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Gerhard Althoff [a], Diego Frezzato [a], Gerd Kothe [a], Diego Frezzato [b], Giorgio J. Moro [b], Marija Vilfan [c], Igor Vilfan [c], Oliver Stauch [d] & Rolf Schubert [d]

[a] Department of Physical Chemistry, University of Freiburg, Albertstr. 21, Freiburg, D-79104, Germany

[b] Department of Physical Chemistry, University of Padova, via Loredan 2, Padova, I-35131, Italy

[c] J. Stefan Institute, Jamova 39, Ljubljana, 1001, Slovenia

[d] Department of Pharmaceutical Technology, University of Freiburg, Hermann-Herder-Str. 9, Freiburg, D-79104, Germany

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TRANSVERSE NUCLEAR SPIN RELAXATION INDUCED BY SHAPE FLUCTUATIONS IN MEMBRANE VESICLES. THEORY AND EXPERIMENTS

Gerhard Althoff, Diego Frezzato, and Gerd Kothe
University of Freiburg, Department of Physical Chemistry, Albertstr. 21, D-79104, Freiburg, Germany

Diego Frezzato and Giorgio J. Moro
University of Padova, Department of Physical Chemistry, via Loredan 2, I-35131, Padova, Italy

Marija Vilfan and Igor Vilfan
J. Stefan Institute, Jamova 39, 1001, Ljubljana, Slovenia

Oliver Stauch and Rolf Schubert
University of Freiburg, Department of Pharmaceutical Technology, Hermann-Herder-Str. 9, D-79104 Freiburg, Germany

Transverse nuclear spin relaxation measurements, employing the Carr-Purcell-Meiboom-Gill (CPMG) sequence, represent a powerful tool to study the dynamics of director fluctuations in liquid crystalline mesophases. Generally, however, the analysis of these experiments requires a slow-motional theory based on the stochastic Liouville equation. In this paper, such an approach is applied to study the shape fluctuations of dimyristoyl-phosphatidylcholine membrane vesicles with controlled size. Analysis of the transverse nuclear spin relaxation rates, measured as a function of the pulse frequency in the CPMG sequence, provides values for the bending rigidity, κ, and the lateral tension, σ, of the membrane vesicles. The results are of major importance in the understanding of the biological membrane function.

Keywords: transverse nuclear spin relaxation; vescicle fluctuations

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1. INTRODUCTION

Transverse nuclear spin relaxation experiments, employing Carr-Purcell-Meiboom-Gill (CPMG) sequences [1], represent a powerful tool for probing slow dynamic processes such as fluctuations of the orientational director in partially ordered phases [2]. Recently, a slow-motional theory has been presented for the analysis of such experiments performed on nematic liquid crystals [3]. In particular, analytical expressions have been derived for the transverse deuteron spin relaxation rates in CPMG sequences on condition that (i) the collective fluctuations of the orientational director constitute a multidimensional Gaussian process and that (ii) the spin Hamiltonian linearly depends on the stochastic variables. This so-called *linear approximation* is strictly valid only in the limit of small amplitude fluctuations and for non-canonical geometries, i.e., for orientations in which the director is neither parallel nor perpendicular to the magnetic field.

In this paper the slow-motional theory [3] is employed in the analysis of pulse frequency dependent transverse ^{31}P NMR relaxation experiments performed on unilamellar membrane vesicles. Particular emphasis is given to the analysis of thermally excited vesicle fluctuations associated with the viscoelastic properties of the bilayer membrane [4]. However, since the damping times of the fluctuation modes extend down to the ms range, the use of a fast-motion theory [4] is no longer justified and one should resort to a slow-motional approach based on stochastic Liouville equation. Such a formal treatment of the dynamic problem has been presented recently [3].

To describe the effect of vesicle fluctuations on transverse nuclear spin relaxation, we adopt a hydrodynamical model developed by Milner and Safran [5] on the basis of Helfrich's theory of the elasticity of lipid bilayers [6]. The model predicts that the amplitudes and damping times of the fluctuation modes depend on the size of the vesicle, the viscosity of the surrounding fluid, and the bending rigidity and lateral tension of the membrane. Thus, analysis of transverse ^{31}P nuclear spin relaxation experiments can provide valuable information on the viscoelastic properties of lipid vesicles [7,8]. Knowledge of these properties is of major importance in the understanding of the biological membrane function.

The paper is organized as follows. In Section two, we briefly discuss the hydrodynamical model used to describe the shape fluctuations of quasi-spherical membrane vesicles. In Section three, we introduce a slow-motional theory for the description of transverse ^{31}P nuclear spin relaxation in CPMG sequences. Particular emphasis is given to the analysis of thermally excited vesicle fluctuations. In Section four, the theory is employed in the analysis of pulse frequency dependent transverse ^{31}P NMR relaxation experiments performed on unilamellar *dimyristoyl-phosphati-*

dylcholine (DMPC) vesicles. The analysis provides values for the bending rigidity and lateral tension of the *DMPC* membranes. In Section five we evaluate the applied relaxation model and examine the grounds for adopting the specific relaxation mechanism. The final Section contains the conclusions of the work with a discussion of future developments opened by the new approach.

2. SHAPE FLUCTUATIONS OF QUASI-SPHERICAL VESICLES

Shape fluctuations of quasi-spherical vesicles are described by the hydrodynamical model of Milner and Safran [5], developed on the basis of Helfrich's theory of the elasticity of lipid bilayers [6]. In this model, the shape fluctuations are characterized by the radial displacement, $u(\theta, \phi) = (R(\theta, \phi) - R_0)/R_0$, where R_0 is the average radius of the vesicle and θ, ϕ denote the polar and azimuthal angle of the considered surface point with respect to an arbitrary reference system (see Figure 1).

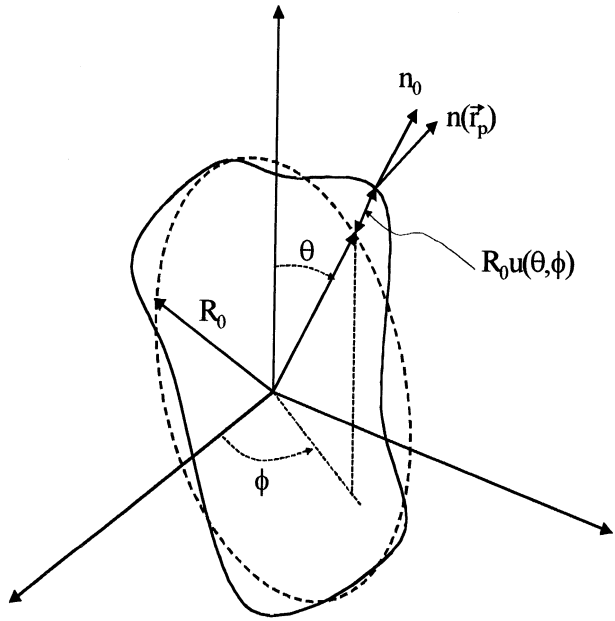

FIGURE 1 Schematic representation of fluctuations of quasi-spherical vesicles. θ and ϕ denote the polar and azimuthal angle of the considered surface point with respect to an arbitrary reference system. $\mathbf{n}(\vec{r}_p)$ represents the *fluctuating* local membrane normal. $R_0 u(\theta, \phi)$ characterizes the radial deviation from the spherical conformation. $\mathbf{n}_0$ indicates the *average* local membrane normal.

It is convenient to expand $u(\theta,\phi)$ in a series of spherical harmonics, $Y_{l,m}(\theta,\phi)$, according to

$$u(\theta,\phi)=\sum_{l}\sum_{m=-l}^{+l}u_{l,m}Y_{l,m}(\theta,\phi). \tag{1}$$

Note that the summation over l in Eq. (1) is extended from $l=2$ to the integer $l_{\max}\approx\pi R_0/a$, where a is an average intermolecular distance [5]. Using the expansion (1), the total elastic free energy of the vesicle can be written as a sum of independent contributions, quadratic in the components $u_{l,m}$ [5,6]. This shows that the spherical harmonics represent normal modes for the description of the thermal fluctuations of quasi-spherical vesicles. Consequently, the time autocorrelation function of the corresponding stochastic variable $u_{l,m}$ can be expressed as

$$\overline{u_{l,m}(0)u_{l',m'}(t)}=\delta_{l,l'}\delta_{m,m'}\overline{u_{l,m}^2}\exp\{-t/\tau_l\}, \tag{2}$$

where the mean square amplitude and relaxation time for each mode are given by [5,6]

$$\overline{u_{l,m}^2}=\frac{k_BT}{\kappa}\frac{1}{(l+2)(l-1)(l^2+l+\sigma)}, \tag{3}$$

$$\tau_l=\frac{\eta R_0^3}{\kappa}\frac{(2l+1)(2l^2+2l-1)}{(l-1)l(l+1)(l+2)(l^2+l+\sigma)}. \tag{4}$$

Here κ is the bending elastic modulus of the membrane, η is the viscosity of the surrounding fluid and σ is the dimensionless lateral tension. Note that the dispersion relation (4) is based on the assumption of strong dissipation arising from viscous damping of the bilayer by the surrounding fluid [5].

The displacement of the membrane $u(\theta,\phi)$ generates a curvature and consequently a deflection of the *fluctuating* local membrane normal, $\mathbf{n}=(n_\theta,n_\phi,n_{R_0})$, away from its average orientation in the radial direction (see Fig. 1). In the limit of small amplitude fluctuations, i.e., $|u(\theta,\phi)|\ll 1$, the transverse components of $\mathbf{n}$ can be approximated by $n_\theta\approx-\nabla_\theta u(\theta,\phi)$ and $n_\phi\approx-\nabla_\phi u(\theta,\phi)$ where ∇_θ and ∇_ϕ are components of the gradient operator in spherical coordinates. The condition $|u(\theta,\phi)|\ll 1$ also ensures that the dynamics of the *fluctuating* local membrane normal constitutes a multidimensional Gaussian process [7]. Evaluation of the partial derivatives and calculation of the time autocorrelation function for n_θ and n_ϕ, using the condition of independent modes, Eq. (2), finally yields

$$\overline{n_\theta(0)n_\theta(t)}=\overline{n_\phi(0)n_\phi(t)}=\sum_{l\geq 2}^{l_{\max}}\sigma_l^2\exp\{-t/\tau_l\}, \tag{5}$$

$$\sigma_l^2 = \frac{k_B T}{8\pi\kappa} \frac{l(l+1)(2l+1)}{(l+2)(l-1)(l^2+l+\sigma)}, \tag{6}$$

where the dependence on the surface position is lost because of the statistical equivalence of all points on the sphere.

3. TRANSVERSE RELAXATION DUE TO VESICLE FLUCTUATIONS

In this Section we outline a slow-motional NMR relaxation model for ^{31}P nuclei in the head group of lipid molecules forming unilamellar vesicles. Particular emphasis is given to the analysis of thermally excited vesicle fluctuations associated with the viscoelastic properties of the bilayer membrane. By analogy with thermotropic liquid crystals, these collective lipid motions can be referred to as director fluctuations.

Let us start by introducing several reference systems of axes. First, we define the magnetic frame (MF) (**X, Y, Z**), in which the chemical shift tensor of the considered ^{31}P nucleus is diagonal. Then, we introduce two molecular coordinate systems, necessary to describe the local lipid motions [7]. For simplicity, we assume that the order tensor and the rotational diffusion tensor of the lipid molecule are colinear and axially symmetric.

To account for the collective lipid motions, we introduce the instantaneous director frame (IDF) ($\mathbf{x}''$, $\mathbf{y}''$, $\mathbf{z}''$), where the z″-axis specifies the *fluctuating* local membrane normal, **n** (see Fig. 1). Due to axial symmetry, the transverse axes of the IDF are arbitrarily chosen. In addition, we introduce the average director frame (ADF) ($\mathbf{x}'$, $\mathbf{y}'$, $\mathbf{z}'$), where the z′-axis specifies the average direction of molecular alignment at each point of the sphere. Obviously, the z′-axis is colinear with the *average* local membrane normal, $\mathbf{n}_0$ (see Fig. 1). By assuming rotational isotropy of the orientational properties about the z′-axis, the orthogonal directions are arbitrarily chosen. Finally, we have to consider the laboratory frame (LF) (**x, y, z**), in which the z-axis is oriented along the static magnetic field $\mathbf{B}_0$. The angle between **z** and the *average* local membrane normal, $\mathbf{z}'$, is denoted by θ_B. Modeling of the vesicle fluctuations is conveniently performed by introducing the ADF in spherical geometry, i.e., $(\mathbf{x}', \mathbf{y}', \mathbf{z}') \rightarrow (\hat{\boldsymbol{\theta}}, \hat{\boldsymbol{\phi}}, \hat{\boldsymbol{R}}_0)$. Here $(\hat{\boldsymbol{\theta}}, \hat{\boldsymbol{\phi}}, \hat{\boldsymbol{R}}_0)$ denotes a set of orthogonal positional-dependent unit vectors in spherical coordinates. Thus, the *fluctuating* local membrane normal can be written as $\mathbf{n}(\vec{r}_p) = (n_\theta(\vec{r}_p), n_\phi(\vec{r}_p), n_{R_0}(\vec{r}_p))$, where $\vec{r}_p$ indicates the position of the probe molecule in the vesicle.

We now specify the spin Hamiltonian for ^{31}P nuclei in the head group of lipid molecules forming unilamellar vesicles. First, we apply the *secular approximation* and neglect all terms containing the spin operators I_x

and I_y. Then, we assume fast molecular motions and average the spin Hamiltonian over the equilibrium distributions for the intramolecular and intermolecular degrees of freedom. Finally, we apply the so-called *linear approximation*, i.e. $n_{R_0}(\vec{r}_p) \approx 1$, and retain only linear contributions of the transverse components of the local director field. With these approximations, the total spin Hamiltonian, representing Zeeman (Z) and chemical shift (CS) interactions in the LF, may be written as

$$\begin{aligned} H &= H_Z + H_{CS}, \\ H_Z/\hbar &= \omega_Z I_z, \\ H_{CS}/\hbar &= [\bar{\omega}_{CS} + \Delta\omega'_{CS} n_\theta(\vec{r}_p)] I_z, \end{aligned} \tag{7}$$

where ω_Z, $\bar{\omega}_{CS}$ and $\Delta\omega'_{CS}$ are characteristic frequencies given by

$$\begin{aligned} \omega_Z &= -\gamma B_0 (1 - \sigma_{iso}), \\ \bar{\omega}_{CS} &= \frac{1}{2} f \gamma B_0 \Delta\sigma S_{MOL} (3\cos^2\theta_B - 1), \\ \Delta\omega'_{CS} &= 3 f \gamma B_0 \Delta\sigma S_{MOL} \ \sin\theta_B \cos\theta_B. \end{aligned} \tag{8}$$

Here $\sigma_{iso} = (\sigma_{XX} + \sigma_{YY} + \sigma_{ZZ})/3$ is the isotropic chemical shift, $\Delta\sigma = \sigma_{ZZ} - (1/2)(\sigma_{XX} + \sigma_{YY})$ specifies the anisotropy of the chemical shift tensor, S_{MOL} denotes the orientational order parameter for the long molecular axis with respect to the IDF and the factor f accounts for the biaxiality of the averaged chemical shift tensor.

It should be noted, however, that knowledge of the quantities $\Delta\sigma$, S_{MOL} and f is not required, since the relevant magnetic anisotropy $\Delta\omega'_{CS}$ can be evaluated from the width, $\Delta\nu_{obs}$, of the experimental ^{31}P NMR powder spectrum, defined as separation of the $\theta_B = 0^\circ$ and $\theta_B = 90^\circ$ singularities [8]. Since both the molecular and the collective motions contribute to the averaging of the CS interactions one can write [7,8]

$$\Delta\omega'_{CS} = 4\pi(\Delta\upsilon_{obs}/S_{ODF}) \sin\theta_B \cos\theta_B, \tag{9}$$

where S_{ODF} is the order parameter of the *fluctuating* local membrane normal, i.e.,

$$S_{ODF} = 1 - 3\sum_{l\geq 2}^{l_{\max}} \sigma_l^2. \tag{10}$$

Note that the expression for H_{CS} in Eq. (7) is obtained within the *linear approximation*, implying that the expansion of the Hamiltonian in terms of transverse components of the local director field is restricted to the lowest order. It must be emphazised that such an approximation holds if (i) the angular amplitudes of the fluctuations are small ($n_{R_0}(\vec{r}_p) \approx 1$, $n_\theta(\vec{r}_p)$, $n_\phi(\vec{r}_p) \approx 0$), and if (ii) only non-canonical geometries are considered, i.e.,

$\theta_B \neq 0°, 90°$ Consequently, the theory cannot account for orientations in which the *average* local membrane normal is either parallel or perpendicular to the magnetic field. In such cases, consideration of second order terms of the director field would be required. Studies along these lines are currently in progress [9].

Within the slow-motional approach adopted here the transverse components $n_\theta(\vec{r}_p)$ constitute the stochastic variables, whose characteristic relaxation frequencies are comparable with the magnetic anisotropies (in angular frequency units). Notice that implicitely we neglect here the effects of translation diffusion of the lipid molecule along the vesicle shell [10], otherwise the probe position, $\vec{r}_p$, has to be included amongst the stochastic variables. Consequently, the angle θ_B is considered as a fixed parameter.

Basis of the slow-motional analysis is the stochastic Liouville equation [11,12], which describes the time dependence of the density matrix under the influence of the Hamiltonian superoperator H^x_{CS} and the evolution operator of the stochastic variables, used to describe the vesicle fluctuations. Recently, such a formal treatment of nematic director fluctuations has been presented for an $I = 1$ spin system exhibiting quadrupolar interactions [3]. The same procedure is followed in the present case referring to an $I = 1/2$ nucleus with anisotropic chemical shift interactions in a CPMG sequence, i.e., $(\pi/2)_x - [\tau - (\pi)_y - \tau]_n$, where τ denotes the pulse spacing [1].

For convenience, we introduce the specific transverse relaxation rate, $R^{CP}_{2,n}(\tau)$, characterizing the decrease of the echo amplitude, $I(2n\tau)$, during cycle n

$$I(2n\tau)/I(2(n-1)\tau) = \exp\{-2n\tau R^{CP}_{2,n}(\tau)\}. \tag{11}$$

Describing the vesicle fluctuations as a multidimensional Gaussian process [13], $R^{CP}_{2,n}(\tau)$ can be specified as [3]

$$\begin{aligned} R^{CP}_{2,n}(\tau) = R^0_2 + \frac{(\Delta\omega'_{CS})^2}{2\tau} \Bigg\{ & -4\sum_{k=0}^{n-2} g(2k\tau) + \\ & + (-1)^n[(-1)^k g(2n\tau - 2\tau) \\ & - 4g(2n\tau - \tau) + g(2n\tau)] \Bigg\}, \end{aligned} \tag{12}$$

where R^0_2 is the contribution from the fast molecular motions [14], and the function

$$g(t) = \int_0^t dt' \int_0^{t'} dt'' \overline{n_\theta(0) n_\theta(t'')} \tag{13}$$

is the double time integral of the correlation function, $\overline{n_\theta(0)n_\theta(t)}$, of the transverse component of the *fluctuating* local membrane normal (see Eq. (5)).

Generally, the dependence of $R_{2,n}^{CP}(\tau)$ on the number of cycles in the CPMG sequence is rather weak [3]. Consequently, one can interpret the experimental data in terms of an asymptotic relaxation rate for an infinite sequence, i.e., $n \to \infty$. In this case the following compact result is obtained

$$R_{2,\infty}^{CP}(\tau) = R_2^0 + (\Delta\omega'_{CS})^2 \sum_l \sigma_l^2 \tau_l [1 - (\tau_l/\tau)\tanh(\tau/\tau_l)], \qquad (14)$$

where σ_l^2 and τ_l are the mean square amplitude and relaxation time of the fluctuation modes (see Eqs. (4) and (6)), and $\Delta\omega'_{CS}$ is the magnetic anisotropy, defined in Eq. (9). This result implies that one can analyze the measured transverse relaxation rate as a superposition of independent contributions evaluated according to the Luz-Meiboom Eq. [15], originally derived within the Redfield limit. It should be mentioned that such an analytical solution of the stochastic Liouville equation is obtained only on condition that (i) the spin Hamiltonian linearly depends on the stochastic variables, and that (ii) the collective fluctuations constitute a multidimensional Gaussian process [13].

The analytical theory has been employed in model calculations for transverse ^{31}P nuclear spin relaxation rates, $R_{2,\infty}^{CP}(\omega)$, induced by vesicle fluctuations. Here ω denotes the pulse frequency, defined as the inverse pulse spacing in the CPMG sequence. Figure 2 shows calculated $R_{2,\infty}^{CP}(\omega)$ dispersion profiles for different geometric and viscoelastic parameters of the vesicles. One sees that $R_{2,\infty}^{CP}(\omega)$ depends linearly on ω^{-1} over a wide frequency range. Within this linear dispersion regime, the magnitude of $R_{2,\infty}^{CP}(\omega)$ is independent of the size of the vesicle R_0 (see Fig. 2a), the viscosity of the surrounding fluid η (see Fig. 2b) and the effective lateral tension σ (see Fig. 2c). The bending elastic modulus κ is thus – apart from the strength of the nuclear interactions – the relevant parameter determining the relaxation rate. Other parameters, R_0, η and σ together with κ determine the frequency at which $R_{2,\infty}^{CP}(\omega)$ levels off to a constant "plateau" value independent of ω.

Our model calculations show that for vesicles with a radius of $R_0 \geq 240\,\text{nm}$, the linear dispersion extends down to $10^4\,\text{Hz}$ regardless of the viscosity and lateral tension varying within reasonable limits. Thus, the bending elastic modulus κ can reliably be extracted from the slope of $R_{2,\infty}^{CP}(\omega)$ vs. ω^{-1}, as demonstrated in Figure 2d. It should be pointed out that η and σ have a distinct effect on the low-frequency cutoff of the $R_{2,\infty}^{CP}(\omega)$ dispersion profile. Note that for $\sigma > 10$ the transition from the linear to the "plateau" regime extends over several decades in ω (see Fig. 2c). Thus, a careful analysis of the low-frequency cutoff of the $R_{2,\infty}^{CP}(\omega)$

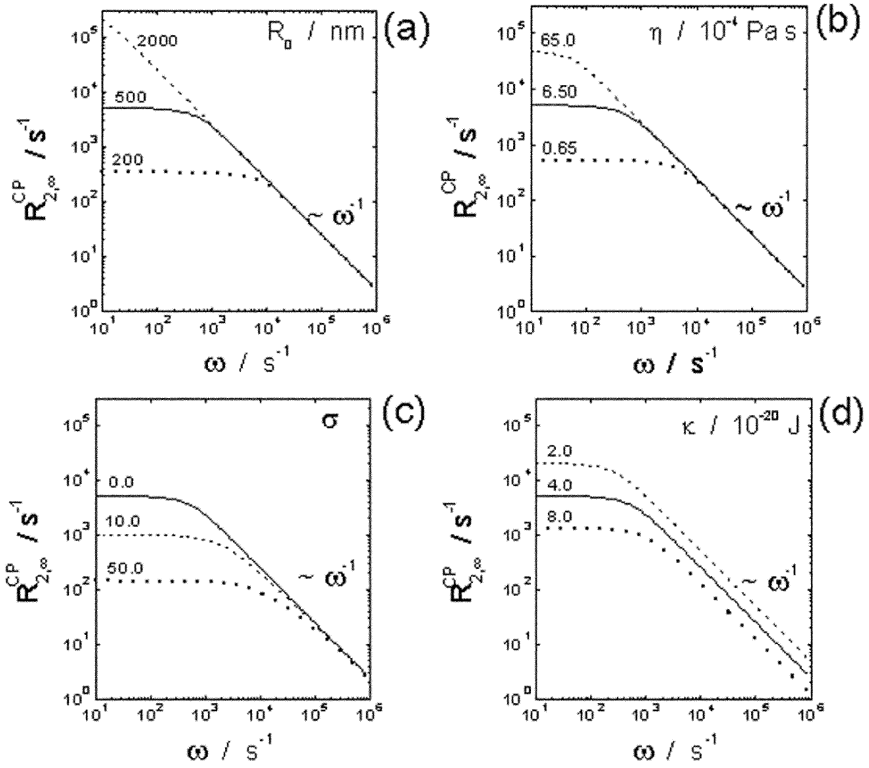

FIGURE 2 Dependence of the transverse ^{31}P nuclear spin relaxation rate $R_{2,\infty}^{CP}(\omega)$ on the pulse frequency ω in a Carr-Purcell-Meiboom-Gill sequence for fluctuations of quasi-spherical vesicles. The dispersion profiles refer to different geometric and viscoelastic parameters of the vesicles. The solid lines are calculated using the following standard parameter set: $R_0 = 500\,\text{nm}$, $\eta = 6.5 \times 10^{-4}$ Pa s, $\sigma = 0$ and $\kappa = 4.0 \times 10^{-20}$ J, $\Delta\nu_{obs}/S_{ODF} = 5\,\text{kHz}$, $a = 1\,\text{nm}$, $T = 313\,\text{K}$ and $\theta_B = 45°$. The dashed and dotted lines refer to the specific parameter values indicated in the graph.

relaxation curve can provide reliable values for the lateral tension σ. It appears that analysis of experimental $R_{2,\infty}^{CP}(\omega)$ dispersion profiles represents a powerful tool for the study of the viscoelastic properties of membrane vesicles.

4. ANALYSIS OF EXPERIMENTAL DATA FROM DMPC VESICLES

It was pointed out in the preceeding Section that the nuclear spin relaxation rates, $R_{2,\infty}^{CP}(\tau)$, depend on the orientation, θ_B, of the *average*

local membrane normal with respect to the magnetic field provided that lateral translational diffusion is too slow to average the relaxation rates of the whole vesicle. The observed ^{31}P NMR spectra clearly show that this averaging does not occur [8].

Vesicles can, therefore, be considered as isotropic powders where all orientations θ_B are represented, and a deconvolution procedure is required to extract the relaxation rate for a particular orientation.

In principle, it is possible to determine the anisotropy of $R_{2,\infty}^{CP}(\tau)$ by Fourier transforming the final echoes of CPMG trains for different number of echoes, n, at each pulse spacing. This yields a set of partially relaxed ^{31}P NMR spectra which can be fitted using the expression

$$R_{2,\infty}^{CP}(\tau)_{\theta_B} = \frac{1}{4}(\cos^2\theta_B - 1)^2 A_0 + 3\sin^2\theta_B \cos^2\theta_B A_1 + \frac{3}{4}\sin^4\theta_B A_2, \quad (15)$$

where A_0, A_1 and A_2 are free adjustable parameters. This general form for the anisotropy of $R_{2,\infty}^{CP}(\tau)$ is exactly correct within the motional narrowing limit. However, Eq. (15) may be usefully applied in any regime, provided that for each individual orientation θ_B the spins relax monoexponentially. This can be expected also in the case of slow vesicle fluctuations except for the canonical orientations $\theta_B = 0°$ and $\theta_B = 90°$, where the *linear approximation* does not apply anymore.

The outlined deconvolution procedure, however, is time and labor intensive and an alternative method has been employed to extract the desired information. For the homogeneous sample studied in this work, it is possible to exploit Eq. (15), so that the amplitude $I(2n\tau)$ of the nth echo in a CPMG experiment is

$$I(2n\tau)/I(0) = \int_0^\pi d\theta_B \sin\theta_B \exp\{-2n\tau R_{2,\infty}^{CP}(\tau)_{\theta_B}\} \quad (16)$$

and the decay curve for a given pulse spacing τ may be fitted by independently varying the four parameters $I(0)$, A_0, A_1 and A_2. In the actual case, the number of fitting parameters is effectively reduced to two. The initial amplitude $I(0)$ can be estimated from the amplitude of the first echoes measured at short pulse spacings, where the initial decay is slow. Furthermore, Eq. (15) predicts a marked anisotropy of $R_{2,\infty}^{CP}(\tau)$ only if not all three terms contribute significantly. As a consequence, there are usually only two of the parameters A_0, A_1 and A_2 to fit.

Figure 3 shows experimental transverse ^{31}P nuclear spin relaxation rates $R_2^{CP}(\omega)$ (filled squares) as a function of the pulse frequency ω for *DMP*C vesicles dispersed in excess of aqueous buffer. The ^{31}P relaxation rates were measured at $T = 313\,\mathrm{K}$ using the standard CPMG sequence with spin-lock proton decoupling [8]. Results are shown for unilamellar

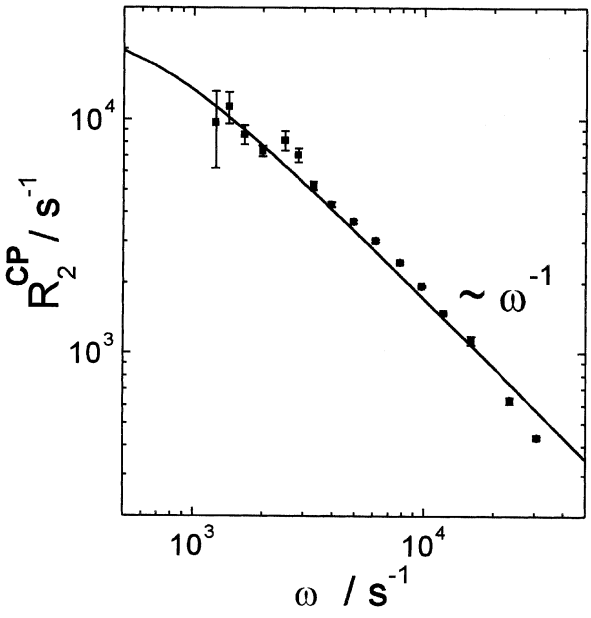

FIGURE 3 Dependence of the transverse ^{31}P nuclear spin relaxation rate $R_2^{CP}(\omega)$ on the pulse frequency ω in a Carr-Purcell-Meiboom-Gill sequence for unilamellar *DMPC* vesicles (■) at 313 K. The dispersion profile refers to a vesicle radius of $R_0 = 425$ nm. The solid line represents a best fit simulation using a slow-motional NMR relaxation model for shape fluctuations of quasi-spherical vesicles. The underlying parameters are listed in Table 1.

DMPC vesicles with a well-defined vesicle radius of $R_0 = 425$ nm. Details about the preparation and the characterization of the sample are described elsewhere [8]. The dispersion profile refers to the $\theta_B = 45°$ orientation, extracted from the CPMG echo decays using Eq. (16). One sees that the relaxation curve exhibits a linear dependence of the transverse relaxation rate on the pulse frequency, i.e., $R_2^{CP}(\omega) \propto \omega^{-1}$, over more than one frequency decade in the kHz regime. Moreover, at low frequencies, a "plateau" appears in the $R_2^{CP}(\omega)$ dispersion plot. This "plateau" is more clearly visible for a *DMPC* sample characterized by the smaller vesicle radius of $R_0 =$ 240 nm (results not shown) [8].

Values for the viscoelastic parameters κ and σ of the *DMP*C vesicles were obtained from a computer analysis of the experimental dispersion profile. The underlying fixed parameters are summarized in Table 1 (columns 1–6). The value for $\Delta\nu_{obs}$ was extracted from the experimental ^{31}P NMR spectrum. The value for η corresponds to the viscosity of water, consistent with the applied hydrodynamic model [5]. The average vesicle

TABLE 1 Parameters Used in the Analysis of Pulse-Frequency Dependent Transverse ^{31}P Nuclear Spin Relaxation Rates from Carr-Purcell-Meiboom-Gill Sequences

Absolute temp. T/K	Observed Splitting[a] $\Delta\nu_{obs}$/ kHz	Viscosity[b] $\eta/10^{-4}$Pas	Vesicle radius[c] R_0/nm
313	5.34	6.5	425
intermol. distance[d] a/nm	director orient.[e] θ_B/deg	bending modulus[f] $\kappa/10^{-20}J$	lateral tension[g] σ
1	45	1.7 ± 0.1	3 ± 1

[a]Extracted from the experimental ^{31}P NMR spectrum.
[b]Viscosity of water.
[c]Determined by dynamic light scattering.
[d]See Eq. (1).
[e]θ_B denotes the angle between the *average* local membrane normal and the static magnetic field.
[f]Bending modulus of the membrane.
[g]Lateral tension in the bilayer.

radius was determined by dynamic light scattering. For the average intermolecular distance a value of $a = 1$ nm has been assumed (see Eqs. (1), (5) and (10)).

Calculated dispersion profiles (see Eq. (14)) were fitted to the experimental profile by varying the parameters κ and σ. In the calculations, contributions from the fast molecular motions have been neglected, $R_2^0 = 0$. Since $\Delta\omega'_{CS}$ is defined in terms of the *a priori* unknown order parameter S_{ODF}, an iterative analysis was performed starting with $S_{ODF} = 1$. The result of the first fit provided an estimate for S_{ODF} according to Eqs. (6) and (10). This value was then employed to calculate a new magnetic anisotropy, $\Delta\omega'_{CS}$, using Eq. (9). The procedure was repeated until convergence was achieved.

The solid line in Figure 3 represents a best fit simulation of the experimental dispersion profile based on the parameter values listed in Table 1 (last two columns). Evidently, the agreement achieved is very good. The uniqueness of the fit was tested by running the fit procedure with different starting values. Within the error limits, the same values for κ and σ were obtained. The cited errors are linear confidence limits, referring to a confidence level of 0.95.

From the "plateau" in the dispersion profile of the DMPC vesicles, an effective lateral tension of $\sigma = 3 \pm 1$ has been extracted. The κ value for the *DMPC* membranes of $(1.7 \pm 0.1) \times 10^{-20}$ J corresponds to previous results obtained by video microscopy and micropipette techniques [16]. It should be noted, however, that the value of κ sensitively depends on the temperature of the sample. For example, κ increases by a factor of two when the temperature is lowered by 10 K [8]. In addition, there is a

significant increase of the bending elastic modulus if H_2O is used instead of buffer [8]. These findings indicate that a comparison of measured κ values is only meaningful if they refer to the same experimental conditions.

5. MODEL VALIDATION

In the previous Section we evaluated viscoelastic parameters of membrane vesicles assuming that vesicle shape fluctuations constitute the dominant transverse relaxation process. It is appropriate at this point to examine in more detail the grounds for adopting this relaxation mechanism. In principle, one might expect that translationally induced rotations [10] of lipid molecules also contribute to the transverse relaxation process. The *DMPC* vesicles investigated in this work display a mean effective rotational correlation time, $R_0^2/6D^{transl}$, of approximately 6 ms. This value places the lateral diffusion process in the slow-motional regime. An appropriate relaxation model has been described recently [7]. Analysis reveals that for vesicle radii $R_0 \geq 200$ nm lateral diffusion across the vesicle shell makes only a minor contribution to the transverse relaxation rates in contrast to previous suggestions [17,18]. Similar is true for contributions from tumbling motion of the vesicle as a whole.

Analysis of the low frequency cutoff of the experimental R_2^{CP} dispersion profiles provides valuable information on the dynamics of the vesicle fluctuations. Based on the assumption that strong dissipation arises from the viscous damping in the surrounding fluid, Milner and Safran derived the dispersion relation given in Eq. (4) [5]. Using this relation, excellent agreement has been obtained between experimental and calculated R_2^{CP} relaxation curves (see Fig. 3). On the basis of this result, one may conclude that other dissipative mechanisms, such as intermonolayer friction [19], are less important in the present case.

Finally, it is appropriate to make a few comments here concerning the strength of inter-bilayer coupling in oligolamellar vesicles. Recent ^{31}P NMR studies show that the relaxation rates $R_2^{CP}(\omega)$ are the same for unilamellar and oligolamellar vesicles [8]. Evidently, the inter-bilayer coupling is weak for fluctuations in the NMR range and has no effect on the measured dispersion profiles. This indicates that the model, developed for the analysis of unilamellar vesicles, might also be used in the case of oligolamellar vesicles.

6. CONCLUSIONS

Transverse nuclear spin relaxation measurements, employing CPMG sequences, can provide detailed information on the slow-motional dynamics in biomembranes. In this paper an analytical relaxation model is

presented for the analysis of such experiments performed on unilamellar quasi-spherical vesicles. The analysis provides reliable values for the bending rigidity, κ, and the lateral tension, σ, of the bilayer membranes. In addition, also oligolamellar lipid dispersions with a broad distribution of vesicle radii can be studied by the NMR technique [2]. This is particularly useful for studies of the modulation of the bending rigidity by different membrane constituents, such as cholesterol, peptides or proteins.

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