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Audio to microwave frequency dielectric study of the pretransition region in DPL–water systems

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The phospholipid–water mixtures are used as model systems to investigate the structure and function of biological membranes. The dielectric behaviour of DPPC–water and DPPE–water systems was explored at audio (⩾2 kHz) and microwave (10 GHz) frequencies versus temperature. Particular care was devoted to the pretransition below the main gel–liquid-crystalline phase transition. A possible interpretation of the pretransition mechanism is given in terms of capillary waves.

1. Introduction

The thermotropic phase transitions in lipid bilayers are actively studied [1] because these systems are important relative to bio-membrane systems [2, 3]. The molecular structure and the temperature dependence of the phospholipid–water mixtures have been investigated by X-ray diffraction [4, 5] and by calorimetric measurements [6]. In general the experimental results show many phases whose structures are dependent on temperature and on the degree of hydration [7, 8]. The gel to liquid-crystalline phase transition arising from a cooperative melting of hydrocarbon chains appears to have direct biological relevance. Actually the transition results in a variation of the spacial structure of hydrocarbon chains and of the charge distribution of head groups, hence in a modification of the transport properties of the membrane. In highly hydrated phospholipid bilayer the calorimetric measurements [9, 10] exhibit pretransition behaviour below the temperature of the main gel to liquid crystal transition. In the pretransition the structural changes of the bilayer are at the moment not well defined.

Janiak *et al.* [11] associate the pretransition with a structural transformation of the bilayer in a two-dimensional lattice, consisting of a lipid lamella modulated by a periodic ripple. The cell parameters of this structure and the ripple periodicity have been reported until now only for two lecithins (dimyristoyllecithins and dipalmitoyllecithins).

Dielectric relaxation behaviour of phospholipid–water mixtures are also commonly used to understand the properties of hydrated bilayers [12–14]. In general the high-frequency dielectric dispersion is attributed to free and bound water, while the dielectric response at radio frequencies is attributed to the motion of the zwitterionic head group with large dipole moments.

Thus far only little attention has been paid to the dielectric properties at audio frequencies, where collective phenomena in the bilayer surface could be properly observed. Actually, for instance, the rotational motion of lipid molecules around their major axis occurs at frequencies of about 10 kHz, as suggested by Westermann *et al.* [15] on the basis of deuterium N.M.R. measurements.

With the purpose of characterizing the pretransition in the lipid–water mixtures, we have measured the dielectric properties of DPPL–H_2O and DPPE–H_2O as a function of temperature from 5 to 70°C, at audio and microwave frequencies. In these frequency ranges we can observe both collective phenomenon and possible hydration effects. Our results show that the audio frequency polarization is relevant around the pretransition temperature. This dielectric behaviour is probably due to the existence in the bilayer of large domains modulated by the propagation of capillary waves. On the other hand, the fact that no appreciable variation in the permittivity was observed at microwave frequencies indicates that any hydration effect should be excluded in the appearance of the pretransition.

2. Materials and methods

The used dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylethanolamine (DPPE) were provided by Sigma Chemical Company with a purity of 98 per cent and 99 per cent respectively. Measurements were done on the two lipids without further purification.

The DPPC, dissolved in chloroform, was inserted in a pyrex glass tube having a narrow constriction. The chloroform was then removed under a nitrogen stream and by drying in vacuum. DPPC–water samples were prepared by incubation of weighted amounts of lipid and distilled deionized water for about 15 hours at a temperature above the main transition temperature. After swelling, the material was forced through the hole by centrifuging, until a homogeneous high viscosity mixture was obtained.

The DPPE and also some DPPC samples were prepared by drying lipids and adding the appropriate amounts of water. The samples were thus vortexed several times for about 1 min at a temperature well above the main transition temperatures of 42°C for DPPC and 60°C for DPPE. These different methods of samples preparation did not change significantly the experimental results.

2.1. *Low-frequency dielectric measurements*

The homogeneous lipid–water mixtures were inserted in a dielectric cell consisting of a cylindrical waveguide excited far below its TM_{01} cut-off frequency mode. The input impedance of the cell was measured by means of a LF analyser HP4219A in the frequency range from 1 kHz to 10 MHz in the temperature interval 5 to 80°C. The cell calibration constants were determined by measurements with various reference liquids.

2.2. *Microwave dielectric measurements*

The dielectric properties of the samples were measured at the frequency of 10 GHz as a function of temperature in the range 0 to 60°C. A glass capillary filled with the lipid–water mixture was inserted along the axis of a cylindrical microwave cavity operating in the TE_{011} mode. The permittivity can be deduced from the changes in the resonance frequency of the resonance peak produced by the sample insertion.

3. Results and discussion

3.1. *Low-frequency dielectric results*

Our low-frequency dielectric measurements on DPPC–H_2O are shown in figure 1. The most interesting feature is represented by a marked change in the permittivity

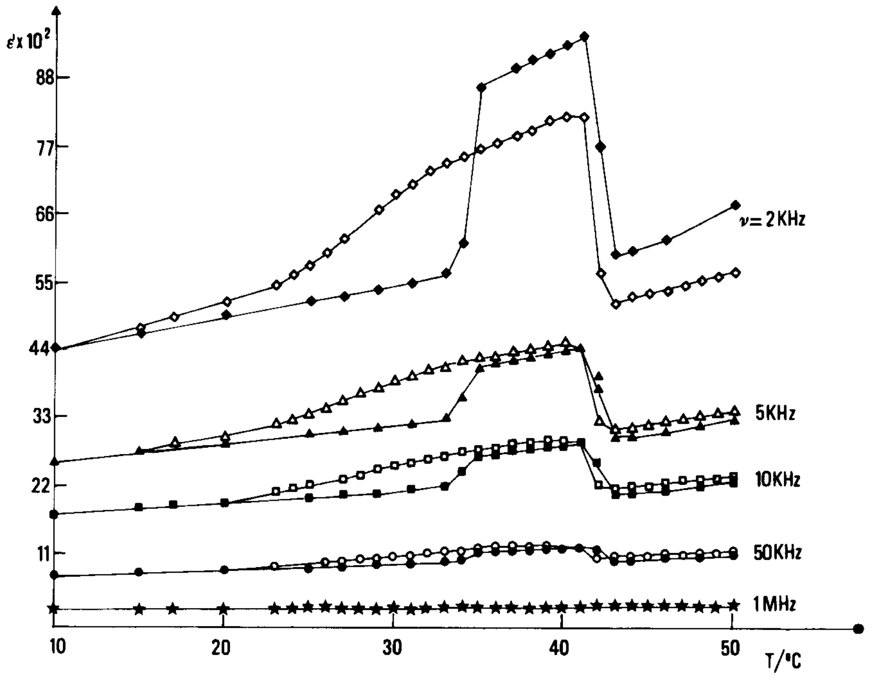

Figure 1. Permittivity ε′ of the DPPC–H_2O mixture as a function of temperature at different frequencies in the range 1 kHz to 1 MHz. The lipid content is 25 % wt. Heating and cooling runs are marked by closed and open symbols respectively. The pretransition temperature for the DPPC is 35°C while the main transition temperature is 42°C.

close to the main gel–liquid-crystalline transition. This anomalous behaviour starts at the pretransition temperature occurring for this sample at about 36°C, as confirmed by calorimetric measurement. Another relevant aspect of permittivity shown in figure 1 is the increase of the size of the 'hump' at the pretransition with decreasing frequency. A possible interpretation of these results might arise from the existence of capillary waves that modulate the polar head orientation. Apparently by raising the temperature up to the pretransition, an onset of capillary modes is excited. This effect seems to be limited to the pretransition temperature interval.

The study of capillary waves in bilayer–fluid interfaces has received considerable attention in the past few years [16, 17]. Recently accurate light scattering measurements [18] have shown that the capillary wave propagation follows the classical hydrodynamic theory, with the bulk values of the viscoelastic properties of these structures, i.e. the surface tension, the shear viscosity and the mass density.

In the case of symmetric membranes subject to fluctuations driven by thermal motion, capillary waves propagate with a frequency determined by the interfacial tension and with a damping factor due mainly to the viscosity of the liquid phase. Actually, it was shown [19] that capillary waves have the usual form

$$F(\omega, t) = \exp[-i(qx + \omega t)], \tag{1}$$

with $q = 2\pi\lambda^{-1}$ the wavevector of wavelength λ and

$$\omega = \omega_0 - i\alpha \tag{2}$$

the complex frequency with α the damping factor. The dispersion equation can be written in the form [20]

$$[s^2 + \tau s + y][1 + 2s]^{1/2} - [\tau s + y] = 0, \tag{3}$$

with

$$s = -(\alpha + i\omega_0)\zeta/2\eta q^2, \; y = \frac{\gamma_0 \zeta}{2\eta^2 q}$$

and

$$\tau = \frac{\gamma' q}{4\eta}.$$

Here η and ζ are respectively the viscosity and the mass density, $\gamma = \gamma_0 - i\omega\gamma'$ the interfacial tension of lipid–aqueous phase with γ_0 the static membrane tension and γ' the shear interfacial tension.

Crilly and Earnshaw [21] have solved equation (3) and shown that above a certain critical value y_c of y, damped oscillation occurs, while below this critical value equation (3) yields two distinct real roots, corresponding to non-propagation modes. Thus, according to equation (3), waves with a wavelength greater than the critical value

$$\lambda_c = \frac{16\pi\eta^2 y_c}{\gamma_0 \zeta} \tag{4}$$

can propagate along the bilayer in the aqueous interface. The evaluation of equation (4) is obtained putting $\gamma' = 0$, because of the weak dependence of ω_0 on λ. Thus typical values of γ' like 10^{-5}–10^{-6} dyn s/cm also leave the result of equation (4) practically unaffected. Assuming as common values $\gamma_0 = 3$ dyn/cm, $\eta = 10^{-2}$ poise, a critical wavelength of 2×10^{-4} cm was obtained. This critical wavelength value corresponds to an upper limit for frequencies of about 150 kHz. It can be seen that the calculated upper limit frequency for capillary waves agrees with the maximum frequency in which the pretransition 'hump' is observed. This fact leads us to suppose that surface waves can propagate along the fluid interface and hence modulate the polar head orientation, thus modifying the spacial arrangement of the polar group.

Further support to this point of view comes from the low-frequency dielectric measurements performed on DPPE–H_2O mixture. This lipid in fact does not display the pretransition in calorimetric studies, but only the main gel–liquid crystalline transistion at about 60°C.

As can be seen in figure 2, the dielectric spectra of DPPE–H_2O are markedly different from those of DPPC–H_2O of figure 1. In the case of DPPE–H_2O in fact the permittivity versus temperature increases monotonically, without the anomalous 'hump' below the main transition temperature, while the change of slope might be due to the co-operative melting of the hydrocarbon chains. The measured dispersion curves at selected temperatures are summarized in figure 3.

3.2. *Microwave dielectric results*

To control if the pretransition is triggered by changes in hydration water, we have measured also the permittivity of the DPPC–H_2O system in the microwave region. At microwave frequencies, the dielectric behaviour is mainly due to the orientational

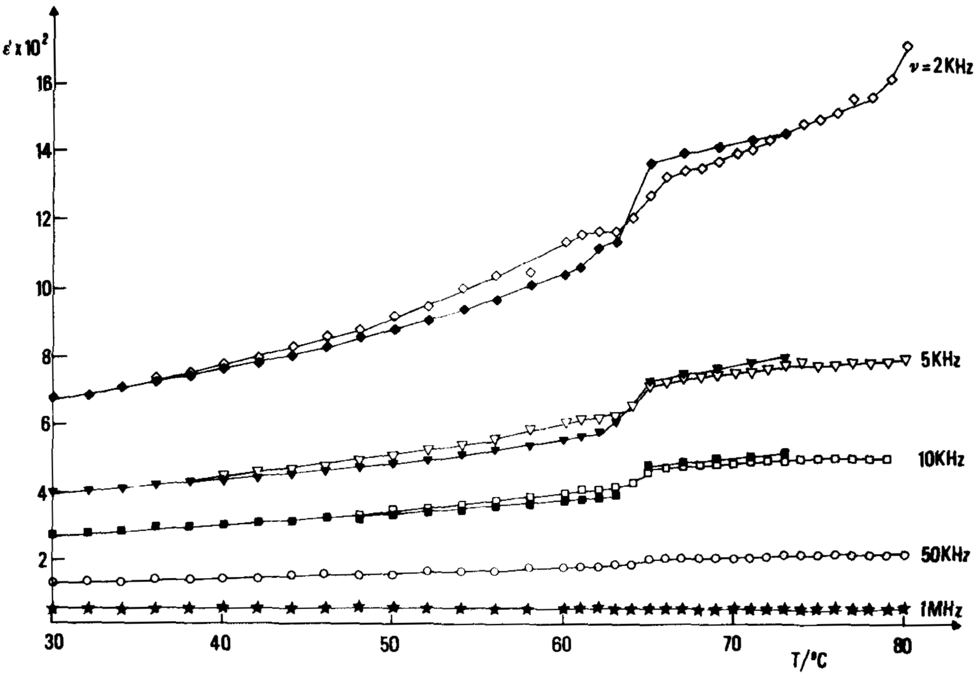

Figure 2. Permittivity ε′ of the DPPE–H_2O mixture as a function of temperature at different frequencies in the range 1 kHz to 1 MHz. The lipid content is 25 % wt. Heating and cooling runs are marked by closed and open symbols respectively. The main transition temperature for the DPPE is 60°C.

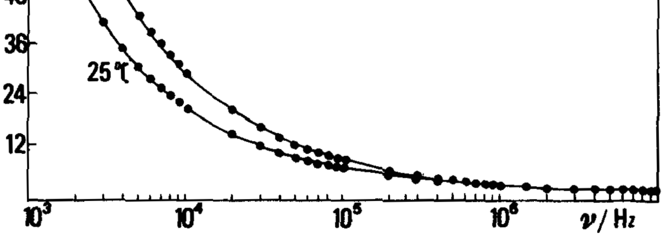

Figure 3. Dielectric dispersion curves around the pretransition temperature of some samples of figure 1.

relaxation of water [22]. This process is characterized by a dipole relaxation time of about $0{\cdot}9 \times 10^{-11}$ s at 20°C, corresponding to a maximum absorption frequency of some GHz. As a consequence, the water molecules bound to the lipid molecules do not contribute to dielectric response. The above 'absence approach' has been widely

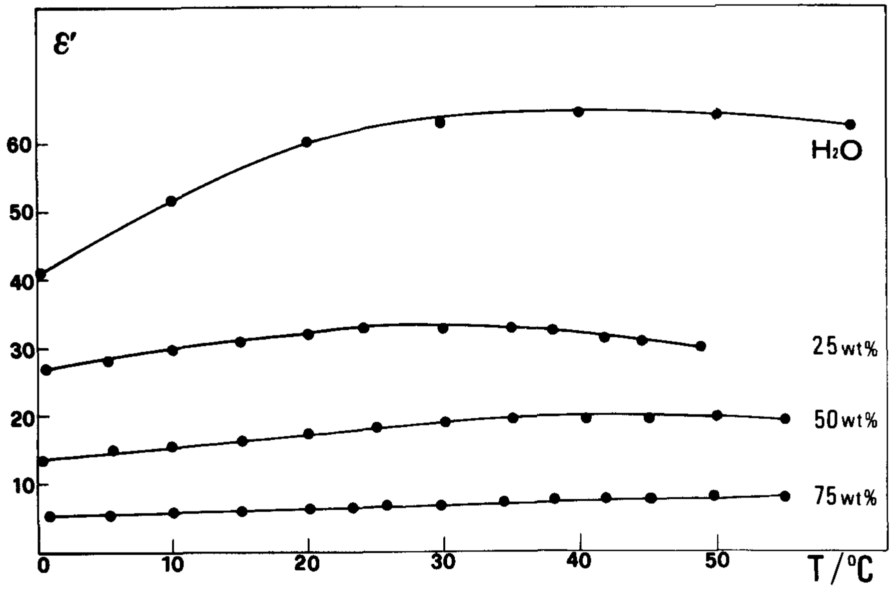

Figure 4. Permittivity ε' of DPPC–H_2O mixture at a frequency of 10 GHz versus temperature, for various lipid contents. The calculated values according to equation (5) are shown by a full line.

adopted to estimate the amount of hydration water in different biological systems [23].

The permittivity of DPPC–water mixtures at three different water concentrations against temperature at 10 GHz is shown in figure 4. The results do not show any anomalous behaviour of the permittivity around the pretransition temperature. The permittivity is quite constant over the whole explored temperature range.

To explain the results of figure 4, taking into account the local anisotropy of the samples due to the lamellar structure, the usual mixture equation [22] must be modified. For this purpose we extend the equation proposed recently by Hamnerius *et al.* [24] to a lipid bilayer with a layer of hydration water of constant thickness

$$\varepsilon' = \frac{2}{3}(\phi_L + \phi_W\varepsilon_W + \phi_{WS}\varepsilon_{WS}) + \frac{1}{3}\frac{\varepsilon_L\varepsilon_W\varepsilon_{WS}}{\phi_L\varepsilon_W\varepsilon_{WS} + \phi_W\varepsilon_{WS}\varepsilon_L + \phi_{WS}\varepsilon_W\varepsilon_L} \quad (5)$$

In equation (5) ε_L, ε_W and ε_{WS} are the permittivities of the lipid, water and hydration water respectively and ϕ_L, ϕ_W, ϕ_{WS} their fractional volumes. By using the typical value $\varepsilon_L = 2{\cdot}5$ [25], and by assuming hydrated water strongly bound with a relaxation time of the order of 10^{-4} s, equation (5) gives the amount of hydration layer and its dependence on temperature.

According to these assumptions, the computed values of permittivities for different DPPC–water mixtures are reported as full lines in figure 4. The agreement was obtained with $\phi_{WS} \sim 0{\cdot}08$ constant in the whole temperature range and roughly independent of the lipid content.

These results suggest that DPPC–water mixture in the lamellar phase is a fully hydrated mixture also at the highest lipid content. Moreover, in this phase no significant influence on the pretransition occurrence can be attributed to the hydration water. This contrasts in part with the dielectric measurements carried out by Enders and Nimtz [26] on multilamellar liposomes of hydrated dimyristoylphosphotidilcholine

(DMPC), where a transition is observed in the microwave dielectric spectrum during the heating runs, making evident strong hysteresis effects at a lower degree of hydration. Moreover, our dielectric experiment show that, at the pretransition temperature, the rippled structure of the lamellar phase suggested by Janiak *et al.* [11] should not be induced by the head group interactions with the hydration water molecules.

4. Conclusion

Our experimental results on the dielectric behaviour of phospholipids–water mixtures support the hypothesis that the region between the pretransition and the main gel–liquid-crystalline transition is involved in a modulation of the bilayer with a probable change in the conformation of hydrocarbon chains [27]. The modulations, propagating like capillary waves on the water–lipid interface, are originated by a tension force rather than a gravity force. The critical wavelength, over which the bilayer ripple propagates, is in good agreement with our qualitative evaluation.

This interpretation is supported also by microwave measurements. In fact, the absence of a discontinuity in the permittivity in the region of interest indicates that the pretransition cannot be triggered by hydration effects.

References

[1] Nagle, J. F., 1980, *A. Rev. phys. Chem.* **31**, 157.
[2] Nagle, J. F., and Scott, H. L., 1978, *Physics Today*, **31**, 38.
[3] Melchior, D. L., and Steim, J. M., 1977, *Biochim. biophys. Acta*, **466**, 148.
[4] Luzzati, V., and Husson, F., 1962, *J. Cell Biol.*, **12**, 207.
[5] Tardieu, A., Luzzati, V., and Reman, F. C., 1973, *J. molec. Biol.*, **75**, 711.
[6] McElhaney, R. N., 1982, *Chem. Phys. Lipids*, **30**, 229.
[7] Scott, H. L., 1975, *J. chem. Phys.*, **62**, 1347.
[8] Enders, A., and Nimtz, G., 1984, *Ber. Bunsenges. phys. Chem.*, **88**, 512.
[9] Knoll, W., and Hohne, G. W. H., 1984, *Ber. Busenges. phys. Chem.*, **88**, 517.
[10] Chapman, D., 1968, *Biol. Membr.*, **11**, 125.
[11] Janiak, M. J., Small, D. M., and Shipley, G. G., 1979, *Biochemistry*, **15**, 4575; 197, *J. biol. Chem.*, **254**, 6068.
[12] Kaatze, U., Henze, R., Seegers, A., and Pottel, R., 1975, *Ber. Bunsenges. phys. Chem.*, **79**, 42.
[13] Shepherd, J. C. W., and Buldt, G., 1978, *Biochem. biophys. Acta*, **514**, 83.
[14] Nimtz, G., Enders, A., and Binggeli, B., 1985, *Ber. Bunsenges. phys. Chem.*, **89**, 842.
[15] Westermann, P. W., Vaz, M. J., Strenk, L. M., and Doane, J. W., 1982, *Proc. natn. Acad. Sci. U.S.A.*, **79**, 2890.
[16] Hansen, R. S., and Mann, J. A., 1964, *J. appl. Phys.*, **35**, 152.
[17] Stone, J. A., and Rice, W. J., 1977, *J. Colloid Interface Sci.*, **61**, 160.
[18] Hard, S., and Neuman, R. D., 1987, *J. Colloid Interface Sci.*, **115**, 73.
[19] Lucassen-Reynders, E. H., and Lucassen, J., 1969, *Adv. Colloid Interface Sci.*, **2**, 347.
[20] Kramer, L., 1979, *J. chem. Phys.*, **55**, 2097.
[21] Crilly, J. F., and Earnshaw, J. C., 1983, *Biophys. J.*, **41**, 197.
[22] Pethig, R., 1979, *Dielectric and Electronic Properties of Biological Materials* (Wiley).
[23] Suggett, A., 1973 *High-Frequency Dielectric Measurement*, edited by J. Chamberlain and G. W. Chantry (I.P.C. Science & Technology Press).
[24] Hamnerius, Y., Lundstrom, I., Paulsson, L. E., Fontell, K., and Wennerstrom, H., 1978, *Chem. Phys. Lipids*, **22**, 135.
[25] Grant, E. H., Sheppard, R. J., and South, G. F., 1978, *Dielectric Behaviour of Biological Molecules in Solution* (Clarendon Press).
[26] Enders, A., and Nimtz, G., 1984, *Ber. Bunsenges. phys. Chem.*, **88**, 512.
[27] Cametti, C., De Luca, F., Maraviglia, B., and Sorio, P., 1985, *Chem. Phys. Lett.*, **118**, 626.