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Molecular dynamics of lipid bilayers studied by incoherent quasi-elastic neutron scattering

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Abstract. — Molecular motions in highly oriented multilayers of dipalmitoylphosphatidylcholine were studied as a function of temperature and hydration using incoherent quasi-elastic neutron scattering (QENS). The short range diffusive motions of the lipid molecules and the chain/headgroup dynamics were evaluated: 1) by measurement of the dependence of the elastic incoherent structure factor (EISF), the line-width \( \Gamma \) and the dynamic structure factors on the scattering vector \( Q \) for two orientations of the sample. The orientations were chosen such that the scattering vector \( Q \) was either predominantly perpendicular or parallel to the membrane normal; 2) by comparing data from protonated and chain deuterated lipids and 3) by the use of instruments of different energy resolution (i.e. time-of-flight and backscattering spectrometers exploring time regimes of \( 10^{-13} \) s to \( 10^{-11} \) s and \( 10^{-11} \) s to \( 10^{-9} \) s respectively). In the fluid phase the time-of-flight spectra revealed a restricted isotropic in-plane and out-of-plane diffusion of the hydrocarbon chain and headgroup protons. The mean displacements range from \( \approx 0.6 \) Å for methylene protons near the glycerol backbone to \( 7 \) Å for protons near the chain ends. These values are obtained for a water content of 23 wt%. The values are somewhat increased at 30 wt% of water. Measurements of the temperature variation of the EISF and the line-width \( \Gamma \) revealed a remarkably high degree of chain dynamics in the gel \( (L_g^-) \)-phase. The total elastic intensity as observed with the backscattering instrument showed that the \( L_u-L_g^- \)-phase transition is only well expressed at \( Q \)-values around \( 1 \) Å\(^{-1} \), while the number and mobility of the chain defects characterized at \( Q = 2 \) Å\(^{-1} \) (possibly gtg-kinks) increase continuously between \( 2 \) °C and \( 70 \) °C. In the time regime explored by the backscattering instrument, motions of the whole lipid molecules are also seen. It was interpreted in terms of a superposition of local in-plane and out-of-plane diffusion and lateral diffusional jumps between adjacent sites as predicted by the free volume model. For a sample containing 12 wt% of water at \( 60 \) °C the diffusion coefficient for the out-of-plane motion is \( D^I = 6 \times 10^{-6} \) cm\(^2\)/s with an amplitude of \( 2.25 \) Å. In-plane the diffusion coefficients range from \( D_{\min} = 1.5 \times 10^{-7} \) cm\(^2\)/s to \( D_{\max} = 6 \times 10^{-6} \) cm\(^2\)/s. The lateral diffusion coefficient is \( D_{ls} = 9.7 \times 10^{-8} \) cm\(^2\)/s in reasonable agreement with FRAP measurements. The strong increase of the lateral mobility with increasing water content yielded an exponential law for the variation of the diffusion coefficient with excess area per lipid (i.e. hydration) in agreement with the free volume model. The out-of-plane motion is characterized by an amplitude of about \( 0.5 \) Å in the time-of-flight time regime and of \( 2-3 \) Å in the backscattering time regime. The origin of this discrepancy could be the thermally excited membrane undulations since their relaxation times of \( \approx 3 \times 10^{-9} \) s (obtained in a separate spin-echo study) agree roughly with the reciprocal line-width of \( 2.5 \times 10^{-9} \) s for the backscattering instrument at \( Q \rightarrow 0 \). The time-of-flight result of \( 0.5 \) Å can be attributed to a dynamic surface roughness.
Important symbols and abbreviations.

RESEARCH CENTRES:
ILL Institut Laue-Langevin, Grenoble, France
KFA Forschungszentrum Jülich, Germany

METHODS:
AFM atomic force microscopy
NMR nuclear magnetic resonance
FRAP fluorescence recovery after photobleaching

MATERIALS:
DPPC L-α-dipalmitoylphosphatidylcholine
DPPC-d62 same lipid as DPPC, but with fully deuterated chains

NEUTRON SCATTERING:
$A_i(Q)$ structure factor for the Lorentzian with index $i$
$D_{\text{lat}}$ diffusion coefficient of long-range lateral diffusion
$D_{\text{min}}^\perp$ in-plane component of restricted diffusion of lipid molecules inside a restricted volume, minimal value, perpendicular to the membrane normal
$D_{\text{max}}^\perp$ in-plane component of restricted diffusion of lipid molecules inside a restricted volume, maximal value
$D^\parallel$ out-of-plane component of restricted motion of lipid, parallel to the membrane normal (diffusion coefficient for relaxational out-of-plane motion)
$\delta(\omega)$ Dirac delta function
$h$ height of the restricting cylinder
EISF elastic incoherent structure factor
HWHM half width at half maximum
$I_{\text{inc}}(Q, t)$ intermediate scattering function for incoherent scattering, Fourier transform with respect to space of the self-correlation function of the proton
$L_i(\omega, \Gamma_i)$ Lorentzian with index $i$ and HWHM $\Gamma_i$
$Q$ momentum transfer vector
$R$ radius of the restricting cylinder/sphere
$S_{\text{inc}}(Q, \omega)$ scattering function for incoherent scattering, Fourier transform with respect to space and time of the self-correlation function of the proton
$\Theta$ scattering angle.

1. Introduction.

Phospholipid membranes in the liquid crystalline phase state represent extremely soft shells with unique physical properties. Their dynamical behaviour is determined by a whole hierarchy of thermal excitations and fluctuations with correlation times ranging from $10^{-12}$ s (corresponding e.g. to the motion of chain defects) to 1 s (corresponding to long wavelength collective excitations of the bilayer membrane).

A large variety of motions in the lipid bilayer is essential for the function of natural membranes: the diffusion of chain defects provides the driving force for rapid transmembrane
transport of water and small solute molecules [1]. The rate of formation of functional enzyme complexes [2] or of elements of the electron transfer chain of mitochondria [3] is controlled by the lateral diffusion of the phospholipids. The collective out-of-plane excitations of the bilayer (the so-called surface undulations) contribute significantly to the repulsion between membranes [4]. Finally, individual out-of-plane motion of single lipid molecules on a short correlation time scale has been postulated very recently as another mechanism contributing to bilayer repulsion [5].

Despite this wide range of implications for bilayer function, our knowledge on bilayer dynamics, in particular on a molecular level, is surprisingly scarce. The best established process, yet, is lateral diffusion, which has been studied by a variety of methods such as fluorescence [6], ultracold neutron scattering [7] and NMR [8]. Processes with correlation times $t < 10^{-8}$ s such as segmental rotational diffusion of lipids and trans-gauche isomerizations of the acyl chains were studied by $^1$H-, $^{13}$C- and $^2$H-NMR relaxation techniques [9] and by Raman scattering [10]. Very recently it was proposed that $^2$H-NMR data can be analyzed in terms of collective motions of the lipids in the bilayer, such as surface undulations [11] and collective director fluctuations about the membrane normal [12, 13].

Incoherent quasi-elastic neutron scattering (QENS) was already applied to membrane systems in the seventies [14], and in a previous study we also established QENS to be a powerful technique for studying lipid membrane dynamics [15]. Neutron scattering offers the distinct advantage of measuring both the correlation times and mean square amplitudes of diffusive molecular processes in the bilayer. Hence, the various dynamical processes of the whole lipid molecules as well as of the hydrocarbon chains and headgroups only can be studied and distinguished using the following techniques:

a) screening the motion of parts of the molecules by partial deuteration;

b) using highly ordered multilamellar systems in order to select the direction of momentum transfer with respect to the membrane normal;

c) choosing an instrument with an appropriate energy resolution in order to probe different time domains.

In the present work we studied the individual motion of single lipid molecules of dipalmitoylphosphatidylcholine (DPPC) in the bilayer (lateral diffusion, acyl chain and headgroup motion) as a function of temperature and water content. Using time-of-flight and backscattering instruments two time domains could be studied.

Local diffusion coefficients and mean square amplitudes of the proton motion were obtained by calculating the wave vector dependent elastic incoherent structure factors (EISF) and line-widths using models of restricted diffusion [16, 17]. The motion of local chain defects in the $L_b$-phase was studied by time-of-flight-spectroscopy.

2. Materials and methods.

2.1 Preparation of oriented multilayers. — L-$\alpha$-dipalmitoylphosphatidylcholine (DPPC) and the same type of lipid with > 95 % deuterated hydrocarbon chains (denoted as DPPC-d62) were commercial products (Avanti Polar Lipids, Alabama, USA). Deuterated water was purchased from Sigma (Sigma Chemie GmbH, Deisenhofen, Germany). The silicon wafers used for the preparation of stacks of ordered multilayers were partly gifts from Wacker Chemie (Wacker-Chemitronic, Burghausen, Germany) and partly purchased from Virginia Semiconductors (Virginia Semiconductors Inc., Fredericksburg, Virginia, USA). The undoped wafers ($\varnothing = 5$ cm) were etched to a thickness of 150 $\mu$m and polished to an accuracy of $\pm 2$ $\mu$m over the whole area. We measured the surface roughness of the naturally grown SiO$_2$ layer on the wafer surface to be $< 4$ Å using atomic force microscopy (AFM).
The sample container was a standard sample holder of the ILL in Grenoble made of aluminum (cf. Fig. 1a). The number of wafers per sample was approximately 10 and the distance between wafers was 25 μm. Before use the wafers were put in 2% Hellmanex solution (Hellma GmbH&Co, Müllheim/Baden, Germany) for 24 h and then rinsed several times with high purity water (Millipore GmbH, Eschborn, Germany).

The preparation of the ordered multilayers consisted of three steps:

1) a dispersion of small unilamellar vesicles of 50-80 nm ⌀ was prepared by sonication as described elsewhere [18];

2) thin layers of the vesicle suspension were deposited onto the silicon wafers. The samples were then dried in a closed chamber, in which the relative humidity was adjusted by saturated salt solutions. This process lasted about two days. The final water content of the sample (between 5 and 20 weight % of water) was adjusted by choosing appropriate salt solutions [19];

3) after being dried the wafers were stacked and deposited in a desiccator, where the relative humidity was again controlled by saturated salt solutions. The samples were finally annealed at 100 °C for 10 hours and cooled down to room temperature at a rate of 0.3 °C per mn. Using thin layer chromatography and high sensitivity DSC we checked that the DPPC did not suffer any degradation due to the preparation procedure.

![Schematic view of the sample](image)

Fig. 1. — a) Schematic view of the sample. The sample holder is made of aluminum. It has lateral dimensions of ⌀ 65 mm, the total thickness is 10 mm and the wall thickness of the windows is 0.5 mm. Stacks of oriented multilayers were prepared between silicon wafers. The distance between wafers was 25 μm and the total number of wafers was ≈ 10, i.e. the total lipid thickness was 250 μm. b) Evaluation of the quality of the bilayer orientation by X-ray measurements of the mosaic spread. Photograph on the top: sample after drying under controlled humidity. Photograph on the bottom: same sample after annealing at 100 °C for 4 h. c) Evaluation of the mosaic spread of an annealed DPPC sample by measuring the angular width of first and second order Bragg reflection using the SV4 triple axis spectrometer at KFA, Jülich, Germany. The angle γ is the difference of the actual angle of incidence to the Bragg angle. The dips at the wings of the curves are due to sample shadows.
Fig. 1 (Continued).
Fig. 1 (Continued).

2.2 Evaluation of the Degree of Orientation. — Special care was taken in order to achieve a high degree of sample orientation, which is essential for a quantitative analysis of the QENS-spectra. Essential preparative requirements are: a) a low surface roughness of the wafers, b) the formation of multilamellar lipid layers from unilamellar vesicles, and c) the annealing of the sample at a temperature of \( \approx 100 \, ^\circ C \) and subsequent slow cooling. The quality of the sample orientation was monitored by measurements of the mosaic spread of the samples by X-ray or neutron scattering. An example of the improvement of sample orientation by annealing at \( 100 \, ^\circ C \) is shown in figure 1b. The most convenient technique for evaluating the mosaic spread is wide angle neutron scattering, since it allows simultaneous measurements of water content and mosaicity of the sample. An example is shown in figure 1c. The intensity of the first and second order Bragg peaks are plotted as a function of the angle \( \gamma \). From the line-widths of the reflections one obtains a mosaic spread of \( \delta \gamma = \pm 1.25 \, ^\circ \). The mosaic spread being small, it was not considered any further in data evaluation. A large mosaic spread would make it difficult to distinguish between motions parallel and perpendicular to the membrane normal.

2.3 Evaluation of Water Content. — Measurements of the water content of the samples were performed by several techniques: the Karl-Fischer-titration method, measurements of the phase transition temperature and the repeat distance of the multilayers by X-ray or neutron scattering. For the first method a Mitsubishi moisture meter (CA-02) was used. The disadvantage was that the measurement could only be performed after the neutron scattering experiment, as the sample had to be destroyed. The second technique was based on the comparison of the (chain melting) transition temperature of the sample with the known phase diagram of the lipid-water-system [20]. Below 20 wt% of water the multilayers undergo a direct transition from the solid \( L_\beta \)-phase to the liquid-crystalline \( L_a \)-phase. It extends, however, over a coexistence regime of about 5 \( ^\circ C \) and care has to be taken to determine the onset and the endpoint of the transition. The onset of the transition was in some cases determined by measuring the elastic intensity of the quasi-elastic spectrum at a fixed scattering angle as a function of temperature [21]. The transitions were also determined by
measurements of the bilayer repeat distance or the observation of the 4.2 Å wide angle reflection as a function of temperature following Büldt and Wohlgemuth [22]. As the measurement of the elastic intensity does not give precise results it was only used in connection with the Karl-Fischer-titration method.

2.4 QENS-EXPERIMENTS. — For the low resolution experiments the time-of-flight spectrometer (IN5) at ILL, Grenoble was used. The instrument resolution was ΔE = 63 μeV, the wavelength of the incident neutron beam 6.0 Å.

The high resolution experiments were performed on the backscattering spectrometer (IN10) at ILL. The instrument resolution was ΔE = 1 μeV, the wavelength 6.275 Å.

The quasi-elastic spectra were taken for two orientations of the samples: the membrane normal, \( \mathbf{n} \), forming an angle of 45° and 135° with the incident beam direction, corresponding to a momentum transfer predominantly parallel or perpendicular to the membrane normal (cf. Fig. 2). In the former case (45°-orientation) the out-of-plane motion, and in the second case, the in-plane-motion of the lipids can be predominantly observed. Standard ILL procedures for background and cell correction as well as normalization to a vanadium sample were used [23].

2.5 FITTING PROCEDURES. — The dynamic structure factor \( S_{\text{inc}}(Q, \omega) \) of each motion considered is represented by a sum of Lorentzians (plus an elastic line for a motion restricted in space). If a superposition of independent motions contribute to the scattering, the resulting dynamic structure factor is the convolution of the corresponding structure factors for each motion. Two types of fitting procedures are applied.

![Diagram](image)

**Fig. 2.** — Orientation of the sample with respect to the neutron beam direction and definition of scattering angle \( \theta \) and vector \( \mathbf{Q} \). The case of 45°-orientation is shown. \( \mathbf{k}_1 \) and \( \mathbf{k}_s \) are the wave vectors of the incident and scattered neutron beam, respectively, \( Q_1 \) and \( Q_\perp \) denote the components of the scattering vector in the direction parallel and perpendicular to the membrane normal (which usually coincides with the long molecule axis). The shaded arches on the circle indicate detector positions.
2.5.1 *Single spectrum fitting.* — Measured QENS-spectra are fitted by a superposition of an elastic line and one or two Lorentzians, which is a first approximation to the correct theoretical function. Additionally, a broad background can be considered. An example is shown in figure 3. In this case two Lorentzians are clearly required to fit the experimental spectrum both in the centre and at the wings. The instrument resolution is taken into account by convolution with the theoretical function, and the final function is:

\[
S_{\text{inc}}(Q, \omega) = S_{\text{instr}} \otimes \{ I \cdot [A_0(Q) \delta(\omega) + A_1(Q)L_1(\Gamma_1, \omega) + A_2(Q)L_2(\Gamma_2, \omega)] \} + \text{BG} \tag{1}
\]

where \(\otimes\) symbolizes a convolution of the instrument resolution function \(S_{\text{instr}}(Q, \omega)\) with the superposition of the sum of the elastic line \(A_0(Q)\delta(\omega)\), the quasi-elastic lines \(A_1(Q)L_1(\omega, \Gamma_1)\) and \(A_2(Q)L_2(\omega, \Gamma_2)\). BG denotes the background, and \(L_1(\omega, \Gamma_1)\) and \(L_2(\omega, \Gamma_2)\) denote normalized Lorentzians with line-widths \(\Gamma_1\) and \(\Gamma_2\), respectively. \(I\) is the absolute intensity measured in counts, normalized to the monitor count rate. Normalization requires \(A_0 + A_1 + A_2 = 1\). This procedure yields good results for the elastic incoherent structure factor (EISF or \(A_0(Q)\)), if the resolution function has sharp features and can thus be well distinguished from the Lorentzians. This is the case for IN5. Good results are also obtained, if the line broadening is sufficiently large in comparison to the width of the resolution function.

2.5.2 *Simultaneous fitting procedure.* — This procedure can be applied, if one has prior information concerning the dominant motional processes contributing to the incoherent scattering. It is usually used as a refinement of single spectrum fitting. The theoretical dynamic structure factor \(S_{\text{inc}}(Q, \omega)\) can usually be expressed as a sum of Lorentzians. However, in most cases only a few terms of the resulting series

\[
A_0(Q) \delta(\omega) + \sum_i A_i(Q)L_i(\Gamma_i, \omega) \tag{2}
\]

have to be taken into account, as the \(A_i(Q)\) go to zero for high orders. In the present experiments both programmes developed by Bée (FIT5MB, FIT0MB, [24]), which are available at the ILL, and the program PIFIT from the research centre in Jülich [25] were applied. A detailed description of the programs FIT5MB for time-of-flight data and FIT0MB for backscattering data can be found elsewhere [26].

In order to get the correct intensity for a given \(Q\) and temperature \(T\), the experimental dynamic structure factor has to be divided by the Debye-Waller factor \(\exp(-\langle u^2 \rangle Q^2)\), which accounts for intramolecular vibrations (e.g. C-C- and C-H-stretching or H-C-H-rocking vibrations). It has been determined by measuring the total integrated intensity

\[
I(Q, T) = \int S(Q, \omega, T) \, d\omega \tag{3}
\]

as a function of the scattering vector. An example for a time-of-flight experiment is shown in figure 4. From the slope of the \(\ln \{I(Q, T)\}\) *versus* \(Q^2\) plot one obtains a mean square amplitude of \(\langle u^2 \rangle \approx 0.09 \pm 0.03 \text{ Å}^2\).

For the backscattering spectra (IN10) the slopes of the \(\ln \{I(Q, T)\}\) *versus* \(Q^2\) plots are, by about an order of magnitude, larger than for the time-of-flight spectra. This is due to the fact that the energy window of IN10 does not include fast motions visible at IN5. Thus the total integrated intensity for the IN10 window does not contain all diffusive motions and therefore cannot be used to determine the Debye-Waller factor.
Fig. 3. — Demonstration of a single spectrum fitting procedure for a time-of-flight spectrum of a DPPC multilayer at $T = 60\,^\circ\text{C}$ (L$_\alpha$-phase). a) Fitting of the experimental line (++) by an elastic line (--) and one Lorentzian (-----). Note the deviation of the theoretical line from the experimental data at the wings of the spectrum. b) Fitting of the same spectrum by the same procedure as described in a), but this time an additional broad line is considered. A good fit is also achieved at the wings using 2 Lorentzians.
3. Experimental results and spectral analysis.

In this chapter some measurements of the dynamic structure factors of multi-layers of DPPC and DPPC-d62 performed on the low-resolution time-of-flight (IN5) and the high resolution backscattering (IN10) spectrometer are presented and analyzed. Both single and simultaneous fitting procedures were applied to determine the parameters of the model functions. Special emphasis was put on the evaluation of the elastic incoherent structure factor (or EISF), as it yields information on the type of motion and its spatial displacement. By comparing the EISF for the two sample orientations the degree of anisotropy of the motion can be explored.

The data was not corrected for D$_2$O, as its contribution to the total scattering is negligible in most cases. Considering the maximum hydration of 20 wt% of water, the incoherent scattering from D$_2$O amounts to 0.7 % of the incoherent scattering from protonated DPPC. As the coherent scattering will also contribute to the quasi-elastic scattering, its contribution has to be taken into account. Thus the total contribution of D$_2$O amounts to 3 % of the total scattering from DPPC. The D$_2$O contribution being even lower for samples with lower hydration, it is really negligible for protonated DPPC. Only in the case of DPPC-d62 samples D$_2$O has a significant contribution (the total scattering is 10 % of the incoherent scattering of DPPC-d62). However, the coherent contribution of the chains is twice that value, thus the data from DPPC-d62 was not taken for further conclusions on its own (see also paragraph on chain deuterated lipid in Sect. 3.1).

3.1 TIME-OF-FLIGHT SPECTROSCOPY OF DPPC IN $L_\alpha$-PHASE: MEASUREMENT OF IN- AND OUT-OF-PLANE DIFFUSION. — In figure 5a the $Q$-dependence of the EISF of DPPC-multilayers in the $L_\alpha$-phase at a temperature of 60 °C is shown for both orientations of the membrane.
normal with respect to the incident beam direction. In figures 5b and c the line-widths (HWHM) corresponding to the narrow and broad line of the same experiment are plotted. As mentioned in section 2.5.1 both lines were necessary to fit the spectra. For all spectra taken, the broad lines show nearly no variation with $Q$, thus in the forthcoming plots, the line-widths shown are the narrow lines only. The large error in the broad line results from the limited energy window observable. The cut-off on the energy loss side was chosen so as to avoid contributions from phonon modes to the quasi-elastic spectrum. In figure 6 the EISF of fully protonated and chain deuterated DPPC for the 135°-orientation are compared.

The most important features of figures 5 and 6 are:

1) the EISF for the two orientations agree within experimental error, i.e. the motion of the protons is essentially isotropic. Neither can an anisotropy be seen for the line-widths;
2) the EISF does not vanish at the largest observed $Q$-values;
3) the $Q$-dependence of the width of the narrow line is not strong (see, however, Sect. 3.3.1, Fig. 12c) for a much more pronounced $Q$-dependence of the narrow line). For the

![Graph](image)

**Fig. 5.** — a) $Q$-dependence of elastic incoherent structure factor (EISF) of DPPC in the L$_c$-phase for a sample orientation of 45° (○) and 135° (●) as obtained by the time-of-flight spectrometer IN5. The drawn curves were obtained by fitting the data with the model of local diffusion inside spheres of radii ranging from $R_{mn} = 0.26 \pm 0.21 \text{ Å}$ to $R_{max} = 4.42 \pm 0.43 \text{ Å}$ for 45°-orientation and from $R_{mn} = 0.45 \pm 0.20 \text{ Å}$ to $R_{max} = 4.54 \pm 0.27 \text{ Å}$ for 135°-orientation. The broken lines indicate the result obtained if the same sphere radius is taken for all protons. Even by eye the quality of fit is worse than in the first case. The curve (------) was obtained by fitting all data points, yielding a result of $R = 1.81 \pm 0.10 \text{ Å}$, for the curve (-----) only the first 7 data points were taken, yielding $R = 2.33 \pm 0.20 \text{ Å}$. b) $Q$-dependence of line-widths $\Gamma$ for the same data as used in figure 5a). From the two Lorentzians necessary for a good fit only the narrow line is shown, the bars indicate the error in the line-width determination. c) $Q$-dependence of the broad line for the same data as described in figure 5a). The large error results from the limited energy window available (the full energy window used is < 2 meV, note that the half width half maximum is already 0.5-0.8 meV).
Fig. 5 (Continued).
Fig. 6. — Comparison of $Q$-dependence of EISF for protonated DPPC and chain-deuterated DPPC-d62, measured by the time-of-flight spectrometer IN5 at 135°-orientation and $T = 60 ^\circ C$. The drawn lines are the result of a best fit using the model described in the text (fitting parameter: DPPC: $R_{\text{min}} = 0.45 \pm 0.20 \, \text{Å}, R_{\text{max}} = 4.54 \pm 0.27 \, \text{Å}, \text{DPPC-d62: } R_{\text{min}} = 0.69 \pm 0.15 \, \text{Å}, R_{\text{max}} = 4.66 \pm 0.28 \, \text{Å}$).

width of the broad line no definite conclusion concerning the $Q$-dependence can be drawn due to the large error.

4) The $Q$-dependencies of the EISF of protonated and chain deuterated lipids are remarkably similar.

The data was interpreted in terms of two different models for the lipid motion: a) a combination of rotation of the lipid molecule about the normal plus an out-of-plane diffusion inside a box (model I) and b) a superposition of in- and out-of-plane diffusion of the protons inside a restricted volume (model II). A sketch of the models is shown in figure 7.

In view of the data in figures 5 and 6, model I can be ruled out, as the average distance of the protons from the rotational axis yielding best fit is $r = 1.8 \, \text{Å}$. This is certainly much smaller than the average distance of the protons from the central long axis of the molecule. The distance would be reasonable, however, if the spectra were dominated by the torsional motion of each hydrocarbon chain, but this process has been ruled out by the similarity of spectra for protonated and chain deuterated lipids. It is this similarity that suggests that the motion of the whole molecule dominates the scattering. The isotropy of the motion could be explained by model I assuming a set of parameters that lead to similar amplitudes for in-plane and out-of-plane motion. However, this is a very stringent requirement and other mechanisms lead to a more natural way of explaining this isotropy (e.g. kink diffusion).

Model II considered the well-known diffusion inside a cylinder or a sphere [15, 16]. The difference of the theoretical scattering laws of a sphere and a cylinder as a restricting volume, respectively, was not significant, reflecting the isotropy of the motion. However, the original model of one restricting volume for all protons could not account for the slow decrease of the
Fig. 7. — Sketch of the two models introduced in section 3.1. Model I assumes a rotation of the whole molecule plus an out-of-plane diffusion inside a box. Model II assumes that each proton is diffusing inside a restricted volume (the volumes may be of different size for the different protons) plus a diffusion of the whole molecule inside a restricted volume.

EISF. Better agreement was achieved by considering a distribution of several radii/heights. This was achieved by allowing each proton to diffuse inside a restricted volume, while assuming a different radius for each proton and adding up all protons with the correct weight. For simplicity the increase between minimal and maximal radius was supposed to be linear. The QENS results of Bée [27] on the alkyl chains of copper(II) alkanoate complexes suggest that a nonlinear distribution of radii does not affect the results dramatically. This is also verified by a preliminary attempt to improve the simulation using a distribution similar to the $^2$H-NMR order profile. Additionally, in order to determine the functional dependence more precisely, further experiments with e.g. partially deuterated chains would have to be carried out.

The present approach of model II can explain the isotropy of the motion, as the restricting volume is nearly isotropic. The model can be interpreted in terms of an increased mobility along the chain starting at the relatively immobile glycerol backbone to the very dynamic methyl groups. The similarity of spectra for protonated and chain deuterated lipids is still not fully understood, but also for the protons of the headgroup different mobilities are expected depending on the distance to the glycerol backbone. However, as the model implicitly assumes the dynamical independence of the protons, it is not suitable for a detailed discussion of the dynamic part of the spectra.
Fig. 8. — a) Comparison of EISF of pure DPPC and DPPC: NaCl 4:1. The symbols correspond to experimental data, the drawn curves are obtained by a fit according to the model described in the text. The sample of pure DPPC contained 23 wt% of water, the sample with NaCl 30 wt% of water. The following parameters were found as a best fit: DPPC: 45°: \( R_{\text{min}} = 0.26 \pm 0.21 \) Å, \( R_{\text{max}} = 4.42 \pm 0.43 \) Å, 135°: \( R_{\text{min}} = 0.45 \pm 0.20 \) Å, \( R_{\text{max}} = 4.54 \pm 0.27 \) Å, DPPC: NaCl 4:1: 45° \( R_{\text{min}} = 1.11 \pm 0.20 \) Å, \( R_{\text{max}} = 5.83 \pm 0.44 \) Å, 135° \( R_{\text{min}} = 0.78 \pm 0.21 \) Å, \( R_{\text{max}} = 7.59 \pm 0.50 \) Å. b) \( Q \)-dependence of line-widths for the same samples as in figure 8a). Note that the line-width is higher for the sample containing NaCl for both orientations.
The chain deuterated spectra were fitted once by assuming that the lipid is fully protonated (i.e. 80 protons) and once with the correct number of 18 protons (headgroup and glycerol backbone) for chain deuterated lipid. In both cases the resulting $R_{\text{min}}$ and $R_{\text{max}}$ agreed within error, which indicates that already a small number of non-equivalent protons is sufficient to explain the observed spectra. Thus, prior information on the structure of the molecule has to be used for data evaluation in order to obtain reasonable results. It should also be mentioned that the coherent scattering of chain deuterated lipid cannot be neglected. This contribution is especially strong around a $Q$-value of 1.5 Å$^{-1}$, which corresponds to the correlation peak (often called 4.2 Å peak) of the chains. Thus, for a true measurement of the headgroup motion a method to deduct this coherent contribution has to be found.

In summary for a sample containing 23 wt% of water at a temperature of 60 °C the following parameters were found as the result of a best fit:

- **protonated lipid**: 45°-orientation:
  - $R_{\text{min}} = 0.3 \pm 0.15$ Å
  - $R_{\text{max}} = 5.7 \pm 0.3$ Å

- **135°-orientation**:
  - $R_{\text{min}} = 0.4 \pm 0.2$ Å
  - $R_{\text{max}} = 6.3 \pm 0.35$ Å

- **deuterated lipid**: 45°-orientation:
  - $R_{\text{min}} = 0.7 \pm 0.3$ Å
  - $R_{\text{max}} = 6.8 \pm 0.6$ Å

- **135°-orientation**:
  - $R_{\text{min}} = 0.6 \pm 0.2$ Å
  - $R_{\text{max}} = 6.6 \pm 0.35$ Å.

**Dependence of fast lipid motion on degree of hydration.** — The water content of ordered DPPC multilayers may be remarkably increased by adding monovalent salt, e.g. NaCl. Thus at a DPPC to NaCl molar ratio of 4 : 1 well ordered multilayers may be prepared up to 30 wt% of water. In figure 8a the EISF-vs.-Q-plot and in figure 8b the $\Gamma$-vs.-Q-plot of DPPC with 23 wt% of water and of DPPC : NaCl 4 : 1 with 30 wt% of water are shown. The decay of the EISF and the increase of $\Gamma$ are much faster for the sample containing NaCl.

**3.2 Backscattering spectroscopy.** — In figures 9 to 11 we present results concerning the slow motions (characterized by relaxation times of $10^{-8}$ to $10^{-10}$ s), as obtained with the backscattering spectrometer. Protonated and chain deuterated samples have been used at different degrees of hydration (23, 12 and 8 wt% of water), again for the two orientations of the sample with respect to the incident beam as defined in figure 2. In order to make the results comparable to IN5 nearly the same wavelength (6.275 Å) as on IN5 was used, thus yielding a similar $Q$-range.

Figure 9 compares the EISF of protonated and deuterated samples at 23 wt% of water. Figure 10 shows the decay of the EISF for three degrees of hydration at the 45°-orientation. The same data but for the 135°-orientation exhibits no remarkable differences. Figure 11 exhibits the line-widths as a function of $Q$ for chain deuterated and protonated samples at 8 wt% of water.

The results can be summarized as follows:

1) in contrast to the time-of-flight data the EISF decays rapidly to zero;

2) at 23 wt% of water the motion appears isotropic for all samples, at 12 wt% of water the protonated sample is still isotropic, whereas for the deuterated one an anisotropy can be seen. At 8 wt% of water the EISF for the two orientations is different for both samples. The difference between the protonated and the deuterated sample at 12 wt% of water should be considered with care, as the error in water determination is about 2%;

3) with decreasing water content the EISF is decreasing more slowly with $Q$, indicating that the motion becomes more restricted in space;
LIPID BILAYER DYNAMICS STUDIED BY QENS

Fig. 9. — Comparison of EISF of protonated and chain-deuterated samples for both orientations, measured at the backscattering spectrometer IN10 at $T = 60 \, ^\circ\text{C}$. The samples contain 23 wt% of water.

Fig. 10. — $Q$-dependence of EISF for three different degrees of hydration using protonated DPPC, as determined with the backscattering spectrometer. The orientation is 45°, for the 135°-orientation there are no major differences in the hydration dependence.
ODPC, protonated, 45°. DPPC, protonated, 135° + DPPC-d62, 45° X DPPC-d62, 135°

30 ~ $x$

Fig. 11. — $Q$-dependence of the line-widths of the backscattering spectra of DPPC and DPPC-d62 containing 8 wt% of water. Note that the line-widths at large $Q$ is higher for the 135°-orientation than for the 45°-orientation in both cases.

4) for all samples the line-width is larger for the 135°-orientation than for the 45°-orientation at least for $Q$-values > 1 Å⁻¹. This suggests a higher mobility of the lipids in the plane of the membrane than perpendicular to it.

The backscattering data could not be analyzed by the single spectrum fitting procedure. Because of the rapid decay of the EISF with $Q$ no unique solution could be found. Only more data at low $Q$ would allow an assessment of the uniqueness of the fits.

Therefore a simultaneous fitting procedure was applied, considering a superposition of: 1) long-range lateral diffusion, 2) a local in-plane diffusion inside a circular area and 3) a local out-of-plane diffusion of the lipid molecules. For the local motion (2 and 3) a cylinder with radius $R$ and height $h$ was assumed as restricting volume. In order to account for the increased flexibility along the hydrocarbon chain a linear distribution of diffusion coefficients between two limiting values $D_{\text{min}}$ and $D_{\text{max}}$ for the local in-plane diffusion was assumed. The implicit assumption of independent motion of the protons for the above model is of course not valid for molecular models and their dynamics. Thus, the resulting diffusion coefficients should be handled with care. However, to get a first idea of the motion, it provides good results and can be understood in terms of an increased mobility of the protons towards the bilayer centre. For saturated lipids such as DPPC this gradient of mobility along the acyl chain is suggested by the orientational order parameter profile obtained by ²H-NMR.

For the case of DPPC containing 12 wt% of water we found, for instance, the following fit parameters:

\[ R = 3.5 \text{ Å} , \quad h = 4.5 \text{ Å} \text{ (i.e. an amplitude of 2.25 Å)} \]

long-range lateral diffusion : \( D_{\text{lat}} = 9.7 \times 10^{-8} \text{ cm}^2/\text{s} \)
local diffusion perpendicular to the membrane normal : \( D_{\text{min}} = 1.5 \times 10^{-7} \text{ cm}^2/\text{s} \)
to the membrane normal : \( D_{\text{max}} = 6 \times 10^{-6} \text{ cm}^2/\text{s} \)
local diffusion parallel to the membrane normal : \( D_{\parallel} = 2.1 \times 10^{-6} \text{ cm}^2/\text{s} \).
3.3 Temperature Dependence of Lipid Motion. — The rather high intensity of the \textit{time-of-flight} spectrometer enabled measurements of the EISF and the line-widths as a function of temperature within reasonable time. On the backscattering spectrometer the temperature dependence of the elastic intensity was recorded, which provides essentially a measure of protons that are immobile on the time scale of the instrument or have motional amplitudes too small to be observed at the chosen \(Q\)-value.

3.3.1 Softening of the hydrocarbon chains observed with the \textit{time-of-flight} spectrometer. — Figure 12 shows an example of a temperature scan recorded at the \textit{time-of-flight} spectrometer for a DPPC sample. In figure 12a the EISF obtained at the 45\(^\circ\)-orientation for various temperatures are presented. Figure 12b compares the EISF as a function of \(Q\) for two temperatures (one below and the other one above the phase transition) and both orientations. Figure 12c shows the \(Q\)-dependence of the line-widths for the same spectra as used in figure 12a. The phase transition temperature was 56 °C for the sample used in figures 12a and c and 41 °C for the sample used in figure 12b.

The most remarkable features of figure 12 are:

1) at temperatures below the lipid phase transition, the EISF decays remarkably fast with increasing \(Q\) (e.g. the curve for 2 °C);

![Time-of-flight Study](image)

Fig. 12. — a) Time-of flight study of the temperature dependence of membrane dynamics of DPPC-multilayers with a phase transition at \(T = 56 ^\circ\text{C}\) (12 wt\% of water). The EISF for the 45\(^\circ\)-orientation as a function of temperature for the \(L_p\)-phase (2 °C, 25 °C, 35 °C, 50 °C) and the \(L_d\)-phase (60 °C) are shown. The drawn curves are the result of best fit to the model of restricted diffusion inside a sphere. The parameters are listed in table I. b) Comparison of EISF of DPPC in \(L_d\)-phase \((T = 2 ^\circ\text{C})\) and \(L_p\)-phase \((T = 60 ^\circ\text{C})\) for both orientations, as seen with the \textit{time-of-flight} spectrometer. The sample contained 23 wt\% of water, i.e. \(T_c = 41 ^\circ\text{C}\). The parameters yielding best fit were: \(T = 2 ^\circ\text{C}: 45 ^\circ\) \(R_{\text{min}} = 0.17 \pm 0.03\ \text{Å},\ R_{\text{max}} = 1.35 \pm 0.06\ \text{Å},\) \(135 ^\circ: R_{\text{min}} = 0.05 \pm 0.03\ \text{Å},\ R_{\text{max}} = 1.81 \pm 0.08\ \text{Å},\) \(T = 60 ^\circ\text{C}: 45 ^\circ\) \(R_{\text{min}} = 0.26 \pm 0.21\ \text{Å},\ R_{\text{max}} = 4.42 \pm 0.43\ \text{Å},\) \(135 ^\circ: R_{\text{min}} = 0.45 \pm 0.20\ \text{Å},\ R_{\text{max}} = 4.54 \pm 0.27\ \text{Å}.\) c) Variation of \(Q\)-dependence of line-widths for the same data as used in figure 12a.)
2) the differences between the EISF-vs.-$Q$-plots for the two orientations are small, i.e. the motion is isotropic even at 2 °C;

3) there is no distinct change in the decay of the EISF at the phase transition;

4) even for some temperatures below the phase transition, e.g. 35 °C, the line-widths show a $Q$-dependent behaviour (cf. Fig. 12c).

The data has been analysed in terms of a superposition of local in- and out-of-plane diffusion as described in section 3.1. The curves obtained by the fitting procedures are also shown in figure 12. Good agreement with the experiments could only be achieved by
assuming not only one radius/height for the restricting cylindrical/spherical volume, but a distribution of these parameters. It was found that there is little difference in using a sphere or a cylinder as a restricting volume.

The parameters giving best fit to the data in figure 12a are listed in table I.

Table I. — Parameters used for theoretical curves in figure 12a. They are the result of best fit for the model of diffusion inside a sphere, using a distribution of radii. Minimal and maximal radius are given, all values in between have equal weight.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>( R_{mn} ) (Å)</th>
<th>( R_{max} ) (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.19 ± 0.04</td>
<td>1.54 ± 0.06</td>
</tr>
<tr>
<td>25</td>
<td>0.18 ± 0.06</td>
<td>2.37 ± 0.07</td>
</tr>
<tr>
<td>35</td>
<td>0.18 ± 0.08</td>
<td>2.75 ± 0.10</td>
</tr>
<tr>
<td>50</td>
<td>0.29 ± 0.13</td>
<td>3.78 ± 0.23</td>
</tr>
<tr>
<td>60</td>
<td>0.72 ± 0.09</td>
<td>4.44 ± 0.20</td>
</tr>
</tbody>
</table>

3.3.2 L\(_{β}\)-L\(_{α}\)-phase transition as observed with the backscattering spectrometer. — The astonishingly high degree of hydrocarbon chain motion in the gel-phase is also revealed by measurements of the elastic intensity of the backscattering spectra as a function of temperature, but at fixed \( Q \)-values. The elastic intensity is the total number of counts at \( \omega = 0 \). Such measurements can be performed reasonably fast. They enable the determination of the phase-transition temperature, and thus of the water content, and at the same time give insight into the temperature induced softening of the hydrocarbon chain region.

Some typical results are shown in figure 13 for protonated DPPC. The most remarkable findings are:

1) at \( Q \)-values around 1 Å\(^{-1} \) the intensity decreases abruptly at the L\(_{β}\)-L\(_{α}\)-phase transition, irrespectively of the water content;
2) the phase transition is not manifested at very small \( Q \)-values (≈ 0.2 Å\(^{-1} \));
3) at high \( Q \)-values (≈ 1.9 Å\(^{-1} \)) the phase transition can still be seen for the sample containing 8 wt% of water, but for the sample with more water (12 wt% of water) the intensity decreases continuously with increasing temperature.

4. General discussion.

The aim of the present study was to explore the application of QENS to the separation and quantitative analysis of the manifold of thermal excitations within bilayer membranes. In the previous section we showed how the spectra can be formally analysed by fitting either single spectra or all spectra in the \( Q \)-range studied simultaneously. However, often more than one model can fit the data, especially when using a system with a large number of degrees of freedom such as the one studied. To overcome this ambiguity it is necessary to compare the results of the fitting procedure with data from other techniques and theoretical predictions. This is the purpose of the present section.
4.1 Dynamics of Lα-phase of the bilayer.— The spectra and the EISF could be reasonably well fitted by a superposition of local in- and out-of-plane diffusion and a long-range lateral diffusion. The latter can be neglected at low resolution.

The time-of-flight spectrometer can resolve motions in the regime $10^{-11}$ to $10^{-13}$ s, where the membrane dynamics are dominated by chain defect motion (gauche-trans isomer motion). The model of rotation of the whole molecule could be ruled out, as the interpretation of the time-of-flight spectra with such a model yields physically meaningless parameters (see Sect. 3.1). Moreover, long-range lateral and rotational diffusion of the whole lipid molecule are too slow to be observed with time-of-flight spectroscopy. The relevant correlation times for rotational motions as suggested by $^2$H- and $^3$P-NMR measurements are of the order of $\tau_c \approx 10^{-9}$ s$^{-1}$ [28]. However, contributions of torsional librations of the single chains cannot be ruled out. Implicitly they are contained in the chain dynamics such as motions of extended chain defects (e.g. jogs, [29, 30]).

Another interesting question is to find a correlation between our parameters and the number of kinks along the chain. One approach is to consider reorientations of bonds as defined by Helfand [30], which are to be expected in the observed time scale and should lead to a similar behaviour of headgroup and chain motion. The model of bond reorientation should lead to a static picture of the chain similar to model II of section 3.1 (i.e. a superposition of in and out-of-plane diffusion inside a restricted volume), as the probability of kink formation increases towards the end of the chain [31] and thus leads to a larger volume the proton can diffuse in. Furthermore it is better suited to a dynamic analysis, as it can take into account that the protons are not independent.

The remarkable difference of the time-of-flight results obtained for DPPC with and without 25 mole% of NaCl could be due to a direct effect of the ions or to a lower packing density of the lipids (owing to a higher degree of hydration). We favour the latter explanation, as the scattering of the chains amounts to nearly 80%, and NMR experiments by Seelig [32] suggest that monovalent cations affect only the orientation of the headgroup.

The change of packing density with hydration has already been seen in X-ray studies [33]. As will be discussed below (Sect. 4.3), the results of the backscattering experiments support the assumption that the packing density plays a major role for the dynamic behaviour of the lipids.

One interesting result of the present work is that for the interpretation of both low and high resolution data a model using the same parameters for all protons was not sufficient. A distribution of spheres/diffusion coefficients is necessary to fit the data, indicating the different spatial extent the proton can explore with increasing distance from the headgroup. It has the same origin as the well-known decrease of the local chain order parameter with increasing distance from the headgroup ($^2$H-NMR, [34]). The decomposition of the in-plane motion into short-range and long-range in-plane diffusion is consistent with the free volume model of diffusion in membranes [35-37]. The minimum value $D_{\text{min}}$ obtained from the backscattering spectra can be interpreted in terms of the diffusion coefficient characterizing the lateral diffusional in-plane motion of the lipid molecule in its solvent cage.

The non-negligible out-of-plane diffusive motion of the lipid molecules has been interpreted previously in terms of a diffusional motion of individual molecules in the direction of the bilayer normal. This interpretation was based on the simultaneous occurrence of such a motion in the time-of-flight and backscattering spectra with amplitudes of $\approx 2$ Å and 6 Å, respectively. These amplitudes were obtained, if all protons were assumed to be equivalent (i.e. only one restricting volume for all protons). In the present refined analysis we find much smaller out-of-plane amplitudes, namely 0.5 Å for the time-of-flight and 2.25 Å for the backscattering regime. The small value of 0.5 Å is the minimal radius obtained from
model II, and we assumed that this can be attributed to the motion of the whole molecule. Thus the difference in amplitudes is due to the fact that the present analysis distinguishes between motions parallel to the membrane normal which are due to intra-molecular dynamics from motions of the whole molecule. In a recent separate spin-echo-study (unpublished results, to be published in a forthcoming paper [38]) we found collective bending undulations of the multilayers at 19 wt% of water up to wavevectors of $Q = 0.125 \text{ Å}^{-1}$. The corresponding relaxation time of $3.1 \times 10^{-9}$ s is of the same order of magnitude as the resolution of the backscattering spectrometer. Therefore it is more likely that the out-of plane motion observed in the slow time regime is a consequence of the collective membrane undulations. The small but remarkable out-of plane motion in the fast time regime is however much faster than expected for undulations. Assuming a $I \sim Q^3$-dependence the relaxation times obtainable with time-of-flight spectroscopy ($> 10^{-11}$ s) correspond to undulation wavelengths smaller than the bilayer thickness. Thus this fast motion corresponds most likely to an out-of plane diffusional motion of individual molecules.

4.2 Dynamics in $L_\beta$-Phase and Softening of Chain Dynamics during $L_\beta$-$L_c$-Phase transition. — One of the most interesting prospects of time-of-flight measurements is that they can give insight into the onset of chain dynamics at the gel-to-liquid crystal phase transition of membranes. As a further example figure 13 shows a series of plots of the elastic intensity versus temperature. For 8 wt% of water the results of three $Q$-values are given ($Q = 0.2 \text{ Å}^{-1}$, $Q = 0.9 \text{ Å}^{-1}$, $Q = 1.9 \text{ Å}^{-1}$), for 12 wt% of water only the data at $Q = 1.9 \text{ Å}^{-1}$ is shown.

In accordance with figure 12 one finds a substantial chain mobility below the phase transition. The slope of the intensity- vs. $-T$-plot increases strongly going from small to medium scattering vectors, suggesting that the chain dynamics in the gel state is determined by the mobility of small amplitude defects e.g. gtg-kinks, as expected. The mean square displacement of the protons of these kinks is of the order of $\langle x^2 \rangle \approx (2.8 \text{ Å})^2$ and should thus show indeed in the plot for $Q = 1.2 \text{ Å}^{-1}$. Remarkably the phase transition cannot be observed for $Q = 1.9 \text{ Å}^{-1}$ in the $I$-$T$-plot for medium water contents (12 wt% of water), while it becomes visible at lower degrees of hydration (8 wt% of water). This could be a consequence of the increase in packing density with decreasing water content and agrees with previous results from Raman spectroscopic studies [10] and also from the change in quadrupolar splitting as observed with $^2\text{H}$-NMR (König, unpublished results).

The continuous softening of the gel phase revealed by figure 13 is in accordance with the results of figure 12. Both the EISF-vs.-$Q$-plots and the minimal and maximal radii of the proton diffusional motion (Tab. I) do not change abruptly at the phase transition. This suggests that the number of gauche-trans conformers as a function of temperature does not exhibit a sudden increase at $T_c$.

The local softening of the gel phase by a temperature induced increase of the defect density could also explain the so-called pre-critical behaviour of lipid lamellae. This was postulated by Jähnig [39] and experimental evidence was provided by ultra-sonic measurements [40] and more recently by Hatta on the basis of AC-calorimetry [41].

4.3 Coupling of Local and Long-Range Lateral Diffusion and the Effects of Hydration. — The backscattering spectrometer can resolve slower motions than the time-of-flight spectrometer. The fact that the EISF in figures 9 and 10 is decreasing much faster than for the time-of-flight data (e.g. Fig. 5a) suggests that a new type of motion is resolved. The close similarity of DPPC and DPPC-d62 spectra suggests that the spectra are determined by the motion of the whole molecule.
Fig. 13. — Plot of the elastic intensity as a function of temperature, as recorded on the backscattering spectrometer. Three different $Q$-values ($0.2 \text{ Å}^{-1}$, $0.9 \text{ Å}^{-1}$, $1.9 \text{ Å}^{-1}$) are shown. The three upper curves correspond to a sample containing 8 wt% of water, the lowest one (full triangles) to a sample containing 12 wt% of water.

Table II. — The local in-plane diffusion coefficient obtained for backscattering spectra of DPPC multilayers with different degrees of hydration. The data was fitted using one Lorentzian only and no elastic contribution. As data at $Q$-values $> 1 \text{ Å}^{-1}$ dominate the fit, mainly local diffusion and next neighbour jumps can be seen.

<table>
<thead>
<tr>
<th>type of DPPC</th>
<th>degree of hydration (wt% of water)</th>
<th>diffusion coefficient (in cm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>protonated</td>
<td>30</td>
<td>$1.6 \times 10^{-6}$</td>
</tr>
<tr>
<td>protonated</td>
<td>23</td>
<td>$1.5 \times 10^{-6}$</td>
</tr>
<tr>
<td>protonated</td>
<td>12</td>
<td>$8.4 \times 10^{-7}$</td>
</tr>
<tr>
<td>protonated</td>
<td>8</td>
<td>$4.9 \times 10^{-7}$</td>
</tr>
<tr>
<td>deuterated</td>
<td>23</td>
<td>$1.4 \times 10^{-6}$</td>
</tr>
<tr>
<td>deuterated</td>
<td>12</td>
<td>$6.1 \times 10^{-7}$</td>
</tr>
<tr>
<td>deuterated</td>
<td>8</td>
<td>$3.4 \times 10^{-7}$</td>
</tr>
</tbody>
</table>
The \( Q \)-dependencies for EISF and line-widths differ much more for the two orientations at low water content than at high water content. This could mean that in-plane and out-of-plane motion are affected in a different way by dehydration.

The value of the in-plane diffusion coefficient can be estimated by fitting the spectra with one Lorentzian (and no elastic contribution) and plotting the resulting line-width vs. \( Q^2 \). The diffusion coefficients for the 135°-orientation obtained by this procedure are given in table II. Their value is mainly determined by data in the \( Q \)-range from 1-2 \( \text{Å}^{-1} \), i.e. predominantly local diffusion or next neighbour jumps can be seen.

The resulting values of the in-plane diffusion coefficient are nearly by an order of magnitude higher than the values of \( D_{\text{lat}} \) obtained by the simultaneous fitting procedure (\( D_{\text{lat}} = 9.7 \times 10^{-8} \text{ cm}^2/\text{s} \) at 12 wt\% of water). This is due to the dominance of local lateral diffusion in the line-width determination, whereas local and long-range diffusion were considered separately in the simultaneous fit. In a recent paper Tabony et al. [42] also reported astonishingly high values of the lateral diffusion coefficient of the order of \( 10^{-6} \text{ cm}^2/\text{s} \). These high values are also a consequence of the \( Q \)-range that was used for data evaluation.

The systematic decrease of the local in-plane diffusion coefficient with decreasing water content can have two origins: the increase of lateral packing density and the friction between adjacent bilayers.

First we consider the effect of packing density. The observed superposition of local and long-range lateral diffusion is an experimental verification of the free volume model of lateral diffusion. According to the free volume model, the diffusion coefficient is of the form [36, 6]

\[
D_{\text{lat}} = kT/f \cdot \exp \left\{ -\gamma \frac{A^*}{(A_{\text{cage}} - A_{\text{mol}})} \right\}
\]

(4)

Fig. 14. — Lipid diffusion in DPPC-multilayers. The diffusion coefficient of the lipids has been measured for four different degrees of hydration (30 wt\%, 23 wt\%, 12 wt\% and 8 wt\% of water). Data are plotted according to the free volume model as \( \ln \langle D \rangle \) vs. \( 1/(A_{\text{cage}} - A_{\text{mol}}) \), where \( A_{\text{cage}} - A_{\text{mol}} \) is the free area per molecule.
where \( f \) is the frictional coefficient characterizing the viscous drag of adjacent bilayers on the diffusing molecules. \( f \) is determined both by the internal friction within the bilayer and the friction exerted by adjacent bilayers. \( A_{cage} - A_{mol} \) is the free area per molecule (\( A_{cage} \) is the area of the total solvent cage the molecule can diffuse in, \( A_{mol} \) is the area per molecule for closest packing). \( A^* \) is a critical free area and is defined as the minimum value of the free area required for diffusional jumps of the test molecule. \( \gamma \) is a geometrical factor, varying between 0.5 and 1, which accounts for overlapping free volumes. The exponential term has been verified experimentally [43].

The effect of the lateral packing density can be estimated by considering the known data and its hydration dependence [33] for the area per molecule. In figure 14 a plot of \( \ln (D) \) versus \( 1/(A_{cage} - A_{mol}) \) is shown. A straight line fit using

\[
\ln (D_{lat}) = \ln (kT/f) - \gamma A^*/(A_{cage} - A_{mol})
\]

yielded \( A_{mol} = 42.5 \text{ Å}^2 \), \( kT/f = 4.085 \times 10^{-6} \text{ cm}^2/\text{s} \), \( \gamma A^* = 17.57 \text{ Å}^2 \).

The area for closest packing \( A_{mol} \) is in good agreement with X-ray data [44], yielding \( A_{mol} = 42.7 \text{ Å}^2 \). A value of \( \gamma A^*/A_{mol} = 0.4 \) compares well with other data [6, 36].

The friction coefficient is calculated to be \( f = 1.115 \times 10^{-8} \text{ erg s/cm}^2 \).

Thus the results can be interpreted purely by an effect of packing density. However, the friction between two opposing bilayers mediated by the water film is predicted to depend on the thickness of the water layer [45]. This effect should only play a role when long-range lateral diffusion is regarded, but the values considered here are dominated by data in the \( Q \)-range 1-2 Å\(^{-1}\).

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