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Determination of Orientational Order Parameters from ^{2}H NMR Spectra of Magnetically Partially Oriented Lipid Bilayers

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ABSTRACT The partial orientation of multilamellar vesicles (MLVs) in high magnetic fields is known to affect the shape of ^{2}H NMR spectra. There are numerical methods for extracting either the orientational order parameters of lipid molecules for a random distribution of domain orientations in the sample, or the distribution of orientations for a known set of spectral anisotropies. A first attempt at determining the orientational order parameters in the presence of an unknown nonrandom distribution of orientations is presented. The numerical method is based on the Tikhonov regularization algorithm. It is tested using simulated partially oriented spectra. An experimental spectrum of a phospholipid-ether mixture in water is analyzed as an example. The experimental spectrum is consistent with an ellipsoidal shape of MLVs with a ratio of semiaxes of ~3.4.

INTRODUCTION

The techniques of solid-state NMR have been applied to a wide range of ordered and partially ordered systems, such as solids and liquid crystals. Deuterium NMR (^{2}H NMR) in particular has provided many insights into the structure, symmetry, and molecular motions in biological and model membranes.

In this class of systems, the anisotropic nature of molecular motions results in an incomplete motional averaging of the orientation-dependent second-rank tensor interactions, such as anisotropic chemical shift, nuclear dipole-dipole, and quadrupolar interactions. Phospholipid molecules of a bilayer membrane in the liquid crystalline (L_α) phase undergo a rapid, axially symmetrical reorientational motion about the bilayer normal. Because of the axial symmetry, it is customary to separate out the effect of this motion on the observed quadrupolar splitting by writing

$$\omega(x, \theta) = \frac{3}{2}\,\omega_Q S_{CD} P_2(\cos\theta) = x P_2(\cos\theta) = x\,\frac{3\cos^2\theta - 1}{2} \tag{1}$$

where $\omega_Q = e^2qQ/\hbar \simeq 2\pi \times 167$ kHz is the quadrupolar coupling constant, θ is the angle between the axis of symmetry of the motion and the external magnetic field, and $S_{CD} = 1/2\,\langle 3\cos^2\gamma - 1\rangle$ is the orientational order parameter, where γ is the angle between the C—^{2}H bond and the axis of symmetry of the reorientational motion. Here the angle brackets $\langle \ldots \rangle$ denote an average over the molecular motions that are fast on the NMR time scale, i.e., compared to $(\omega_Q)^{-1}$. For nuclei other than ^{2}H, a similar description could be made in terms of, for example, a chemical shift anisotropy in place of the quadrupolar splitting. The essential consequence of the highly symmetrical nature of some of the fast motions in phospholipid bilayers is that a spectroscopic observable ω is related to some generalized time-averaged anisotropy parameter x, through $P_2(\cos\theta)$ scaling.

Different parts of the molecule may move differently, with different mean orientations and degrees of reorientation, and may, therefore, have different order parameters. In phospholipids, $S_{CD}(n)$ of the hydrocarbon chains decreases with increasing carbon number n, i.e., with increasing distance from the glycerol backbone, giving rise to a characteristic order parameter profile. This order parameter profile and, in particular, changes in it induced by chemical or physical means have become an important tool for obtaining both structural and dynamical information in membrane biophysics.

For a fixed θ, e.g., for a lipid bilayer squeezed between flat glass plates, the observed pattern of quadrupolar splittings provides a direct measure of the order parameter profile. Extracting the same information from a "powder" sample that consists of domains of different bilayer orientations is considerably more difficult, because the observed quadrupolar splitting is directly affected by changes in both S_{CD} and θ. When all orientations in a powder are equally probable, i.e., when the orientational distribution function is simply $p(\theta) \propto \sin\theta$, then for each carbon position a superposition of contributions from different θ values results in a characteristic Pake doublet lineshape. In such a case, a numerical procedure ("de-Pake-ing") can be used to obtain the spectrum for a single θ value, and thus to extract the order parameter profile.

A random orientational distribution in a powder sample is realized when ordering due to the presence of an external magnetic field is negligible. However, the magnetic susceptibility of phospholipid bilayers is anisotropic, with a neg-

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ative anisotropy of the magnetic susceptibility,

$$\Delta\chi = \chi_{\|} - \chi_{\perp} < 0 \tag{2}$$

When a small domain of a bilayer of area A, thickness d, and bilayer normal $\vec{n}$ is placed in a strong external magnetic field $\vec{H}$, a magnetic moment is induced, which then interacts with the field. The resulting torque,

$$\tau = \Delta\chi Ad(H \cdot n)(H \times n) \tag{3}$$

orients the domain preferentially so that $\vec{n} \perp \vec{H}$ is favored. Because of the quadratic dependence on the strength of the external magnetic field, the effect on the distribution of bilayer domain orientations is considerable. With the advent of higher magnetic fields in NMR spectroscopy, dramatic experimental evidence has appeared that the existence of such partial magnetic ordering in biological and model membrane systems must be taken into account.

Such ordering distorts the interpretation of NMR lineshapes, their moments, and the relaxation times. In particular, extraction of the orientational order profile is strongly affected. As shown below, significant errors are introduced if standard dePakeing methods are applied to partially ordered systems.

Two experimental techniques have been proposed to avoid the orientational ordering in liposomes placed in a high magnetic field. In the so-called spherically supported vesicles, pioneered by Bayerl and co-workers (Bayerl and Bloom, 1990), lipid bilayers are deposited on spherical glassy beads 0.5–1.0 μm in radius. While multilamellar vesicles of a mixture of dipalmitoylphosphatidylcholine and cholesterol in an aqueous solution are elliptically deformed if exposed to a magnetic field of 9.4 T, the spherically supported vesicles of the same composition do not show any orientational distortion. Recently, Gawrisch et al. (1997, private communication) suggested the use of a double-tuned hopping-coil NMR probe to prevent lipid powder samples from orienting in high magnetic fields. In some initial experiments using a standard slow-spinning magic angle spinning NMR probe (spinning rate ~100 Hz, slow enough to avoid an averaging of anisotropic quadrupolar interactions), the orientational ordering was dramatically reduced. This suggests that a simultaneous spinning around two orthogonal axes would time-average any orientational torque on the lipid molecules to zero. Unfortunately, for financial and technical reasons, this experiment has not yet been done.

In this work, an alternative method of dealing with magnetically partially ordered systems is proposed. We introduce a numerical procedure that is capable of extracting the oriented spectra, and thus the order parameter profiles, from the NMR spectra of partially oriented lipid samples. The ability of the algorithm to extract correct order parameter profiles from strongly magnetically distorted powder spectra is demonstrated using numerical simulations. Analysis of an experimental spectrum of a phospholipid-ether mixture in water is then presented as an example.

THEORY

Distribution functions *g*(*x*) and *p*(*θ*)

In general, a system has more than a single inherent time-averaged anisotropy (more than a single order parameter) and thus it is appropriate to introduce the anisotropy distribution function $g(x)$, which contains all of the physical information of interest. For example, the order parameter profile $S_{\mathrm{CD}}(n)$ extracted from the ^{2}H NMR spectra is directly related to such a distribution function, but our approach is not restricted to this case. Clearly, for a system that can be adequately described by a single anisotropy parameter, e.g., anisotropy of the chemical shift for a spin-(1/2) system, $g(x)$ is a δ-function.

On the other hand, a powder sample contains domains of different orientations, and the observed powder spectrum is a superposition of contributions from all of these domains. As a result, a single fixed anisotropy parameter x corresponds to a continuous lineshape function, $s_x(\omega)$, in the frequency domain. To determine $s_x(\omega)$, it is essential to consider also the orientation distribution function $p(\theta)$, which represents the probability of encountering a domain oriented at an angle θ with respect to the external magnetic field in a given sample.

For an oriented sample, the orientation distribution function reduces to $p(\theta) = \delta(\theta - \theta_0) = \delta(\theta)$ (where, for simplicity, we chose $\theta_0 = 0$), and the corresponding lineshape is simply $s_x(\omega) = \delta(\omega - x)$. Clearly, the position ω of the spectral line is a direct measure of the anisotropy. In the case of ^{2}H NMR, the matters are somewhat complicated by the presence of two symmetrical transitions; in this case, $s_x(\omega) = \delta(|\omega| - x)$, and thus only the magnitude (but not the sign) of the anisotropy can be determined from the spectrum.

For a random uniform powder distribution, $p(\theta) \propto \sin\theta$, and the following well-known lineshape function is obtained:

$$s_x(\omega) = [3x(x + 2\omega)]^{-1/2} \tag{4}$$

For a single quadrupolar splitting, the powder spectrum $s_x(\omega) + s_{-x}(\omega)$ has the so-called Pake doublet lineshape.

Powder spectrum as a mapping *g*(*x*), *p*(*θ*) → *S*(*ω*)

Clearly, both distribution functions must be accounted for when considering experimental powder spectra. The two expressions

$$S(\omega) = \int g(x)\left[p(\theta)\frac{\partial\theta}{\partial\omega}\right]\mathrm{d}x \tag{5}$$

$$= \int p(\theta)\left[g(x)\frac{\partial x}{\partial\omega}\right]\mathrm{d}\theta \tag{6}$$

represent two equivalent descriptions of the same mapping of the two distribution functions $g(x)$, $p(\theta)$ onto the powder spectrum $S(\omega)$. The two expressions simply reflect which of the two points of view is being taken. Equation 5 is a

$g(x)$-weighted superposition of lineshape functions, one for each anisotropy x. On the other hand, Eq. 6 is a $p(\theta)$-weighted superposition of spectra from the oriented domains that constitute the powder sample, one for each orientation θ. In both cases, ω, x, and θ are related through the fundamental scaling relationship of Eq. 1. Thus, Eq. 5 implies $\theta = \theta(x, \omega)$ and Eq. 6 implies $x = x(\theta, \omega)$.

Analysis of the experimental data corresponds to obtaining the inverse of the mapping described by Eqs. 5 and 6, i.e., a solution of an integral equation with the kernel function defined in both cases by the expression in square brackets. Reliable numerical methods of solving such integral equations are known. Two possible experimental situations have received considerable attention in the literature.

If $p(\theta)$ is known, and thus an appropriate lineshape function can be calculated, $g(x)$ can be extracted from the powder spectrum. This is precisely the case of "dePakeing" (Bloom et al., 1981), a numerical procedure of extracting an oriented spectrum (and, thus, $g(x)$) from a powder spectrum. Several dePakeing algorithms have been reported and successfully applied to a variety of model and biological membrane systems (Sternin et al., 1983; Whittall et al., 1989; Schäfer et al., 1995). The explicit assumption made by all algorithms is that of a uniform random orientation distribution, which corresponds to $p(\theta) \propto \sin\theta$, but because this assumption is often satisfied, dePakeing has been widely used as a reliable method of obtaining order parameters of the system. Clearly, applying the standard dePakeing method to the spectra of partially oriented systems inevitably leads to gross systematic errors in $g(x)$.

If, instead, $g(x)$ is known, $p(\theta)$ can be determined. One example is the extraction of the orientational distribution of the local director in a nematic liquid crystal (mixture M5) with a small amount of chiral dopant confined to the cylindrical channels of Anopore (Al_2O_3) filters (Schäfer and Stannarius, 1995). Isotropic chemical shifts, chemical-shift anisotropies, and relative line intensities determined previously for 18 inequivalent carbon positions are used as inputs, and the orientational distribution of the nematic director is extracted. Monitoring this distribution through changes in temperature or in the direction and strength of the external magnetic field provides direct experimental access to the molecular ordering mechanism in a liquid crystal.

Finally, the case of determining both $g(x)$ and $p(\theta)$ simultaneously has not yet been addressed. Because of the interdependence of ω, x, and θ, this general case is a considerably more difficult problem. A reliable method of obtaining physical information (contained in $g(x)$) from the experimental spectra measured under the conditions of partial magnetic ordering in a high external magnetic field is clearly needed. Fortunately, it is possible to make considerable progress in this direction by modify a dePakeing algorithm to extract both $g(x)$ and a limited number of parameters that characterize $p(\theta)$.

The Tikhonov regularization algorithm

To emphasize that x is an independent variable, we introduce the kernel function $C(x, \omega)$ in place of the lineshape function $s_x(\omega)$. With that, Eq. 5 becomes

$$S(\omega) = \int_{-\infty}^{+\infty} g(x)C(x, \omega)\mathrm{d}x \tag{7}$$

One can write

$$C(x, \omega) = \begin{cases} p(\theta)\dfrac{\partial\theta}{\partial\omega}, & \text{for } -x/2 < \omega \leq x \\ 0, & \text{otherwise} \end{cases} \tag{8}$$

where

$$p(\theta)\frac{\partial\theta}{\partial\omega} = -\frac{p[\theta(x, \omega)]}{[2(x-\omega)(x+2\omega)]^{1/2}} \tag{9}$$

if the dependence on $\theta(x, \omega)$ as well as the scaling relationship of Eq. 1 are put in explicitly. Knowing $p(\theta)$ completely determines $C(x, \omega)$.

A discrete set of experimental data, S_j^σ, $j = 1 \ldots m$, is always incomplete, i.e., is known at a limited number of frequency sampling points ω_j, and is affected by the noise σ_j:

$$S_j^\sigma = S_j + \sigma_j \tag{10}$$

where the "exact data" $S_j = S(\omega_j)$ are completely determined by $g(x)$ through Eq. 7.

For a well-defined kernel function $C(x, \omega)$, i.e., if $p(\theta)$ is known in advance, numerical analysis of such data should provide the distribution function $g(x)$. However, for noisy data one can only expect to obtain approximations, $\tilde{g}(x)$. In general, obtaining reasonable approximations from noisy relaxation data is a rather ambitious undertaking. Equation 7 is a Fredholm integral equation of the first kind and thus belongs to the class of so-called ill-posed problems. That is, for any set of noisy data $\{S_j^\sigma\}$, infinitely many approximations $\tilde{g}(x)$ result in equally good fits $S_j[\tilde{g}(x)]$ to the input data in the least-squares or maximum error sense. Almost all of those approximations are in fact wrong, as can be demonstrated by using the Riemann-Lebesque theorem (Bertero et al., 1980). For this reason, simple least-squares or linear programming algorithms are not appropriate for dealing with such integral equations.

However, the Tikhonov regularization technique (Tikhonov and Arsenin, 1977; Groetsch, 1984) is known to provide reliable solutions of such integral equations by minimizing the least-squares error with respect to $g(x)$ under additional constraints, e.g., a certain degree of smoothness of $g(x)$. Successful dePakeing of powder spectra has already been achieved by this method (Schäfer et al., 1995). For m input data points, the regularization algorithm minimizes the following expanded least-squares expression with respect to $g(x)$:

$$\Phi[g(x)] = \sum_{j=1}^{m} [S_j^{\sigma} - S_j]^2 + \lambda \|g''\|^2 \tag{11}$$

subject to $g(x) \geq 0$. The first term in Eq. 11 is the usual least-squares term, which guarantees compatibility of the fit with the data. The Tikhonov regularization approach introduces the second term, which controls the smoothness of the approximation. Here λ is the regularization parameter, and g'' denotes the second derivative of the distribution function $g(x)$.

A good estimate for λ is essential for the quality of the solution. Too small values for λ result in artificial, physically meaningless structures in $\tilde{g}(x)$, whereas too large a λ tends to oversmooth the shape of $\tilde{g}(x)$ and to suppress information. Honerkamp and Weese have developed a self-consistency (SC) method for the estimation of regularization parameters that proved successful in many numerical algorithms related to Fredholm integral equations (Honerkamp and Weese, 1990; Weese, 1992; Schäfer and Bauch, 1995; Schäfer and Stannarius, 1995). The SC method guarantees that the approximation $\tilde{g}(x)$ is in the vicinity of the true distribution function $g(x)$.

Once the optimum value of the regularization parameter is determined from the SC method, minimizing $\Phi[g(x)]$ yields $\tilde{g}(x)$.

$p(\theta)$ of partially ordered systems

The above algorithm assumes that $p(\theta)$ is well defined. Although this is true for the random orientational distribution of powder samples, $p(\theta) \propto \sin(\theta)$, this assumption does not hold if magnetic ordering occurs in a membrane system. This is a much more difficult situation, where $p(\theta)$ and, consequently, the integral kernel $C(x, \omega)$ itself are not exactly known. The algorithm outlined above cannot be applied directly because both $g(x)$ and $p(\theta)$ need to be determined by the data analysis. Thus a generalization of the regularization method is required.

A general representation of $p(\theta)$ could be made in terms of the orthogonal Legendre polynomials

$$p(\theta) \propto \sin(\theta) \times \sum_{i=1}^{\infty} A_i P_i(\cos \theta) \tag{12}$$

Because $p(\theta)$ is completely defined by the coefficients A_i, one could try to calculate simultaneously both $\tilde{g}(x)$ and the set of $\tilde{A}_i$ from the data S_j^{σ}. However, this general approach increases the complexity and size of the mathematical optimization problem beyond a reasonable limit. Whereas the calculation of $\tilde{g}(x)$ from the data S_j^{σ} is a linear optimization problem if $p(\theta)$ is available, a combination of linear and nonlinear optimization techniques has to be applied if $\tilde{g}(x)$ and $\tilde{A}_i$ are to be calculated. That leads to an increase in computation time and to numerical instability.

Instead, one could try a simple, physically reasonable model for $p(\theta)$ and attempt to determine (a limited number of) parameters of such a model simultaneously with $\tilde{g}(x)$. Restricting the model to a single parameter κ clearly represents the simplest possible case. The expectation is that a magnetic ordering effect can be successfully described by a physically justified one-parameter function $p_{\kappa}(\theta)$ that approximates the true $p(\theta)$ when the value of κ is chosen appropriately. The parameter κ can be thought of as describing the degree of magnetic distortion of the original sin θ distribution. For each fixed value of κ, $p_{\kappa}(\theta)$ is well defined, and thus $\tilde{g}(x)$ can be determined from the data S_j^{σ} by using the Tikhonov regularization algorithm. Sweeping an appropriate range of values for κ, the minimum of the least-squares error sum

$$\Psi(\kappa) = \sum_{j=1}^{m} [S_j^{\sigma} - S_j(\tilde{g}(x), \kappa)]^2 \tag{13}$$

provides an optimum estimate $\tilde{\kappa}$. Of all functions $p_{\kappa}(\theta)$, the one closest to the true $p(\theta)$ is thus determined, leading to the best approximation $\tilde{g}_{\tilde{\kappa}}(x)$ within the limits of the model defined by $p_{\kappa}(\theta)$. Note that this approach is very general and is not restricted to a certain one-parameter model function $p_{\kappa}(\theta)$. The entire procedure can be repeated using different model functions, to determine the best one.

The following three one-parameter models for $p(\theta)$ are examined to illustrate the power of our approach. $p_L(\theta)$ arises from the truncation of the series expansion of Eq. 12 after the first two even-numbered terms:

$$p_L(\theta) \propto \sin \theta \times (1 + \kappa_L \cos^2 \theta) \tag{14}$$

The random orientation distribution can be recovered by setting $\kappa_L = 0$, and the decrease in spectral intensity observed at the shoulders ($\theta = 0°$) of the Pake doublet spectra of ^{2}H NMR can be modeled by negative κ_L values.

The second model function arises in the extreme case of a completely uncorrelated orientation of domains within the membrane. A Boltzmann distribution of energies of interaction between the membrane domains and the magnetic field, proportional to $\cos^2 \theta$ as can be seen from Eq. 3, yields

$$p_B(\theta) \propto \sin \theta \times \exp[\kappa_B \cos^2 \theta] \tag{15}$$

Again, $\kappa_B = 0$ corresponds to the random distribution of orientations, whereas $|\kappa_B| \gg 1$ leads to a perfect order. If the anisotropy of the magnetic susceptibility $\Delta\chi$ is negative, $\kappa_B \leq 0$ is to be expected.

The third model function arises if a continuous ellipsoidal deformation of a spherical liposome is considered (Pott and Dufourc, 1995). This is just the other extreme—a completely correlated orientation of membrane domains originally arranged in a spherically symmetrical manner:

$$p_E(\theta) \propto \sin \theta \times [1 - (1 - \kappa_E)\cos^2 \theta]^{-2} \tag{16}$$

Here κ_E is the square of the ratio of the semiaxes, i.e., $\kappa_E = 1$ means spherical symmetry and $\kappa_E > 1$ describes an ellipsoidal deformation, with the long axis parallel to the magnetic field.

For small values of κ_B and $(1 - \kappa_E)$, the corresponding distributions $p_B(\theta)$ and $p_E(\theta)$ coincide with $p_L(\theta)$. This can be seen if the terms in the square brackets in Eqs. 15 and 16, respectively, are expanded in $\cos^2 \theta$ up to the first order and if one assumes that $\kappa_L = \kappa_B = 2(1 - \kappa_E)$. Thus, in the limit of a small deviation from the random distribution of orientations, all three models should yield similar results.

SIMULATIONS

Extensive numerical studies were conducted to test the algorithm under different simulated experimental conditions. An illustration of the artifacts arising when a standard dePakeing algorithm is applied to samples that are partially oriented in the presence of a strong external magnetic field is shown in Fig. 1. An experimentally measured order parameter profile is used as input, a simulated powder pattern is generated for both a spherical and an ellipsoidal geometry of a liposome, and a standard ("spherical") dePakeing is applied to reextract the original data. The orientational order profile extracted from an ellipsoidally distorted liposome exhibits a considerable deviation from the "true" one, whereas the points extracted from the simulated spectrum of a spherical liposome are faithful to it. The error is significant, even at relatively low ratios of the semiaxes of the ellipsoid, $\kappa_E = 5$, much less than the experimentally observed $\kappa_E \simeq 20$ (Qiu et al., 1993). The observed error is much greater than the changes in the order parameter profile associated with, for example, an L_α-to-H_{II} phase transition (Sternin et al., 1988), and thus a correct physical interpretation of the obtained order parameter profile is not possible.

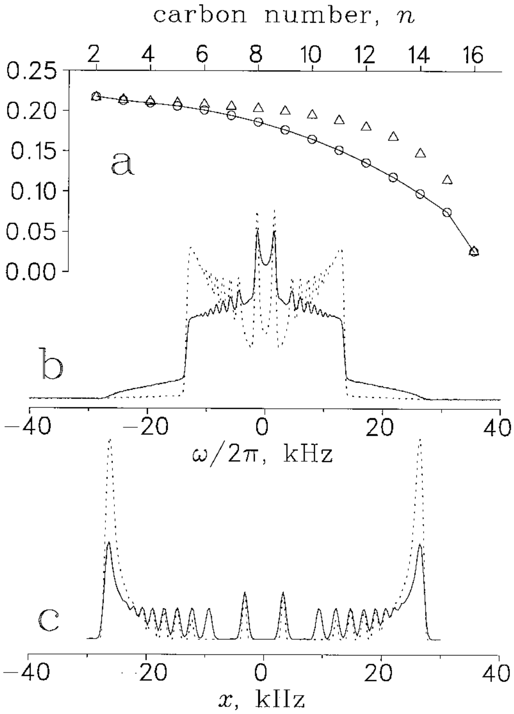

FIGURE 1 (*a*) *Solid line*: A smoothed order parameter profile obtained by dePakeing the ^{2}H NMR powder spectra of POPC-d_{31} liposomes, from Lafleur et al. (1989). The discontinuity in the smooth curve at the $n = 16$ carbon position is due to a different averaging mechanism of the terminal CD_3 group. (*b*) Powder spectra simulated from the smoothed order parameter profile in *a*, using Gaussian lineshapes 500 Hz in width. The solid line describes a spherical vesicle, $\kappa_E = 1$, and the dashed line corresponds to an ellipsoid with $\kappa_E = 5$. Random noise is added to simulate an experimental signal-to-noise ratio of ~1000 for the central peaks attributed to the terminal CD_3 group. (*c*) The oriented spectra obtained by dePakeing the simulated powder spectra from *b*, using the Tikhonov regularization algorithm and forcing $\kappa_E = 1$ (i.e., a spherical vesicle). Line types are the same as in *b*. The smoothed order parameter profiles extracted from these spectra are shown as the discrete symbols in *a*: ○, $\kappa_E = 1$ simulation; △, $\kappa_E = 5$.

The dePakeing algorithm does "report" when the process of extraction of an oriented spectrum has failed in some way. For the original iterative procedure, this takes the form of spectral artifacts of nonphysical negative amplitude (Sternin et al., 1983). For the linear inversion and regularization algorithms that explicitly restrict the result to positive spectral intensities only, $g(x) > 0$, the indication that a nonphysical oriented spectrum has been obtained is no longer present in the oriented spectrum itself, but rather, in much larger systematic misfit values, $S_j^\sigma - S_j$ in Eq. 11, reported by the algorithms. An example is shown in Fig. 2 *a*. If the restriction $\kappa_E = 1$ is now removed, and the algorithm described above is applied to the data, assuming the orientational probability distribution of Eq. 16 and allowing κ to vary, the true oriented spectrum is obtained. Consequently, the true order parameter profile is determined (not shown). At the same time, the systematic misfit reported by the algorithm essentially disappears, as seen in Fig. 2 *b*. In fact, experience has shown that the presence of a systematic misfit is a reliable indicator of convergence to nonphysical oriented spectra, and thus of the inadequacy of a particular model being evaluated. This is the reason why

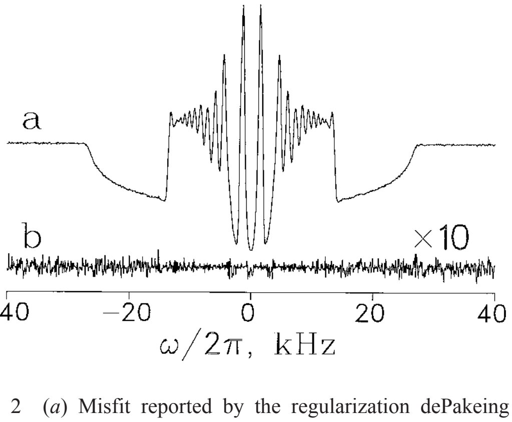

FIGURE 2 (*a*) Misfit reported by the regularization dePakeing algorithm, which is restricted to $\kappa_E = 1$, when applied to the powder spectrum of Fig. 1 *b* simulated by using $\kappa_E = 5$. When κ_E is allowed to vary, the algorithm extracts an oriented spectrum indistinguishable from the true one (shown in Fig. 1 *c*), whereas the systematic misfit in *b* is essentially eliminated.

a minimum in the misfit function $\Phi(\kappa)$ of Eq. 11 is used as the criterion for obtaining the estimate $\tilde{\kappa}$. A large misfit, even at the minimum of $\Phi(\kappa)$, should be interpreted as a sign that the chosen model for $p(\theta)$ is not adequate.

Fig. 3 shows how monitoring the misfit functions $\Phi(\kappa)$ for each of the three trial models of $p(\theta)$ given by Eqs. 14, 15, and 16 allows one to select the best one. Because our input spectrum was simulated by using the ellipsoidal model, it is gratifying that the lowest minimum of $\Phi(\kappa)$ is determined by the algorithm to correspond to $p_E(\theta)$, and that it occurs at $\tilde{\kappa}_E = 5$, precisely the value used in the simulation. The other two trial orientational distributions, $p_L(\theta)$ and $p_B(\theta)$, are clearly rejected, because their respective minima in $\Phi(\kappa)$ are considerably higher than that of $p_E(\theta)$.

AN EXPERIMENTAL EXAMPLE

A water dispersion of a mixture of 1,2-dimyristoyl-sn-3-phosphatidylcholine deuterated in all of the chain positions (DMPC-d_{54}) and a dioxyethylene-mono-dodecyl-ether ($C_{12}E_2$) was used to test our numerical procedure experimentally.

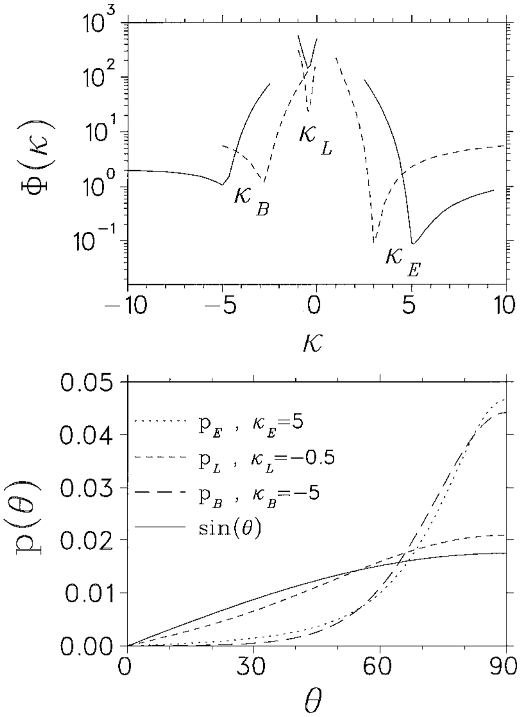

FIGURE 3 (*Top*) Minimizing $\Phi(\kappa)$ yields $\tilde{\kappa}$, for each of the three trial functions $p_L(\theta)$, $p_B(\theta)$, and $p_E(\theta)$, described by Eqs. 14, 15, and 16, respectively. The lowest minimum corresponds to the best model, as expected for the powder spectrum simulated, assuming an ellipsoidal shape of the liposome. Two sets of functions are shown, for data simulated with $\kappa_E = 5$ (*solid lines*) and $\kappa_E = 3$ (*dashed lines*), and in both cases the lowest minimum reports the correct model and the correct κ value. (*Bottom*) The three trial orientation dependence functions, calculated for their respective best approximations of $\tilde{\kappa}$, as determined from the three minima above, are shown to illustrate the ability of the algorithm to resolve different shapes of $p(\theta)$. The random distribution function, $p(\theta) = \sin\theta$, is also shown for comparison.

Materials and experimental procedure

DMPC-d_{54} was purchased from Avanti Polar Lipids (Alabaster, AL), $C_{12}E_2$ from Nikko Chemicals (Tokyo, Japan), and deuterium-depleted water (99.97% ^1H_2O purity) from Aldrich Chemical Co. (Milwaukee, WI). Lipid purity was verified by thin-layer chromatography. The molar fraction of $C_{12}E_2$ was 0.16 of the mixture, and water was added in excess (100 mol of H_2O per mol of lipid + $C_{12}E_2$ mixture).

NMR spectra were obtained with a Bruker MSL 500 spectrometer in a field of 11.7 T (^{2}H Larmor frequency of 76.7 MHz), using a broadband probe with a cylindrical rf coil 5 mm in diameter. Quadrupolar echo pulse sequence was used (Davis et al., 1976) with a 90° pulse length of 2.5 μs, $\tau = 40$ μs, and a repetition time of 450 ms. The spectrum was acquired as 4096 complex data points, with a 1-μs dwell time and a receiver bandwidth of 2 MHz.

A Bruker B-VT 1000E temperature control unit maintained the mixture in the L_α phase at 313 ± 0.5 K, with the temperature of the main phase transition at ~291 K (phase diagram to be published).

DMPC, $C_{12}E_2$, and water were directly dispersed and homogenized above the main phase transition temperature by vortexing, and the mixture was allowed to equilibrate for 5 days, monitored by differential scanning calorimetry. Similar preparations are known to yield multilamellar vesicles 1–5 μm in diameter, as measured by electron microscopy.

Fig. 4 *a* shows a typical experimental powder spectrum of a perdeuterated phospholipid in the L_α phase. The spectrum exhibits reduced spectral intensity in the shoulders, charac-

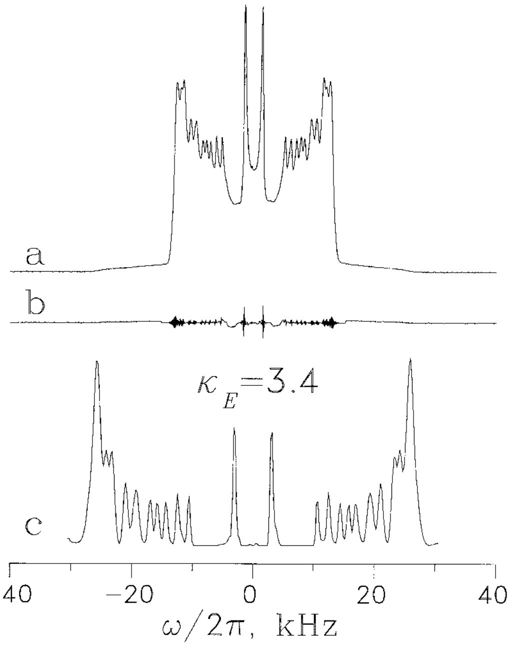

FIGURE 4 (*a*) A powder spectrum of DMPC-d_{54}/$C_{12}E_2$ mixture in excess water at 313 K. (*c* and *b*) The dePaked spectrum and the reported misfit, respectively, extracted by the regularization dePakeing algorithm for the ellipsoidal orientational model with $\kappa_E = 3.4$, which corresponds to the minimum of $\Phi(\kappa)$ (see Fig. 5).

teristic of a partially oriented system, but the degree of spectral distortion is not great. This spectrum was analyzed by using the regularization dePakeing algorithm for all three orientational models described earlier, and Fig. 4 *c* is the dePaked spectrum that corresponds to the lowest minimum of $\Phi(\kappa)$, namely that of the ellipsoidal model of Eq. 16, with $\kappa_E = 3.4$. The misfit reported by the algorithm is shown in Fig. 4 *b*. The small systematic misfit seen in the shoulder section of the spectrum is indicative of the limitations of the model employed, and probably indicates that a distribution of vesicle shapes with a range of κ_E values is present in the sample.

As discussed above, for this relatively small deviation from a random orientational distribution, it is somewhat difficult to distinguish between different model functions for $p(\theta)$. Fig. 5 shows the misfit functions $\Phi(\kappa)$ for each of the three trial models used in the dePakeing of the experimental spectrum of Fig. 4 *a*. Although the ellipsoidal model exhibits the lowest minimum at $\kappa_E = 3.4$, the minimum associated with the Boltzmann model is relatively close, indicating that both models may provide adequate approximations to the true orientational distribution function $p(\theta)$. However, the presence of a deviation from a random distribution function is clearly detected by using either of the two models, and thus a true oriented spectrum and, hence, a true averaged order parameter profile can be extracted.

CONCLUDING REMARKS

The method presented here is the first attempt to obtain simultaneously the order parameters (anisotropies) of the system as well as the distribution of domain orientations present in the sample from purely spectroscopic data. Magnetically induced partial ordering of lipid bilayers is currently seen as a distortion that obscures the true orientational order parameters of the lipid molecules, because the commonly available dePakeing algorithms rely on an explicit assumption of a random orientational distribution function. On the other hand, to measure the magnetic ordering effects, one must rely on a set of independently measured anisotropies of the system.

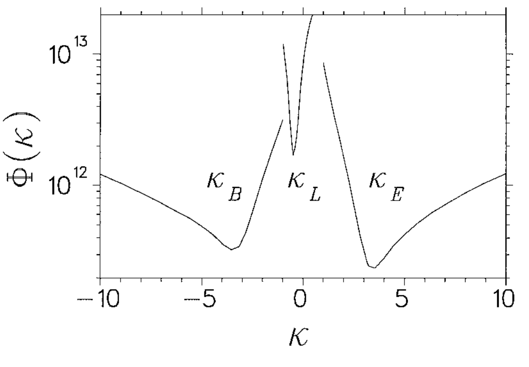

FIGURE 5 $\Phi(\kappa)$ calculated for each of the three trial functions $p_L(\theta)$, $p_B(\theta)$, and $p_E(\theta)$, as applied to the analysis of the experimental spectrum of Fig. 4 *a*. The lowest minimum corresponds to the ellipsoidal shape of the liposome with the ratio of the semiaxes $\kappa_E = 3.4$.

Complex experimental techniques, such as the spherically supported vesicles of Bayerl et al., have been developed to counteract the magnetic ordering in bilayer systems. The proposed numerical method has the obvious advantage of not requiring any special sample manipulation. In fact, previously acquired data can now be reanalyzed to extract the true orientational order parameters. Furthermore, the "distortion" introduced by the magnetic ordering can be used to extract information on the shape distributions and, hence, on the bulk magnetic properties of the lipid bilayer. Higher magnetic field strengths, often preferable for spectroscopic reasons, need not be avoided because of the changes in the observed lineshape due to magnetic ordering of bilayer domains.

The method requires high spectroscopic fidelity and is fairly intensive computationally. Depending on the size of the data set, a calculation for a single κ value may take several minutes on a midsize workstation (Silicon Graphics' Indigo R4000). Currently it is only possible to extract a limited number of parameters describing the orientational distribution function. However, the approach is very general, and with further advances in NMR and computer technology, the method could be extended to extract a more detailed description of orientational distributions.

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