maximum possible gradient strength from being available.

To demonstrate the functioning of the described setup we have chosen a sample composed of the lipid 1-monooleoly-*rac*-glycerol (monooleoin, MO) and deuterated water, which forms a bicontinuous cubic liquid-crystalline phase. A bicontinuous cubic liquid-crystalline phase (cubic phase) is composed by a minimal-surface-forming lipid bilayer that is interlaced by water channels. Because of the bilayer nature, cubic phases can be considered as models for the lipid membrane. Besides, some applications of cubic phases have been reported. For example, cubic phases have shown to be effective matrices for membrane-protein crystallization[16] and also their role in the function of cells has been discussed. Furthermore, some very interesting applications of cubic phases as drug release systems have been reported.[17]

Here, the diffusion properties of molecules, embedded in the lipid bilayer of a cubic phase, make it ideal for inspecting the experimental setup. The diffusion coefficient of lipids in cubic phases is of the same order as those of lipids in membranes, but the diffusion process can be best characterized as pseudo-three-dimensional.[17] The lipids diffuse fast along the curved minimal surfaces. As a consequence, the spin−echo attenuation is purely Gaussian. Therefore, any deviation from the linear behavior of the semilogarithmic plot of the spin−echo attenuation vs the squared gradient strength would be caused by imperfections in the experimental setup.

As an added advantage, the ^{1}H MAS spectra of cubic phases are well resolved.[18] The line width observed is on the order of 1−3 Hz, which makes the spectra highly sensitive for the detection of field instabilities caused by gradients, eddy current effects, and other distortions of the spectra, which can be introduced if pulsed field gradients with high strengths combined with long durations are applied.

The experiments were performed on a Bruker Avance 400 MHz wide-bore spectrometer by using a double-resonance narrow-bore HR MAS probe. The probe was inserted into the Bruker micro-imaging DIF2.5 system in such a way that the sample is located in the center of the gradient system.

Diffusion studies were performed using the stimulated echo (STE) sequence with eddy current delay. Gradients were applied as a pair of bipolar sine-shaped gradients separated by a 180° pulse with a duration of 1 ms each.[19] The maximum gradient strength at the system used was 2.6 T/m. In Figure 2, spectra observed using single-pulse excitation and spectra measured using the STE sequence applying gradients with maximum strength are compared. No influences on line shape and signal position even at high gradient strengths are observed. The semilogarithmic plot of the echo

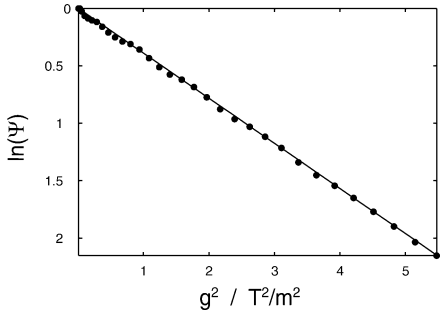

Figure 3. Semilogarithmic plot of the echo attenuation of the lipid signals. $\Delta = 200$ ms. The solid line is the linear fit. A diffusion coefficient of 17 x 10^{-12} m^2 s^{-1} was calculated.

attenuation of the lipid signals is shown in Figure 3. No significant deviation from the linear behavior is seen. The diffusion coefficient obtained is comparable to those recently published.[20]

The obtained results indicate that the proposed setup is quite useful for studying diffusion processes in lyotropic liquid crystalline phases. Here, we restricted ourselves to an effective δ of 2 ms, but an increase of this length is easily possible, also if molecules in membranes shall be studied. A doubling of δ would result in a 4-fold stronger decay of the echo attenuation, thus allowing the study of slower diffusion processes and also of short-time diffusion processes. The application of this setup to investigate other systems such as cells, lipid membranes, zeolites, and others is straightforward, and it can be expected to provide a useful means to observe diffusion in systems that have not been accessible thus far by PFG NMR in combination with high-resolution spectroscopy.

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Supporting Information Available: Enlarged version of the spectra shown in Figure 2 and additional details of the sample preparation and experimental setup (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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