

# Lateral Diffusion Rates of Lipid, Water, and a Hydrophobic Drug in a Multilamellar Liposome

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**ABSTRACT** The lateral diffusion constants of 1-palmitoyl-2-oleoyl-*sn*-glycero-3 phosphocholine (POPC), water, and ibuprofen were measured in multilamellar liposomes using pulsed field gradient magic-angle spinning (PFG-MAS)  $^1\text{H}$  NMR. The analysis of diffusion data obtained in powder samples and a method for liposome curvature correction are presented. At 322 K POPC has a diffusion constant of  $(8.6 \pm 0.2) \times 10^{-12} \text{ m}^2/\text{s}$  when dehydrated (8.2 waters/lipid) and  $(1.9 \pm 0.1) \times 10^{-11} \text{ m}^2/\text{s}$  in excess water. The diffusion constant of water in dehydrated POPC was found to be  $(4.7 \pm 0.1) \times 10^{-10} \text{ m}^2/\text{s}$ . The radius of curvature is  $21 \pm 2 \mu\text{m}$  for the dehydrated sample and  $4.5 \pm 0.5 \mu\text{m}$  for POPC sample containing excess water. The activation energies of diffusion are  $40.6 \pm 0.4 \text{ kJ/mole}$  for dehydrated POPC,  $30.7 \pm 0.9 \text{ kJ/mole}$  for POPC with excess water, and  $28.6 \pm 1.5 \text{ kJ/mole}$  for water in dehydrated POPC. The diffusion constants and activation energies for a sample of POPC/ibuprofen/water (1:0.56:15) were also measured. The ibuprofen, which locates in the lipid-water interface, diffuses faster than POPC but has a slightly higher activation energy of lateral diffusion. Within certain restrictions, PFG-MAS NMR provides a useful method for characterizing membrane organization and mobility.

## INTRODUCTION

Not only is it highly desirable to measure the lateral diffusion of individual lipids in a membrane without the use of perturbing labels, but it is also of interest to simultaneously measure the diffusion of substances intercalated into the lipid matrix. For instance, many lipophilic drugs are thought to approach active sites of intrabilayer receptors by lateral diffusion through the membrane (Mason et al., 1991). Therefore, to characterize the pharmacokinetics of these substances, it is of great interest to know how fast they move through the lipid phase.

Fluorescence recovery after photo bleaching is used widely for measuring the diffusion in cell membranes, but a major drawback of this approach is the requirement for labeled molecules. Pulsed-field gradient nuclear magnetic resonance (PFG-NMR) is an alternative approach for measuring diffusion on micrometer length scales (Callaghan, 1991; Lindblom and Orädd, 1994; Stilbs, 1987). However, diffusion measurements of substances in lamellar phases have been difficult to perform with PFG-NMR because of the low spectral resolution in these phases. Often labeling, for instance with fluorine, is required to obtain a resolvable signal (Orädd et al., 2002). However, the properties of a compound may be considerably changed by the substitution of a fluorine atom for a hydrogen. Spectral resolution may be improved by using oriented samples with their bilayer normal at the magic angle to the magnetic field.

This requires painstaking preparation to approach complete order (Lindblom and Wennerström, 1977; Roeder et al., 1976). The spectral resolution for oriented samples is still limited. Multipulse homonuclear decoupling techniques have also been used to reduce proton linewidths to measure lipid diffusion with PFG-NMR (Crawford et al., 1980). This approach has advantages for studying anisotropic diffusion (Furó and Dvinskikh, 2002); however, it is an experimentally demanding approach and may not achieve the excellent resolution of resonance signals afforded by magic-angle spinning. A difficulty of these measurements is the large internal magnetic field gradients that exist in heterogeneous samples at high magnetic fields that may give erroneously low diffusion constants (Sørland and Aksnes, 2002).

An alternative tactic for obtaining diffusion measurements using NMR signals of membranes with a resolution close to experiments in liquids is pulsed field gradient magic-angle spinning (PFG-MAS). This approach has been used successfully to monitor the diffusion of an anti-cancer drug in a cubic lipid phase (Pampel et al., 2002).

The analysis of PFG-MAS NMR diffusion measurements on multilamellar liposomes has two obstacles. First, diffusion in multilamellar systems is anisotropic. Substances that are confined to the lipid matrix will move along the plane of the bilayer. The gradient in MAS experiments is applied along the rotation axis. The bilayer normals are oriented at random, and therefore the resulting signal has contributions from lamellae of all orientations relative to the axis of the gradient. Furthermore, apparent movement of substances along the gradient axis may be restricted due to the finite size of liposomes that, depending on preparation procedures, could be in the micrometer range as well. Solutions to both of these complications are presented here, confirming that PFG-MAS NMR is a suitable method for obtaining diffusion measurements on substances in lamellar phases.

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The technique is demonstrated on three samples. The initial measurements were performed on a 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) sample at limited hydration to get large MLVs of low curvature that confine the water to the interbilayer spaces without the formation of large water pockets (Koenig et al., 1997; Gawrisch et al., 1985). Experiments on POPC in excess water were conducted for comparison with results reported for oriented samples (Filippov et al., 2003). Diffusion measurements on a sample of POPC containing the NSAID ibuprofen are also presented to establish the applicability of this method for measurements of lateral diffusion of pharmaceuticals intercalated into bilayers.

## MATERIALS AND METHODS

### Sample preparation

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (5 mg, Avanti Polar Lipids, Alabaster, AL) was hydrated with deionized water and vortex-mixed to homogenize, resulting in a sample of POPC:H<sub>2</sub>O 1:8.2 (mol/mol). After the initial NMR measurements were made, 5  $\mu$ L of D<sub>2</sub>O (Cambridge Isotope Labs, Andover, MA) were added to this sample with a syringe to give a POPC sample in excess water. For the sample containing ibuprofen, POPC and (S)-(+)-4-isobutyl- $\alpha$ -methylphenylacetic acid (Aldrich, St. Louis, MO) were co-dissolved in methanol, dried to a thin film under a stream of dry N<sub>2</sub>, and hydrated with D<sub>2</sub>O (Cambridge Isotope Labs, Andover, MA) to prepare a sample of POPC:ibuprofen:D<sub>2</sub>O 1:0.56:15 (mol/mol/mol). The samples were transferred by centrifugation into 4-mm MAS rotors with inserts (Bruker Instruments, Billerica, MA) to generate a spherical sample volume of 11  $\mu$ L.

### NMR measurements

NMR measurements were performed on a Bruker DMX500 widebore spectrometer (proton frequency of 500.17 MHz) running XWINNMR v3.1. The 4-mm PFG-MAS probe with *z*-axis gradients was operated at a spinning frequency of 5 or 10 kHz. Spectra were obtained at a sweep width of 5000 Hz and are shown in Fig. 1. Spectral assignments were aided by COSY experiments. For each diffusion experiment, measurements were conducted at 16 different values of gradient strength varying from 0.01 to 0.37 T/m with a stimulated echo sequence using sine-shaped bipolar gradient pulses (Cotts et al., 1989) of 5-ms duration. A longitudinal eddy current delay of 5 ms was used. Diffusion times were varied from 15 to 200 ms. At every gradient strength, eight scans were acquired with a recycle delay of 4 s. The temperature was varied from 296 to 331 K.

### Temperature calibration

The temperature was calibrated at MAS spinning frequencies of 5 and 10 kHz by measuring the chemical shift difference between water and choline in a micellar sample of 1,2-dicaproyl-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids; Alabaster, AL) loaded into an 11- $\mu$ L spherical MAS rotor insert. The chemical shift as a function of temperature was measured on the same sample in a 5-mm tube in a high resolution probe whose temperature had been calibrated precisely with a thermocouple.

### Gradient calibration

The gradient strength was calibrated by measuring the diffusion constant of deionized water at 296 K in the same 11- $\mu$ L spherical MAS rotor insert

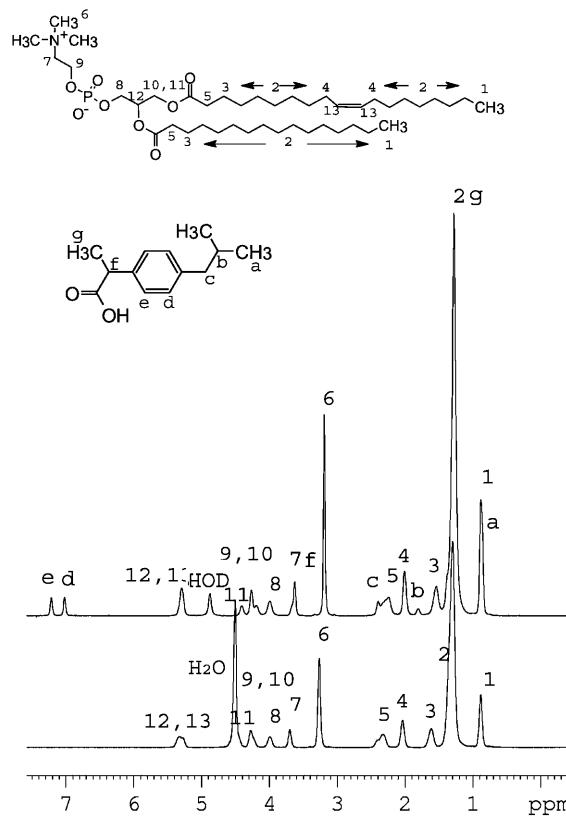


FIGURE 1  $^1\text{H}$  NMR spectra of POPC:H<sub>2</sub>O 1:8.2 (mol/mol) (lower) and POPC:ibuprofen:D<sub>2</sub>O 1:0.56:15 (mol/mol/mol) (upper) with resonance assignments as indicated in the POPC and ibuprofen structures shown above the spectra.

spinning at 10 kHz. A stimulated echo sequence with sine-shaped bipolar gradient pulses was used, with 32 measurements made from 2 to 95% of full gradient strength. The diffusion time was 50 ms and the length of gradient pulses was 1 ms. With  $D_0 = 2.119 \times 10^{-9} \text{ m}^2/\text{s}$  (Price et al., 1999), the effective gradient strength was found to be 0.3875 T/m at 10 Å (see Fig. 2), which is within 10% of the value of 0.35 T/m calculated from a measurement of gradient strength based on line broadening of the water resonance. No influence on the stability of MAS was observed with increasing gradient strength as seen from this calibration conducted on a spinning sample.

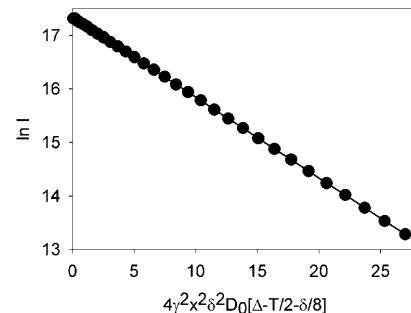


FIGURE 2 The signal attenuation of water as a function of increasing gradient strength gives the effective gradient strength to be 0.3875 T/m at 10 Å.

## RESULTS AND DISCUSSION

### Signal attenuation in powder samples

The diffusional motions of lipids and interlamellar water in multilamellar liposomes are confined to layers with random orientation to the applied magnetic field gradient (Callaghan and Söderman, 1983). If for simplicity it is assumed that these layers have no curvature, the attenuation of NMR signal intensity after application of two gradient pulses separated by a diffusion time,  $\Delta$ , is given by the following equation (Callaghan and Söderman, 1983; Lindblom et al., 1977):

$$\frac{I}{I_0} = \exp(-kD) \int_0^1 \exp(kDx^2) dx, \quad (1)$$

with  $x = \cos(\theta)$ , where  $\theta$  is the angle between the bilayer normal and the axis of the field gradient. The symbol  $D$  is the diffusion constant, and  $k$  is a factor whose exact nature depends on the pulse sequence and on instrumental settings. For the stimulated echo sequence,

$$k = 4\gamma^2 g^2 \delta^2 \left( \Delta - \frac{T}{2} - \frac{\delta}{8} \right),$$

where  $\gamma$  is the gyromagnetic ratio of protons,  $g$  is the gradient strength,  $\delta$  is the gradient pulse length, and  $T$  is the time between the gradient pulses sandwiching the  $180^\circ$  pulses (Fordham et al., 1996). The integral in Eq. 1 can be evaluated to give Eq. 2.

$$\frac{I}{I_0} = \frac{1}{2} \exp(-kD) \operatorname{erf} \left[ (-kD)^{1/2} \right] \left( \frac{\pi}{-kD} \right)^{1/2}, \quad (2)$$

where  $\operatorname{erf}(z) \equiv (2/\pi^{1/2}) \int_0^z e^{-t^2} dt$ .

For numerical analysis of experimental results it is convenient to plot the logarithm of signal intensity,  $\ln(I/I_0)$  vs.  $k$ . The error function  $\operatorname{erf}(z)$  can be developed into a so-called semiconvergent power series. A well-known feature of these series is that for given values of  $z$ , the upper and lower bounds of approximation first converge with increasing number of elements in the series and then diverge again (Afken, 1985). We established that for  $kD \leq 7$  the series can be truncated at the quadratic term in good approximation. The following equation may be used to follow the signal attenuation,  $I/I_0$ , from 0 to 92%:

$$\ln \left( \frac{I}{I_0} \right) = -\frac{2}{3} kD + \frac{2}{45} (kD)^2. \quad (3)$$

The first term of Eq. 3 contains the familiar factor of  $2/3$  that has been cited in previous work as first-order correction for powder samples (Callaghan and Söderman, 1983; Lindblom et al., 1977). In Fig. 3, the attenuation of choline and water resonances in a sample of incompletely hydrated POPC as a function of  $k$  for different diffusion times is fit to Eq. 3.

Though the data for the POPC appear linear, the second term of Eq. 3 is required for an adequate fit to these data. A

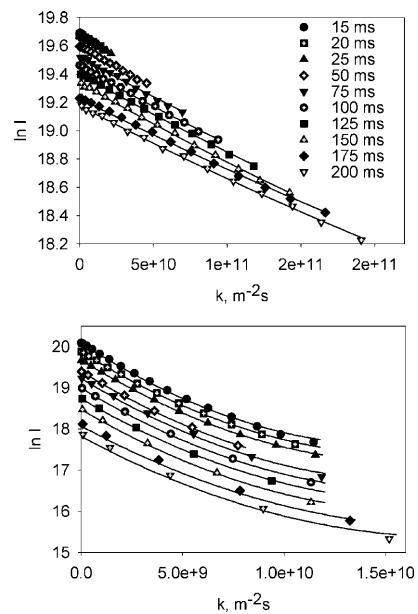


FIGURE 3 A plot of the natural log of the attenuation of the choline (upper) and water (lower) resonances vs.  $k$  for different diffusion times,  $\Delta$ , at 322 K. The lines are fits to Eq. 3. Only points for which  $k_D < 5$  were included in the fit. The intensities on the y-axis have been individually scaled for clarity.

linear fit underestimates the slope systematically with increasing diffusion time because of the increase in barely perceptible curvature of the data. The water data are well fit down to the mathematical limits of the function,  $\sim 10\%$  of initial intensity, indicating that the sample is a homogeneous powder.

### Correction for bilayer curvature

The apparent diffusion constants obtained from these fits are plotted against diffusion time in Fig. 4. The measured diffusion constant of water has a weak dependence on diffusion time because of the influence of curvature. Here it is assumed that  $D = D_{||}$ , i.e., water diffusion occurs parallel

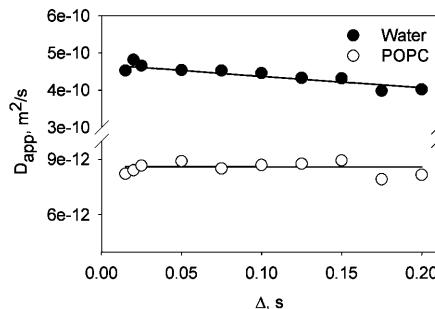


FIGURE 4 A plot of the apparent diffusion constant of water as a function of diffusion time,  $\Delta$ , at 322 K. The fit to Eq. 5 gives an average radius of curvature of  $21 \mu\text{m}$ ,  $D_{\text{water}} = (4.7 \pm 0.1) \times 10^{-10} \text{ m}^2/\text{s}$ , and  $D_{\text{POPC}} = (8.6 \pm 0.2) \times 10^{-12} \text{ m}^2/\text{s}$ .

to the plane of the bilayers. Diffusion through bilayers is much slower, and  $D_{\perp}$  can safely be neglected (Wassall, 1996; Wästerby et al., 2002). Since experiments were conducted on samples with <15 water molecules per lipid, no correction for the presence of bulk water is necessary (Koenig et al., 1997). The true diffusion constant can be extracted from these measurements by assuming an average radius of curvature,  $r$ , that is  $\geq z$ , the length of diffusion along the axis of the field gradient.

For simplicity we assume that all bilayers have the same radius of curvature. On average, lipid and water molecules move during the diffusion time,  $\Delta$ , over a distance  $l = \sqrt{2D\Delta}$ . If diffusion occurs over a surface with the radius of curvature,  $r$ , then the distance  $l$  corresponds to an angle  $\theta$  according to  $l = r\theta$ . The projection of  $l$  on the axis of the field gradient yields the distance,  $z = r \sin(\theta)$ , that molecules travel along the gradient. After substitution of  $\theta$  and  $l$  we get

$$z = r \sin((2D\Delta)^{1/2}/r). \quad (4)$$

In the experiments an apparent diffusion constant,  $D_{\text{app}} = (z^2/2\Delta)$  is measured that, for diffusion over curved surfaces, is smaller than the true diffusion constant,  $D$ . Substitution of  $z$  by Eq. 4 yields the relation between the apparent and true diffusion constants:

$$D_{\text{app}} = \frac{r^2}{2\Delta} \sin^2(2D\Delta)^{1/2}/r. \quad (5)$$

Fitting the water diffusion data acquired at 322 K as a function of diffusion time,  $\Delta$ , to Eq. 5 gives a radius of curvature of  $21 \pm 2 \mu\text{m}$ , a reasonable value for dehydrated liposomes (Gawrisch et al., 1985). The true diffusion constant obtained from the fit is  $(4.7 \pm 0.1) \times 10^{-10} \text{ m}^2/\text{s}$ . The water diffusion constant at 296 K in nonoriented POPC at hydration levels similar to this sample is  $\sim 3 \times 10^{-10} \text{ m}^2/\text{s}$ , agreeing well with this value, considering the difference in temperature (Volke et al., 1994). The same value of  $r$  gave a good fit to the POPC diffusion data at 322 K, with a diffusion constant of  $(8.6 \pm 0.2) \times 10^{-12} \text{ m}^2/\text{s}$ . In fact, for particle sizes  $\geq 10 \mu\text{m}$  at typical lipid diffusion rates and diffusion times of 200 ms or less, there is no measurable influence of curvature on diffusion rates, and the effect of curvature on diffusion may be neglected. Particle sizes an order-of-magnitude larger would be necessary to neglect the influence of curvature on water diffusion.

To check the influence of hydration, the diffusion of POPC bilayers hydrated with excess water was also measured at a spinning frequency of 5 kHz to limit dehydration from centrifugal forces. This POPC sample was found to have a curvature-corrected diffusion value of  $(1.9 \pm 0.1) \times 10^{-11} \text{ m}^2/\text{s}$  at 322 K and an average radius of curvature of  $4.5 \pm 0.5 \mu\text{m}$ . The diffusion constant is in excellent agreement with the value reported for oriented POPC samples with 30 wt %  $^2\text{H}_2\text{O}$  (Filippov et al., 2003). Although our sample originally had greater water content,

the centrifugal forces from MAS most likely reduced water concentration to a comparable level (F. Volke, University of Leipzig, personal communication; and Nagle et al., 1999). As expected, at higher hydration, the diffusion constant increases and the radius of curvature decreases.

## Activation energy

The activation energy for lateral diffusion of water in POPC bilayers was obtained from the temperature dependence of the diffusion rates (Fig. 5). The value obtained for water,  $28.6 \pm 1.5 \text{ kJ/mole}$ , is comparable with previously-reported data for oriented egg PC, where the activation energy steadily decreases from 28.2 kJ/mole at 6.4 waters/lipid to 23.0 kJ/mole at 18.6 waters/lipid (Wassall, 1996). The activation energy for the lateral diffusion of POPC also depends on water concentration. The activation energy obtained for POPC at 8.2 waters/lipid is  $40.6 \pm 0.4 \text{ kJ/mole}$ , and in excess water it was  $30.7 \pm 0.9 \text{ kJ/mole}$ . The latter value is in excellent agreement with data reported by Filippov and co-workers for oriented POPC with 30 wt %  $^2\text{H}_2\text{O}$ , 28 kJ/mole (Filippov et al., 2003). As expected, at higher hydration, the activation energies for both lipid and water diffusion decrease.

## Diffusion of ibuprofen in POPC membranes

The structure of ibuprofen is given in Fig. 1. A hydrophobic molecule, ibuprofen has low partitioning into water and locates in the lipid phase (Barbato et al., 1997). Indeed, the aromatic ring current of ibuprofen induces upfield chemical shifts in the lipid resonances. Furthermore, these shifts (Fig. 6) are more pronounced for upper acyl chain and glycerol resonances, indicating that ibuprofen locates preferentially in the interfacial region of the bilayer.

Diffusion rates of ibuprofen and POPC in a multilamellar environment were measured and the signal attenuation was fit to Eq. 3 and is plotted in Fig. 7. We made the reasonable

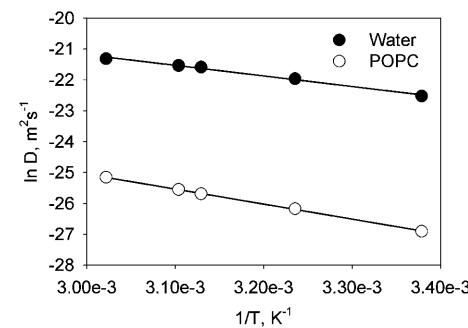


FIGURE 5 The activation energies of lateral diffusion of POPC and water were extracted from the temperature dependence of the diffusion constants. For POPC,  $E_a = 40.6 \pm 0.4 \text{ kJ/mole}$ . The water diffusion constants were corrected for curvature using Eq. 5 and a radius of curvature of  $21 \mu\text{m}$ . For water,  $E_a = 28.6 \pm 1.5 \text{ kJ/mole}$ . Water content is 8.2 waters/lipid.

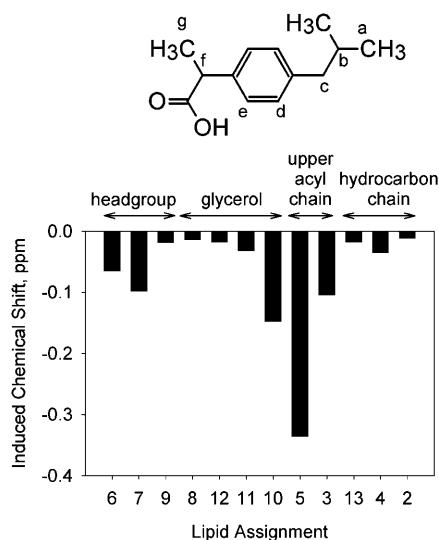


FIGURE 6 A plot of the induced chemical shift of each lipid resonance per mole of ibuprofen. The inset shows the structure of ibuprofen. The signal assignments can be found in Fig. 1.

assumption that average liposome sizes were greater than the  $\sim 10 \mu\text{m}$  necessary to neglect curvature for diffusion rates on the order of  $10^{-11} \text{ m}^2/\text{s}$  or smaller, and made no corrections for the effects of curvature.

In all cases, the ibuprofen diffuses faster than the POPC. The ibuprofen diffusion constant varies from  $(1.05\text{--}5.04) \times 10^{-11} \text{ m}^2/\text{s}$  at 296–331 K compared to  $(6.6\text{--}23.1) \times 10^{-12}$

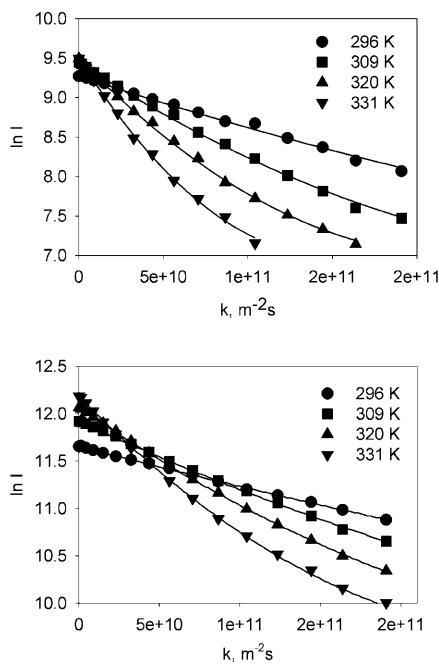


FIGURE 7 A plot of the attenuation of signal intensities of ibuprofen (upper) and POPC (lower) as a function of increasing  $k$  for different temperatures. The data were fit to Eq. 3 to obtain diffusion constants. Only points for which  $k_D < 5$  were included in the fit. Water content is 15 waters/lipid.

$\text{m}^2/\text{s}$  for POPC. At 309 K, the diffusion constant of ibuprofen is  $(2.1 \pm 0.1) \times 10^{-11} \text{ m}^2/\text{s}$ . In just over a second, ibuprofen diffuses  $10 \mu\text{m}$ , the approximate dimension of a cell. For comparison, the diffusion constant of free ibuprofen in phosphate buffer is  $5.5 \times 10^{-10} \text{ m}^2/\text{s}$  (Derrick et al., 2002).

The temperature dependence of the diffusion constants was used to extract activation energies for ibuprofen and POPC (Fig. 8). The activation energy of ibuprofen ( $36.6 \pm 1.3 \text{ kJ/mole}$ ) is slightly higher than that of POPC ( $31.9 \pm 0.3 \text{ kJ/mole}$ ). We propose that the activation energy of ibuprofen diffusion reflects contributions from an energy barrier of temporary association between ibuprofen and POPC. The aromatic-induced changes in lipid chemical shift provide further evidence for these interactions. Such temporary associations are responsible for NOESY cross-relaxation and for induced chemical shift changes observed in samples of aromatic indole derivatives and POPC (Yau et al., 1998). The same interactions thought to drive these indole associations are also present in ibuprofen/POPC systems.

## CONCLUSIONS

PFG-MAS NMR is a reliable approach for measuring diffusion constants of water, lipids, and membrane-associated substances in multilamellar liposomes and is an alternative to oriented sample measurements. The advantages of this method are manifold. Superior resolution of the resonances is obtained, without the need for onerous sample preparation. Measurements can be performed on a very small amount of material, micrograms if necessary, making possible experiments on real cell membranes. Furthermore, the high spinning frequencies average internal field gradients that are likely to perturb diffusion measurements at high field strength (Leu et al., 2000). Finally, because of the high precision in measurements of signal amplitudes of the well-resolved resonances, low errors in the diffusion constant are obtained.

There are also limitations to the method. Diffusion perpendicular to the planes cannot be measured, and it must be assumed that all diffusion is in the bilayer plane. Data

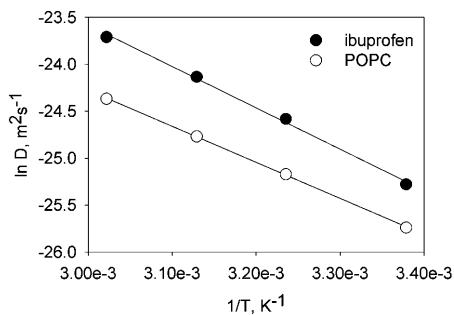


FIGURE 8 The activation energy for POPC and ibuprofen is determined from the temperature dependence of the diffusion constants. The activation energy for POPC is  $31.9 \pm 0.3 \text{ kJ/mole}$  and for ibuprofen is  $36.6 \pm 1.3 \text{ kJ/mole}$ .

analysis according to Eq. 3 is an approximation that works well for analyzing signal decay down to 8%. Analysis of signal decay down to 1% of the initial signal intensity, the typical criterion for assuring homogeneous samples, requires using numerical solutions of Eq. 1 that are less convenient. Currently, PFG-MAS experiments on lipids do not reach such attenuation because of limits in available gradient strength. Therefore, a small fraction of immobile sample would be undetectable in this approach. Additionally, fast MAS spinning has a tendency to dehydrate the sample (F. Volke, University of Leipzig, personal communication; and Nagle et al., 1999), which, as shown, has a dramatic effect on the diffusion rates.

Also, the interpretation of the diffusion data depends on the assumption that the phospholipid bilayers are oriented randomly. Partial orientation of the bilayers may have two origins: anisotropy of the magnetic susceptibility of the bilayers in combination with the high magnetic fields of the NMR instrument (Brumm et al., 1992) and the centrifugal forces from fast spinning. The effects of the magnetic field are ameliorated by MAS (Brumm et al., 1992; Courtieu et al., 1994) and can be safely ignored for most lipid bilayers. Centrifugal forces from spinning will press bilayers against the spherical walls of the rotor inserts and may cause propagation of orientation into the inner volume of samples. Since these forces are strongest for orientation of the surface normal perpendicular to the rotation axis, this may cause a slight preference for alignment of the bilayer normals perpendicular to the rotor axis. We simulated the influence of such sample alignment on signal decay by introducing orientation distribution functions into Eq. 1. Minor alignment results in a modest overestimation of diffusion constants when data are analyzed by Eq. 3. However,  $^2\text{H}$  NMR powder spectra of deuterated POPC taken immediately after fast spinning show no signs of alignment. Though we are confident that orientation of the bilayers did not influence our measurements, the possibility of some degree of orientation cannot be excluded a priori for all samples.

Finally, depending on the pertinent diffusion rates, a correction for the effects of curvature may be necessary. For our experimental settings, as long as particle sizes are  $\geq 10 \mu\text{m}$ , no correction is needed for the diffusion constant of lipids. However, a correction for curvature is essential for fast-moving substances like water except in the case of particle sizes that are larger by one order of magnitude. The availability of stronger gradients would allow for the reduction of diffusion times and would consequently lessen the influence of curvature on data analysis. The dependence of apparent diffusion rates on diffusion time can be used to calculate an effective radius of curvature and extract a “true” diffusion constant.

With the above caveats, this approach provides a valuable tool for measuring the mobility of substances dissolved in membranes. Since lipid lateral diffusion is the major contributor to intermolecular cross-relaxation in lipids, the

diffusion constants will be a valuable tool in the interpretation of NOESY cross-relaxation rates of lipid bilayers (Yau and Gawrisch, 2000; Feller et al., 1999). Furthermore, this method has several other exciting applications. PFG MAS-NMR should be a practical approach for characterizing membrane organization. For instance, the presence of domains can be ascertained by diffusion measurements in lipid mixtures containing cholesterol, as long as an individual resonance for each compound can be resolved. Furthermore, temporary associations of lipids with proteins can be noted by a decrease in the lipid diffusion constant. Moreover, if a resolvable signal is present, the diffusion of proteins themselves could be measured in the same experiment. Finally, the diffusion of membrane-associated molecules in the water space between bilayers could be measured.

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