The ripple phase in a DMPC-water mixture. A dielectric investigation

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The ripple phase in a DMPC–water mixture
A dielectric investigation

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The dielectric behaviour of a DMPC–water mixture has been investigated in the frequency range from 1 kHz to 10 MHz and in the temperature interval from 0 to 40°C over the crystalline–liquid crystalline phase transition temperatures. A model to explain the dielectric characteristics of the DMPC–water mixture as a function of frequency, and in general for each DPL–water mixture which displays a ripple phase, is proposed. The model is based on the assumption that a particular contribution to the total polarizability comes from the ripple deformation of the bilayer. This contribution is evaluated supposing strong interactions between the lipid head groups with the formation of in-phase domains of correlated electric dipoles. A further assumption relates the extension of the domains to a set of spatial modes, called ripplons, thermally activated in the ripple phase. By the ripplon dispersion relation, the dipole domain extensions (hence the polarizability), were made frequency dependent. The final result shows that the anomalous peak which appears in the permittivity at the ripple phase temperature interval is qualitatively well fitted by our model.

1. Introduction

Model membrane systems, like liposomes or bilayer lipid membranes in excess water, have attracted much interest in understanding different structure-function relationships of real biomembranes [1]. In view of the complexity associated with the wide variety of functions performed by these systems, a great deal of interest in the dynamical properties and structural transitions occurring in phospholipid–water mixtures has arisen in recent years and considerable efforts are being made to understand their biological functions, by measuring a large variety of their properties [2].

The phospholipid bilayers exhibit different structures, the most common of which is the lamellar, a repeated bilayer–water structure consisting of bimolecular leaflets of lipids separated by regions of water. The phase diagram [3] of these structures, in the room temperature region and at moderate to high water content (the biologically relevant regime) includes at least three different phases: a high temperature fluid-like disordered L_α phase; a solid-like ordered L_β phase at lower temperatures and an intermediate P_β phase, also known as the ripple phase. The main transition is generally attributed to the transition from the L_β phase, when the acyl chains are frozen in a rigid, extended all-trans conformation, to an L_α phase in which melted hydrocarbon chains form the interior of the bilayer. While the L_α and L_β phase structures are well known, the ripple phase that occurs in highly hydrated bilayers, is not yet sufficiently clear.

Different experimental techniques such as NMR [4], dielectric spectroscopy [5], light scattering [6], electron microscopy [7], X-ray diffraction [8], calorimetric measurements [9], have partially characterized the P_β phase in different phospholipid–water mixtures and to a various extent they have supported the model of the ripple distortion of the lipid lattice. However, the origin of the intermolecular force responsible for the bilayer deformation and hence the role of the lipid chain length and of the zwitterionic head groups is still unclear and thus different interpretations of the experimental results have been proposed. We have associated [10, 11] the ripple phase to a thermally fluctuating rippled deformation of the bilayer that may be Fourier decomposed into a set of spatial modes with a wavevector q. These modes, called ripplons or capillary waves, are driven by the viscoelastic properties of the membrane at the interface with the bathing fluids. Because of the long ripplon wavelengths, radio frequency dielectric spectroscopy can be considered as a really effective tool in the investigation of the structure of the P_β phase. In fact an upper value for ripplon frequency was established [10, 11] by means of dielectric spectroscopy in phospholipid–water systems, namely dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylethanolamine (DPPE) with 75 per cent water content. While the DPPC–water mixture exhibits a ripple phase the DPPE–water mixture does not. Such behaviour was considered as a first indication that the pretransition is associated with a molecular mechanism involving the bilayer head groups.

In order to elucidate further the structural modifications occurring at the pretransition, we undertook the investigation reported here using the dielectric spectroscopy technique on phospholipid–water systems, within the region of existence of the lamellar phase, with different hydrophobic chains. For this purpose we have measured the dielectric properties, as a function of frequency and temperature of phospholipid–water mixtures of dimyristoylphosphatidilcholine (DMPC), which differs from DPPC only by the chain length (14 carbon atoms for DMPC and 16 carbon atoms for DPPC and DPPE). The permittivity measurements were performed on a mixture with 25 per cent wt. of lipid, as in our previous investigation on DPPC- and DPPE-mixtures [10, 11]. The frequency range of these experiments was from 1 kHz up to 1 MHz and was repeated at different temperatures in the vicinity of the crystalline-liquid crystalline phase transition.

The dielectric model which we propose to explain the experimental results is based on the assumption that the polarizability contribution to the total dielectric increment originates from the ripple modulation of the bilayer besides the usual contribution due to the heterogeneity of the system. This contribution is related to the ripplon wavelength through the size of the dipole in-phase domains. Here the anomalous permittivity variation, which we observe in the ripple phase temperature region associated with the ripplon activity, becomes frequency dependent through the ripplon dispersion equation. By comparing the permittivity increment at the pretransition with the prediction of the model, we find good qualitative agreement at different frequencies. This supports the role of ripplon propagation in the P_β phase.

2.   Experimental
2.1. *Materials*
The DMPC samples were obtained by Fluka, specified to be pure and were further purified by dissolving them in methanol with a final drying under vacuum in a

dessicator. The samples were prepared by weighing appropriate amounts of the components into suitable flasks and allowing the mixture to swell for one day at a temperature above the main phase transition temperature. After completion of this procedure, the homogeneous solutions were placed in the sample cell and the dielectric measurements were started immediately. The samples investigated contained 75 per cent wt/wt water of the total sample weight. Due to some ionic impurities, the solutions showed a small DC electrical conductivity of about $10^{-4}\,\Omega^{-1}\,cm^{-1}$ at room temperature.

2.2. *Dielectric measurements*

The spectrum of the permittivity ε' was determined by frequency domain spectroscopy, performing impedance measurements from 1 kHz to 1 MHz by means of a LF Impedence Analyzer HP Model 4192 A. The cell was a cylindrical waveguide excited well below its cut-off frequency. The value of the lumped elements of the equivalent network, representing the cell, was obtained by calibration measurements with standard liquids of known conductivity and dielectric constant. The experimental error in ε' is lower than 5 per cent for frequencies up to 10 KHz and within 1 per cent at frequencies above 10 kHz. The effects of electrode polarization in the low frequency region of the dielectric spectra were checked by measuring standard electrolyte solutions with a conductivity similar to that of the samples investigated. The increase of the permittivity at a frequency of 1 kHz is small and contained however within the quoted experimental error.

2.3. *Calorimetric measurements*

The enthalpy variations were measured by a differential scanning calorimeter, Mettler TA 3000. For each sample, several scans were performed between $-40°C$ and $60°C$ with heating rates between 2·5 aned 8 K/min with a sensitivity of 21 J/s. Figure 1 shows some typical calorimetric transition curves for the systems investigated. All of the curves show the transition which corresponds to the highest enthalpy.
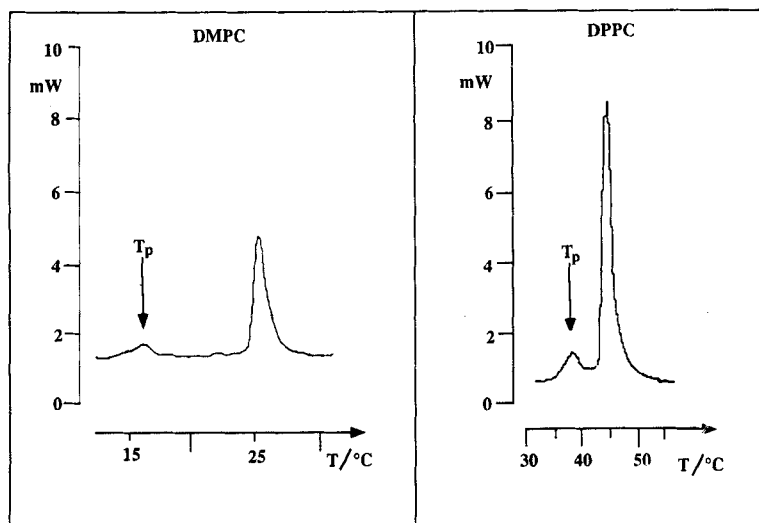

Figure 1. Calorimetric transition curves measured at 5·0 K/min for DPPC– and DMPC–water mixtures with 25 per cent wt of lipids. The arrow marks the pretransition temperature.

3. Results and discussion

The dependence of the DMPC–water mixture permittivity on temperature for different frequencies is reported in figures 2 and 3. In all of the figures, the change (peak) of the permittivity in the temperature interval ranging between the pretransition and the main transition temperature, is clearly visible. The peak amplitudes moreover, increase when the frequency is lowered and disappear at frequencies higher
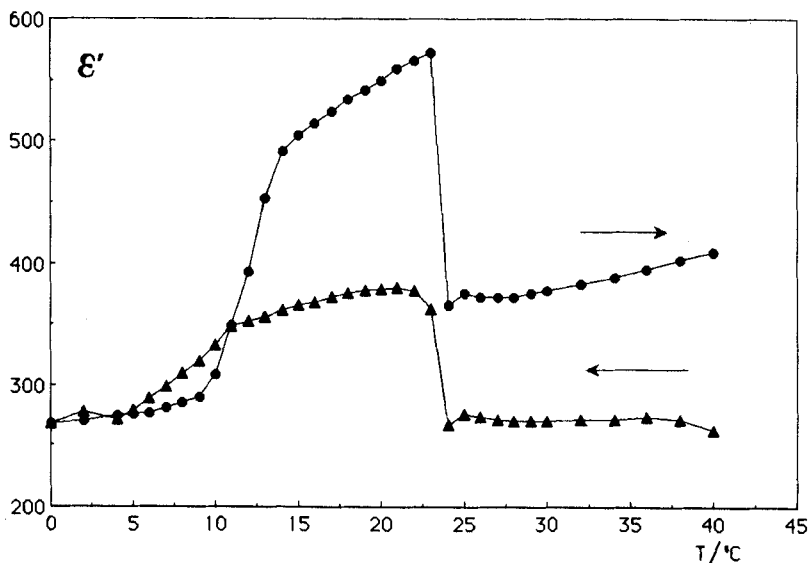
Figure 2. Permittivity ε' of a DMPC–water mixture as a function of temperature at the frequency of 1 kHz. The lipid concentration is 25 per cent wt/wt. ●, heating run; ▲, cooling run. In all runs the temperature has been varied with ten minute intervals for thermal stabilization.

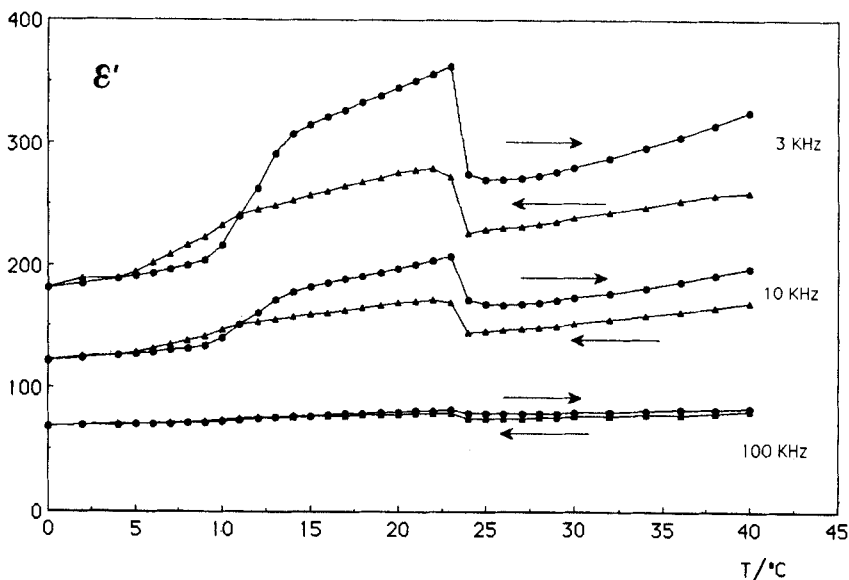Figure 3. Permittivity ε' of a DMPC–water mixture as a function of temperature at three selected frequencies. The lipid concentration is 25 per cent wt/wt. The arrows indicate the heating or cooling runs.

than 100 kHz. This behaviour is very similar to that of DPPC–water mixtures [11], which exhibit a pretransition, while it is different to the DPPE–water mixture which shows no pretransition [11]. In this last case, in fact, besides the lack of the pretransition peak in the calorimetric measurements, there are no anomalous changes in the permittivity between the L_α and L_β phases. Figures 2 and 3 show the permittivity data of cooling and heating runs; in all of the runs the temperature was changed from high to low values or vice versa by allowing ten minute intervals in order to obtain thermal stabilization.

It must be noted that a quite different behaviour occurs with sonicated samples, indicating that the anomalous high dielectric response which we have observed is characteristic of the lamellar phase and it disappears when different structures such as vesicles and liposomes are formed. This finding is consistent with the absence of the low-frequency dielectric dispersion reported by Uhlendorf [12] in very pure aqueous suspensions of phospholipids consisting of unilamellar vesicles, about 15–20 nm in radius.

The data of figures 2 and 3 clearly show that, at least as far as the ripple phase is concerned, the DMPC–water mixtures have the same behaviour as DPPC–water mixtures with respect to both temperature and frequency. This can be considered as an indication that the mechanism involved in the ripple phase originates in the lipid head group interactions rather than in the chain interactions.

Recently, Kaatze *et al.* [13] have observed pronounced temperature-induced effects in suspensions of single shell vesicles due to vesicle fusion resulting in a composite structure of multilamellar liposomes. These effects should be ruled out for phospholipid mixtures in lamellar phase, as those studied in this work, since the temperature produces a considerable reduction of the bilayer thickness, but the basic structure remains unchanged. On the other hand, the experimentally observed temperature-induced changes in the low frequency dielectric constant are too large as to be explained by changes in the bilayer thickness occurring at the main transition temperature.

The dielectric spectra of aqueous solutions of zwitterionic phospholipids reflect, in general, both the reorientational motion of the water molecules and the diffusion motion of zwitterionic groups at the bilayer lipid surface. The thermally-induced transitions in these systems involve structural rearrangements related to the chemical structure of headgroups and to the hydrophobic chains of the phospholipids [2, 14]. This complex phenomenology results in the dielectric properties of the overall system, whereas the magnitude of the different effects observed depends on the frequency of the applied field. Owing to the complexity of the molecular structure at the interface, the evaluation of the dielectric properties may be performed only by using simplified models based on mean field theories and on very crude assumptions. Recent studies [15–17] on radiofrequency dielectric relaxation in phospholipid vescicles have shown that the main contribution to the dielectric dispersions seems to result from the rotation of the polar head groups in a plane parallel to the bilayer, with consequent formation of extended domains of ordered dipoles. A particularly simple model [15, 17], which takes into account the large permittivity values which we observed in the phospholipid–water mixture at low frequency, considers that the cationic head group diffusive motions relative to the anionic group give rise to domains with high orientational correlation of the neighbouring zwitterions. Such alignment results in the formation of large in-phase domains resulting from the cooperative nature of the motion.

The ordinary Maxwell–Wagner effect [18] due to the bulk conductivity and permittivity of the two phases of the heterogeneous system, should occur at frequencies of the order of hundred of kHz, but with a negligibly small dielectric increment, if we assume that $\sigma_p \ll \sigma_m$ and $\varepsilon_p \ll \varepsilon_m$, where σ_p, σ_m, ε_p, ε_m are the conductivities and the permittivities of the lipid and aqueous phase, respectively. Insertion of typical data for 25 per cent wt lipid solution yields the dielectric increment $\Delta\varepsilon \approx 10^{-2}$ and the relaxation frequency $v \approx 2 \times 10^5$ Hz. Consequently, the dielectric properties of the bilayer are characterized by a surface frequency dependent polarizability given by

$$\alpha(\omega) \;=\; \alpha(\infty) \,+\, \frac{g\bar{n}(ze\xi)^2/2kT}{1 + (i\omega\tau)^{1-\delta}}, \tag{1}$$

where g is the orientational correlation factor (the number of neighbouring head groups with the same orientation), $\bar{n}$ is the mean surface number density of the phospholipid molecules in the lamella, ξ measures the extension of the cation motion around the phosphate group and kT is the thermal energy. In equation (1), it is assumed that the polarizability might display the typical Cole–Cole dispersion with an average relaxation time τ and a relaxation time distribution described by the parameter δ. This model has been successfully applied to various phospholipid–water systems [17, 19–20] including monoalkylphosphadiesters [17], DPPC [19] and DMPC [20] in different phases.

If the dielectric properties of the system are described, to a first approximation, by a simple additive mixture equation, the lipid membrane–water interface region contributes to the overall dielectric constant with a dielectric dispersion characterized by a dielectric increment $\Delta\varepsilon$ and a mean relaxation time τ related to the parameters of the model through

$$\Delta\varepsilon \;=\; \frac{4\pi g(ze\xi)^2\,\bar{n}\phi}{6kTd}, \tag{2}$$

$$\tau \;=\; ze\xi^2/ukT, \tag{3}$$

with u the mobility of the cationic head groups, d the bilayer thickness and ϕ the volume fraction of the lipid given by

$$\phi \;=\; [1 + (1 - W)\rho_w/W\rho_L]^{-1}, \tag{4}$$

where W is the weight fraction of the lipid in the sample, ρ_w and ρ_L are the partial specific volumes of water and lipid, respectively. Since the dielectric increment, $\Delta\varepsilon$, and the relaxation time, τ, are related to the factor g and the mobility u according to equations (2) and (3), only the quantities $g\xi^2$ and gu can be obtained from the fit of equation (1) to the measured data. Moreover the knowledge of some physical parameters concerning the structure of the lipid polar region is required. The bilayer thickness, d, in the gel state is assumed to be 62 Å [23], resulting in a surface number density of the zwitterionic head groups of $4\cdot8 \times 10^{14}$ which is in agreement with the value quoted in literature [24]. The diffusion length of the cationic group of the zwitterions is assumed to be 5 Å corresponding to the maximum anion–cation centre distance in the extended length of the phosphorylcholine groups [25]. Consequently, the correlation factor g and the ionic mobility u can be evaluated as a function of temperature.

In figure 4, the bulk permittivity as a function of frequency for the DMPC–water system is shown at three different temperatures. The bulk permittivity is represented
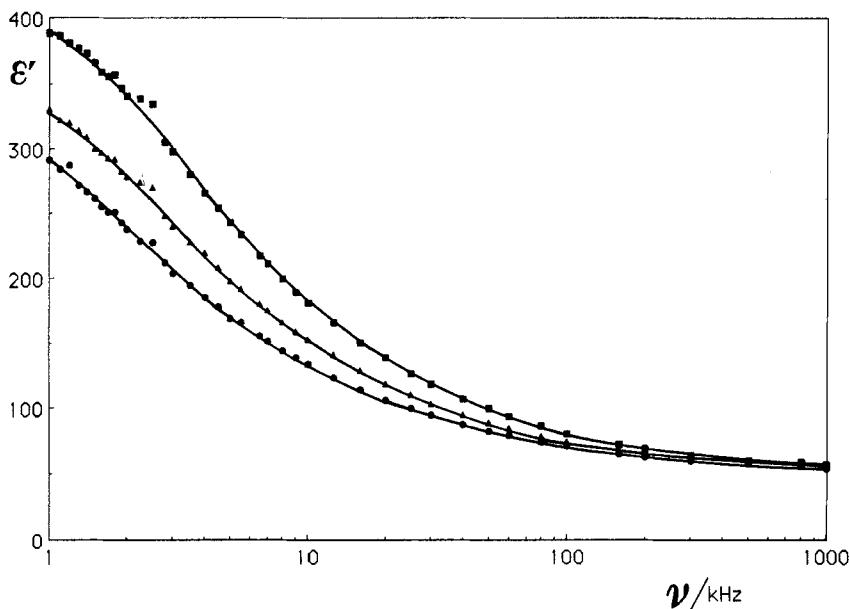
Figure 4. Typical bulk dielectric dispersion curves of a DMPC–water mixture. The permittivity ε as a function of frequency is at three selected temperatures. Full lines are best fits to equation (2); ■, $T = 34°C$; ▲, $T = 18°C$; ●, $T = 9°C$. The lipid concentration is 25 per cent wt/wt.a

by the permittivity data in the whole L_α–L_β temperature interval without the discontinuity associated with the permittivity pretransition peaks of figures 2 and 3. Since the permittivity data outside the peak temperature interval, are well fitted by a linear temperature dependent function (as in the whole L_α–L_β DPPE–water mixture temperature interval [11]), we assume that function to represent the bulk permittivity also in the peak temperature interval (the peak is then a permittivity contribution different to the bulk permittivity.) As we can see in figure 4 a reasonably good fit (full lines) to equation (2) is obtained with a value of g about 80–100 and a value of u of about 3–4 $\times$ 10^4 s/g, depending on the temperature. If in the dielectric data of figure 4 the peaks due to the pretransition are included, the parameter g needs to be increased considerably to obtain a good fit with the experimental data, and the number of the strongly correlated head groups reaches a value about twice as large. Within this model, the pretransition is accompanied by the formation of extended domains with increased structural dipole correlation.

Similarly, if ξ is still assumed to be 5 Å, as the temperature is increased from the pretransition to the main transition temperature, the mobility value decreases from about 3–4 $\times$ 10^4 to about 8–9 $\times$ 10^3 s/g. These values are considerably smaller than those calculated from the limiting equivalent conductivity of unbound cations in aqueous solution and smaller than those observed in other zwitterionic micellar systems. These situations are also confirmed by Kaatze *et al.* [19, 20, 26] who noted that the phospholipid head group mobility is greater if zwitterionics belong to strongly curved micellar surface and tend to decrease for less curved bilayer surfaces.

In the model which we have proposed, the peaks in the permittivity were associated with the existence of capillary waves that modulate the polar head orientation to create a further contribution to the permittivity. We observe that the head group

mobility increases with the curvature of the bilayer surface or that the g factor increases with the radius of the bilayer curvature. Therefore the ripplons, by producing a local curvature of the bilayer surface, must alterate the permittivity in the ripple phase region temperature.

For a symmetric bilayer separation identical fluids, the displacement [27] of the membrane from its equilibrium plane can be written as

$$s = s_0 \exp\{-i(qx - \omega t)\},$$

where q is the real wavenumber ($q = 2\pi/\lambda$), and ω is the complex frequency which are related by the dispersion equation

$$(2\omega^2 \rho/q^3\gamma + 1)(1 - i\omega\rho/\eta q^2) = 1, \tag{5}$$

where γ is the complex membrane tension and ρ and η are the density and viscosity of the aqueous medium bathing the membrane, respectively. Approximate analytic solutions of equation (5) have been derived by Crilly and Earnshaw [27], particularly for the case of a purely real membrane tension γ_0. In the low damping limit and for wavelengths greater than a critical value λ_c, the capillary waves propagate with a frequency v_R

$$v_R = \frac{1}{2\pi} \left\{ \left(\frac{\gamma_0 q^3}{2\rho} \right)^{1/2} - i \left(\frac{\eta q^2}{\rho} \right) \right\}, \tag{6}$$

where the imaginary term is the damping factor of the ripplon wave, $q = 2\pi/\lambda_R$ is the real wavenumber. By assuming, to a first approximation, that the ripplon spectrum is described by a δ function, i.e. the damping factors are longer than the period of the electromagnetic field, the ripplon wavelength is given by

$$\lambda_R = \left(\frac{4\pi^3 \gamma_0}{\rho} \right)^{1/3} v^{-2/3}. \tag{7}$$

The purpose of this model is to introduce, along with the ripple structure induced by the capillary waves, a mechanism responsible for the anomalous increase of the low frequency dielectric constant.

Since the ripplon wavelength can be considered proportional to the local curvature of the bilayer, we suppose that the extension of the in-phase dipole domains, i.e. the correlation length g, is proportional to the wavelength λ_R of the ripplon wave

$$g \sim \lambda_R \bar{n}. \tag{8}$$

Within this assumption the permittivity peak dispersion, at temperatures between the pretransition and the transition temperature, depends on the frequency of the ripplons according to

$$\Delta\varepsilon(v_R) = A v_R^{-2/3}, \tag{9}$$

where the factor A includes all of the numerical constants appearing in equations (6) and (8) for a certain temperature. It should be noticed that equation (9) diverges as $v_R \to 0$. This singularity can be removed by considering an appropriate frequency distribution of the ripplon density (a ripplon spectrum), which, in turn, requires the knowledge of some physical parameters concerning the structure of the lipid polar region and moreover the activation mechanism for ripplon propagation. On the other hand, due to the accuracy of our experimental set-up and the value of the DC ionic
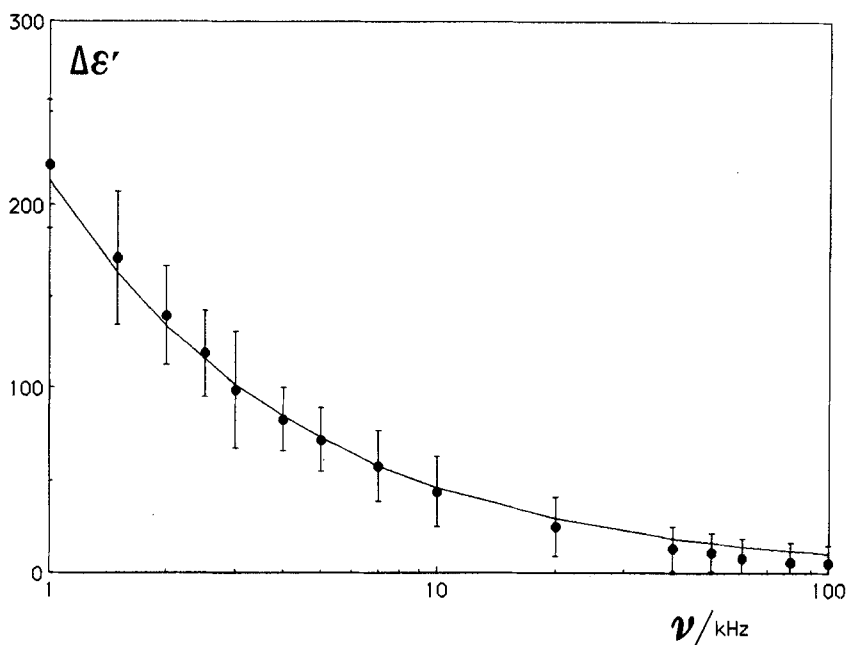
Figure 5. The increment in the permittivity at the pretransition interval as a function of frequency. The temperature is 22°C. The full line represents the calculated value according to the power law $\Delta\varepsilon \sim \omega^{-2/3}$.
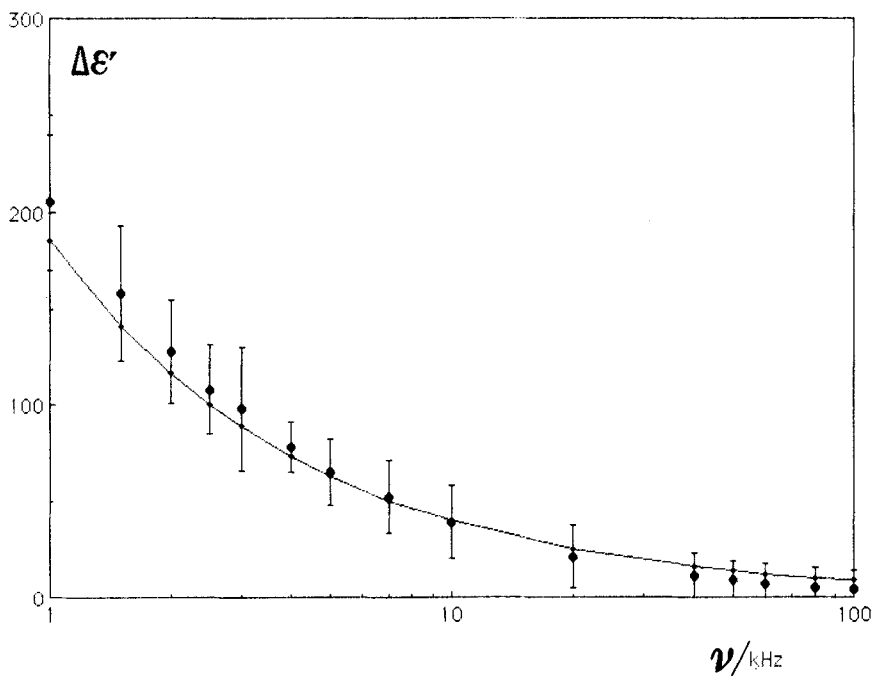

Figure 6. The increment in the permittivity at the pretransition interval as a function of frequency. The temperature is 18°C. The full line represents the calculated value according to the power law $\Delta\varepsilon \sim \omega^{-2/3}$.

conductivity of the samples under test, our experimental investigation is confined to frequencies higher than 1 kHz and this frequency appears to be yet too high to account for the complete behaviour of the permittivity increment as a function of the ripple perturbation. For this reason we limit the validity of equation (9) to frequencies higher than 1 kHz. In figures 5 and 6, the permittivity increments as a function of frequency are reported for two different temperatures. Over the data, the function in equation (9) with two appropriate factors is superimposed. The behaviour of equation (9) and the experimental data corresponding to the dielectric increment in the pretransition–transition temperature interval agree very well. This gives confidence to the interpretation of the increased orientational correlation close to the pretransition temperature as due to the ripple wave modulation.

4. Conclusions

On the basis of all our results the existence of a connection between the structure and dynamics of the ensemble of the polar head groups and the occurrence of the pretransition seems reasonably well established. Moreover we have an encouraging qualitative description of the dielectric behaviour of these systems based on the existence of large ordered domains of polar head groups in the bilayer modulated by the propagation of capillary waves. This model also allows a qualitative explanation of the experimental results. A more quantitative analysis of our data requires a more elaborate model and more careful experiments. This work is in progress.

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