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MOTION OF THE LENGTHENED ZWITTERIONIC HEAD GROUPS OF C_{16}-LECITHIN ANALOGUES IN AQUEOUS SOLUTIONS AS STUDIED BY DIELECTRIC RELAXATION MEASUREMENTS

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Phosphatidyl choline analogues with increased phosphate-trimethylammonium distance were synthesized and aqueous solutions of these bilayer forming phospholipids were prepared. Dielectric spectra of the solutions were measured at several temperatures around the crystalline/liquid-crystalline phase transition temperature of the samples. The observed data are treated in terms of a Debye relaxation function and also of a relaxation function based on a theoretical model of the aqueous solutions of multibilayer vesicles. As a noteworthy result, a pronounced cooperativity effect in the diffusive motions of the zwitterionic head groups emerges. The degree of cooperativity depends on the radius of curvature of the multibilayer vesicles and also on the length of the phospholipid zwitterions. The values for the mobility of the trimethylammonium group are of the same order of magnitude as those for the mobility of whole phospholipid molecules in its lateral diffusive motion. Indications for a phase transition at a temperature above the main transition temperature are found with solutions of C_{16}-lecithin analogues with 9 and 10 methylene groups between the phosphate and the trimethylammonium group.

1. Introduction

The structural properties and functional characteristics of phospholipid bilayers are largely determined by the combined action of the hydrophobic properties of the aliphatic inner part of the membrane and of the molecular mechanisms at the hydrophilic membrane surface. In the past decade the hydrocarbon phase of phospholipid double layers has been extensively studied by various experimental and theoretical methods. Only recently, however, interest has been directed towards the physical state of the membrane surface [1–15].

Among the experimental techniques which are sensitive to surface properties of bilayers, dielectric relaxation spectroscopy takes advantage of the fact that many of the phospholipids have a zwitterionic head group. The electric dipole moment of such head groups is used as a natural molecular mark in dielectric relaxation studies. No artificial spectroscopic labels have thus to be introduced into the sample.

It has been shown by dielectric relaxation spectroscopy [15], that there exists a high degree of cooperativity (orientational correlation) in the molecular motions of the phosphorylcholine groups in aqueous solutions of dimyristoylphosphatidylcholine (C_{14}-lecithin). The strong correlation of zwitterion orientations appears to be restricted to bilayer structures since the effect is dramatically smaller in micellar lysolecithin solutions and also with other zwitterionic amphiles which form micelles in water [16]. As another result of the previous dielectric relaxation measurements a substantial reduction in the mobility of the cationic trimethylammonium group of the phosphorylcholine zwitterion emerges if it is incorporated in the surface of C_{14}-lecithin bilayers instead of lysolecithin micelles. It seems to be interesting in this connection to look for factors other than shape of the multimolecular aggregates which control the orientational correlation

Table 1
Data of the solutes and solutions. Values for the molar weight M_u of the solutes given for the unhydrated phospholipids. The crystalline/liquid-crystalline phase transitions temperature T_t has been determined at decreasing temperature [22]. Values for the molarity c and the volume fraction v refer to room temperature. The dc conductivity σ has been found by fitting of the relaxation function $R_D(\nu)$ (eq. (1)) to the experimental permittivity spectra

Phospholipid (shorthand notation)	Formula	M_u [g/mol]	T_t [°C] ±1°	c [mol/l] ±1%	v ±10%	σ [mS/cm] ±1%	Sonication
C_{16}-lecithin	$C_{40}H_{80}NO_8P$	734.0	41.0	0.129	0.095	0.253 (24.4°C)	strong
C_{16}-PN_5-lecithin	$C_{43}H_{86}NO_8P$	776.1	37.3	0.095	0.074	0.144 (23.4°C)	weak
C_{16}-PH_6-lecithin	$C_{44}H_{88}NO_8P$	790.1	37.1	0.137	0.108	0.298 (21.8°C)	weak
C_{16}-PN_7-lecithin	$C_{45}H_{90}NO_8P$	804.2	37.0	0.097	0.078	0.099 (22.9°C)	weak
C_{16}-PN_9-lecithin	$C_{47}H_{94}NO_8P$	832.2	36.8	0.058	0.048	0.137 (23.7°C)	weak
				0.106	0.088	0.195 (39.4°C)	
C_{16}-PN_{10}-lecithin	$C_{48}H_{96}NO_8P$	846.2	34.8	0.098	0.083	0.129 (22.3°C)	weak

and dynamics of the phospholipid polar head groups.

In this communication we are reporting the results of our dielectric relaxation studies on aqueous solutions of various 1,2-dipalmitoyl-sn-glycerol-3-phosphoryl-N,N,N-trimethylalkanolamines (C_{16}-PN_n-lecithins, $n = 2, 5, 6, 7, 9$, and 10) with a different number of methylene groups between the anionic phosphate group and the cationic trimethylammonium group. The dependence of the complex permittivity of the samples, $\epsilon(\nu) = \epsilon'(\nu) - i\epsilon''(\nu)$, on frequency, ν, has been determined in the range 10^5 to about 3×10^7 Hz by time domain reflectometry. The measurements were carried out at several temperatures around the crystalline/liquid-crystalline phase transition temperature, T_t, of the aqueous phospholipid system.

The observed frequency dependent permittivities are phenomenologically described by a simple Debye relaxation function and the parameters of this function are discussed with respect to both, its variation with temperature and with zwitterion length. The measured $\epsilon(\nu)$ data are also discussed on the basis of a theoretical model of the solutions in order to get insight into the molecular mechanisms in the motions of different dipolar head groups at the membrane surface.

2. Materials

2.1. Synthesis of the C_{16}-PN_n-lecithin

The 1,2-dipalmitoyl-sn-glycerol-3-phosphorylcholine (C_{16}-lecithin) analogues with stepwise increase in the number of CH_2 groups between phosphate and trimethylammonium were prepared from 1,2-dipalmitoyl-sn-glycerol by phosphorylation with the respective bromoalkylphosphoric acid dichlorides to yield 1,2-dipalmitoyl-sn-glycerol-3-phosphoric acid bromoalkylesters as described elsewhere [17]. The bromoalkylesters were converted to the phosphorylcholine analogues by amination with trimethylamine [17,18]. The crude amination products were purified by chromatography. The pure C_{16}-lecithin analogues were obtained in yields of about 60 per cent based on 1,2-dipalmitoyl-sn-glycerol.

The starting 1,2-dipalmitoyl-sn-glycerol (Rf = 0.2) was free of the 1,3-isomer (Rf = 1.3) as shown by thin layer chromatography on boric acid plates [19] with the solvent system chloroform/hexane/diisopropylether 5 : 4 : 1 (parts of volume). The molar ratio of fatty acid/phosphate/vicinal diol [19] was close to that expected (expected: 2 : 1 : 1; found: 1.98 : 1.00 : 1.02 for C_{16}-PN_5-lecithin and 1.97 : 1.00 : 1.01 for C_{16}-PN_{10}-lecithin). There was no indication for fatty acid migration, 1,2-dipalmitoyl-sn-glycerol to 1,3-dipalmitoyl-glycerol, during the phosphorylation step.

Phosphate content was analyzed according to Eibl and Lands [20] with the reagent kit supplied by Serva (Heidelberg, Germany). Vicinal diol was determined by perjodate analysis by measuring the formed iodate directly as introduced by Eibl and Lands [21].

The synthesis and results of the elemental analysis of the C_{16}-lecithin analogues are described in detail in ref. [17]. The normal C_{16}-lecithin had been purchased from Koch–Light (Colnbrook, England) and was used without additional purification.

2.2. Preparation of the solutions

The solutions were prepared by addition of water to a weighed out amount of phospholipid. The water used was doubly distilled and sterilized by UV radiation during distillation. The electrical dc conductivity σ of the pure H_2O was smaller than 2×10^{-3} mS/cm ($= 2 \times 10^{-6} \Omega^{-1}$ cm^{-1}).

The crude solutions were stored at 60°C for about 12 hours to allow for swelling. The solutions of the C_{16}-lecithin analogues got homogeneous by weak ultrasonic irradiation. The aqueous sample of normal C_{16}-lecithin had to be strongly sonicated for some minutes in order to get homogeneous.

Due to some unavoidable ionic impurities of the phospholipid materials the dc conductivity of the aqueous solutions was larger by a factor of about 100 than that of the pure solvent. Conductivity values and some other data of the solutes and solutions are presented in table 1.

3. Measurements

3.1. Experimental method

The dependence of the complex (relative) permittivity of the solutions, $\epsilon(\nu) = \epsilon'(\nu) - i\epsilon''(\nu)$, on the frequency, ν, has been determined by fast response time domain reflectometry (TDR). A sequence of fast rising step-voltage pulses was applied to a specimen cell by a coaxial transmission line and the time-dependent electrical response of the cell to the exciting pulses was observed. The signals in the transmission line were probed by a sampling device, analog/digital converted, and stored on a digital tape. The frequency spectrum of the complex permittivity was calculated from the stored data by means of a digital computer.

3.2. Apparatus

The fast rising pulse signals as produced by a tunnel-diode step generator were characterized by a rise time of 35 ps, a duration of 36 μs and a repetition frequency of 17.6 kHz. The step-voltage pulses were fed to a specimen cell which consists of a coaxial line/circular cylindrical waveguide transition [23], the waveguide being excited below its cut-off frequency by the Fourier components of the pulses. The main advantage in the use of such an open circuit termination as a sample cell is the fact, that no change in the experimental set-up is needed in order to fill the cell. For this reason, reference measurements on pure water and with the empty cell can be performed without any changes in the contacts of the line system.

The apparatus as well as the methods of data acquisition and processing are described in full particulars elsewhere [24].

3.3. Uncertainties in the permittivity data

Errors in the complex permittivity values are mainly due to imperfections of the apparatus, to the limited time window in the measurements, to the uncertainty in the determination of the zero point of time scale and to temperature fluctuations in the sample. Usually, complex permittivity data obtained by TDR studies are more reliable around the central part of the relaxation spectral region than at the ends of the spectrum. On grounds of test measurements on reference liquids and of numerical simulations the precision of one permittivity data can be generally characterized by an estimated uncertainty of less than 2% in the values of $\epsilon'(\nu)$ and of less than 5% in the values of $\epsilon''(\nu)$. The latter figure, however, may be a little greater in the high and low frequency part of the spectral range under consideration.

3.4. Realization of the temperature program

The measuring program for each solution was performed at falling temperature with a fifteen minutes interval between successive points of measurements allowing for thermal stabilization of the sample. After completion of a series of measurements the liquid was heated up and one of the initial data points was measured again in order to look for eventual alterations of the solution. Only those series of measurements in which the complex permittivity spectrum was sufficiently independent of time (deviations between the original data and those obtained by repetition of the measurement smaller than the experimental uncertainty in the $\epsilon(\nu)$ values) are presented here.

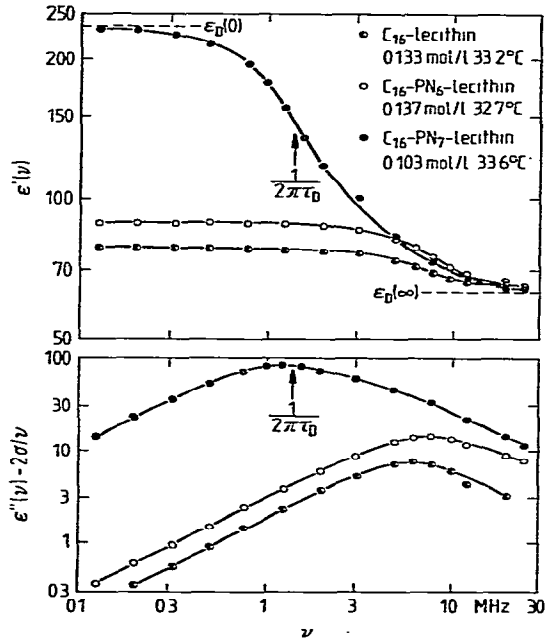

Fig. 1. Real part of the permittivity, $\epsilon'(\nu)$, and negative imaginary part excluding contributions of ohmic conductance loss, $\epsilon'' - 2\sigma/\nu$, plotted versus frequency, ν, for solutions of normal C_{16}-lecithin and of the PN_6 and PN_7 C_{16}-lecithin analogues.

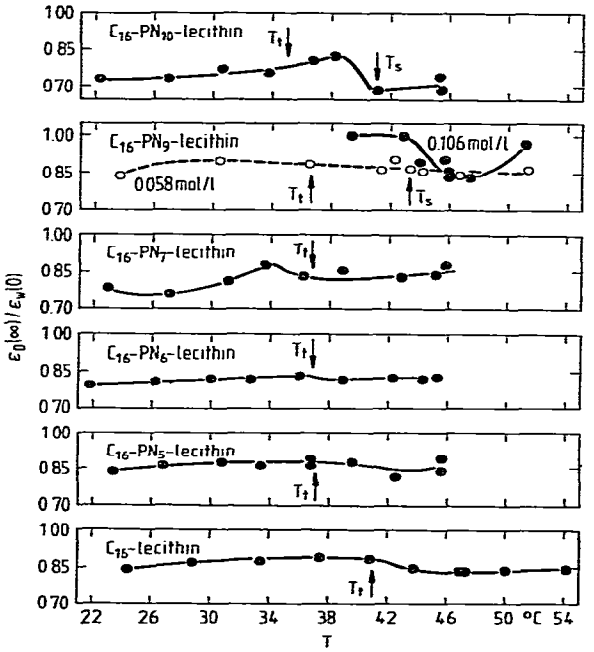

Fig. 2. The extrapolated high frequency permittivity $\epsilon_D(\infty)$ of the Debye relaxation term (eq. (1)), normalized to the static permittivity $\epsilon_w(0)$ of pure water at the same temperature, plotted versus temperature T for the C_{16}-PN_n-solutions.

4. Results in terms of a Debye relaxation function

4.1. Empirical description of the measured complex permittivity spectra

In fig. 1, the dependence of the quantities $\epsilon'(\nu)$ and $\epsilon''(\nu) - 2\sigma/\nu$ on the frequency ν is displayed in the range 0.1 to 30 MHz for the aqueous C_{16}-PN_2-lecithin (normal C_{16}-lecithin) and for solutions of the analogues C_{16}-PN_6lecithin and C_{16}-PN_7-lecithin. The term $2\sigma/\nu$ has been subtracted from total $\epsilon''(\nu)$ in this presentation of experimental data in order to omit contributions due to ohmic conductivity σ as caused by slight ionic impurities.

Within the limits of experimental error the measured complex permittivity spectra $\epsilon(\nu) = \epsilon'(\nu) - i\epsilon''(\nu)$ can be analytically represented by the function

$$R_D(\nu) = \epsilon_D(\infty) + \frac{\epsilon_D(0) - \epsilon_D(\infty)}{1 + 2\pi i\nu\tau_D} - \frac{2i\sigma}{\nu}. \qquad (1)$$

Since the dielectric relaxation of the solvent water has to be expected to occur at frequencies well above 30 MHz (see fig. 1 in [15]), the extrapolated high frequency permittivity $\epsilon_D(\infty)$ reflects two different contributions to the static permittivity $\epsilon_D(0)$ of the solution, namely those resulting from fast solute and solvent distortion polarization mechanisms and those resulting from the orientation polarizability of the water. The second term on the right hand side of eq. (1) represents a "Debye" relaxation which is characterised by one discrete relaxation time τ_D. The results of previous dielectric studies on aqueous C_{14}-lecithin solutions [15] suggest this relaxation to be essentially due to the restricted diffusive motions of the zwitterionic phospholipid head groups. The term $2i\sigma/\nu$ in eq. (1) represents conductivity contributions to the measured total imaginary part $-\epsilon''(\nu)$ of the complex permittivity.

The values for the parameters of function (1) have been found by fitting $R_D(\nu)$ to the experimental complex permittivity spectra by means of a non-linear least-squares fitting procedure. Values for the conductivity

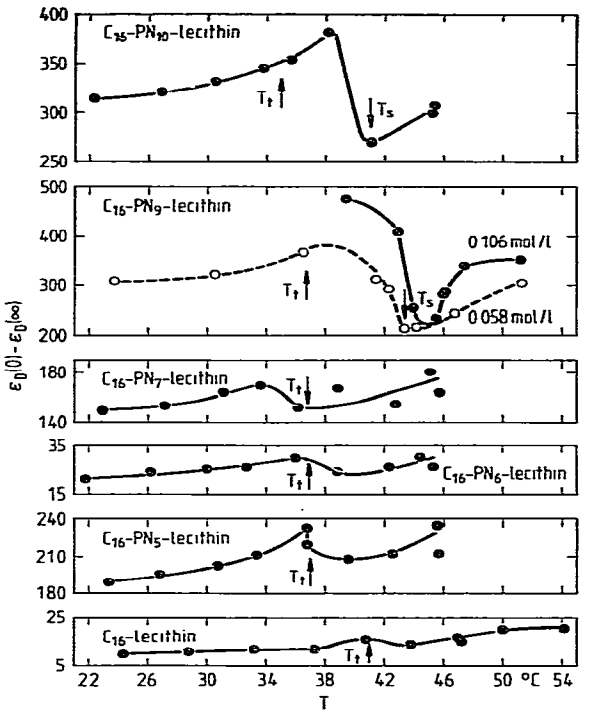

Fig. 3. The relaxation strength $\epsilon_D(0) - \epsilon_D(\infty)$ of the Debye relaxation term (eq. (1)) as a function of temperature T for the various phospholipid solutions.

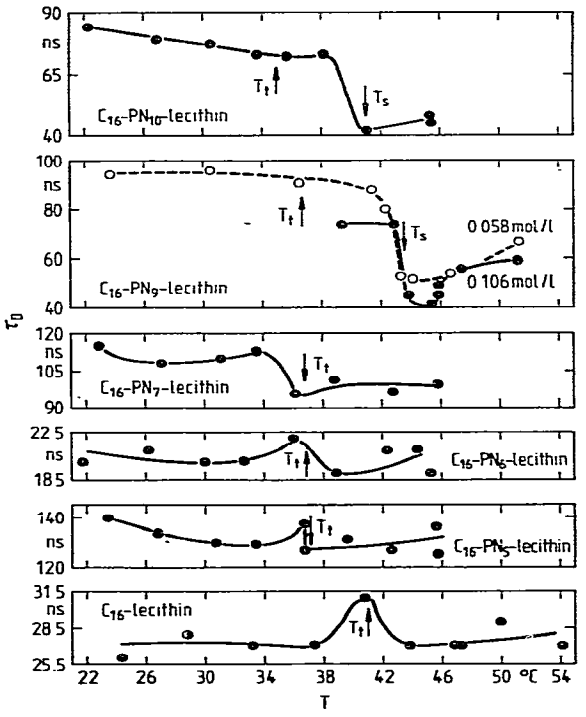

Fig. 4. The Debye relaxation time τ_D of the $R_D(\nu)$ function (eq. 1) plotted versus temperature T for the solutions of C_{16}-lecithin analogues.

σ obtained thereby are given in table 1 for one temperature. The values for the other parameters of the $R_D(\nu)$ function are presented in figs. 2, 3, and 4 and will be discussed below.

4.2. Parameter values of the relaxation spectral function

In fig. 2, a plot of the ratio $\epsilon_D(\infty)/\epsilon_w(0)$ versus temperature T is given for the various solutions. $\epsilon_w(0)$ denotes the static permittivity of pure water at the respective temperature T. The values for $\epsilon_w(0)$ have been taken from fig. 2 in ref. [15] where data from Mason et al. [25], Malmberg et al. [26], and from this laboratory are collected. The $\epsilon_D(\infty)/\epsilon_w(0)$ ratios are around 0.85 reflecting the dilution of the polar solvent and the accompanying action of internal depolarizing electric fields within the solution.

The crystalline/liquid-crystalline phase transition temperature for the various phospholipids, T_t, as obtained at decreasing temperature is also indicated in the plots shown in fig. 2. These T_t values have been determined by light scattering measurements on pure aqueous phospholipid solutions and, additionally, by fluorescence methods using admixed N-phenylnaphthylamine as a label [22]. The $\epsilon_D(\infty)/\epsilon_w(0)$ versus T relations for solutions of C_{16}-lecithin, C_{16}-PN_5-lecithin, C_{16}-PN_6-lecithin, and C_{16}-PN_7-lecithin are either smooth curves or show small changes at T_t. With the C_{16}-PN_{10}-lecithin sample and the 0.106 molar C_{16}-PN_9-lecithin solution, however, a distinct step-like change in the $\epsilon_D(\infty)/\epsilon_w(0)$ values emerges at a temperature T_s well above T_t. As demonstrated by figs. 3 and 4, the other parameters of the Debye relaxation function show a similar behaviour.

The relaxation strength $\epsilon_D(0) - \epsilon_D(\infty)$ is plotted versus temperature T in fig. 3 while the dielectric relaxation time τ_D is displayed in dependence of temperature in fig. 4. These plots clearly show step-like changes in

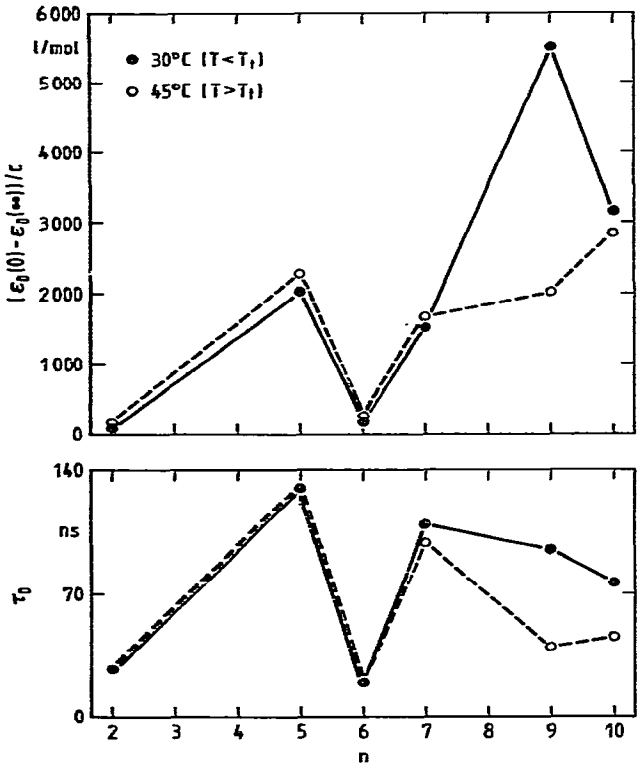

Fig. 5. The molar dielectric increment $(\epsilon_D(0) - \epsilon_D(\infty))/c$ and the relaxation time τ_D of the Debye relaxation term (eq. (1)) plotted versus the number n of methylene groups between the phosphate and the trimethylammonium group for temperatures 30°C and 45°C.

the $\epsilon_D(0) - \epsilon_D(\infty)$ and τ_D values of the C_{16}-PN_9-lecithin and C_{16}-PN_{10}-lecithin solutions at $T_s > T_t$. The relative decrease in the parameter values of these solutions at T_s is substantially greater than the change in the data of the other solutions at T_t. The occurrence of strong step-like changes in the parameters of the Debye relaxation spectral function at T_s may be taken to indicate a transition in the physical state of the bilayer surface formed by dipolar phosphoryl-N,N,N-trimethylnonanolamine and phosphoryl-N,N,N-trimethyldecanolamine groups, respectively.

In fig. 5, the values for the molar dielectric increment $(\epsilon_D(0) - \epsilon_D(\infty))/c$ and the relaxation time τ_D are plotted versus the number n of methylene groups between the anionic phosphate and cationic trimethylammonium group of the phospholipids. The non-monotonic behaviour of the $(\epsilon_D(0) - \epsilon_D(\infty))/c$ versus n and τ_D versus n relations, especially the small values of the relaxation parameters at $n = 6$, suggest the formation of special zwitterion conformations at the surface of the phospholipid bilayers. This aspect will be taken up below in section 6.

5. Theoretical model of the solutions

5.1. Outline of the model

The description of the experimental data by the $R_D(\nu)$ function (eq. (1)) allows for the discussion of general trends in the dielectric properties and the relaxational behaviour of the various phospholipid solutions. To enable statements on the microdynamics of the solutions it is desirable, however, to relate the measured permittivity spectra to molecular parameters. For this purpose internal depolarizing fields and Maxwell–Wagner effects of the dielectrically heterogeneous phospholipid solutions have to be taken into account.

At high water content lecithins form vesicles which consists of a series of concentric, roughly spherically shaped closed bilayers, each separated from the next by a layer of water [27,28]. Aggregation to such vesicles is illustrated, for instance, by electron micrographs of aqueous samples of normal C_{16}-lecithin (C_{16}-PN_2-lecithin) [29] and of the C_{16}-PN_4-lecithin analogue [22]. In our theoretical model of the solutions the multilaminar vesicles are assumed to be exactly spherically shaped as shown in fig. 6, and any distribution in the size parameters is neglected for simplicity. Those parameters include the ratius r_1 of the water containing core, the thickness d_v of the interlamellar solvent layers, and the number N of phospholipid bilayers per aggregate.

In the frequency range under consideration, the solvent is simply characterized by the complex permittivity

$$\epsilon_v(\nu) = \epsilon_w(0) - i2\sigma_v/\nu \,, \qquad (2)$$

where $\epsilon_w(0)$ denotes the temperature dependent static permittivity of water and σ_v the solvent conductivity. The interior of a solute bilayer, essentially consisting of hydrocarbon chains but also including the anionic phosphoryl groups, is treated as a homogeneous dielec-

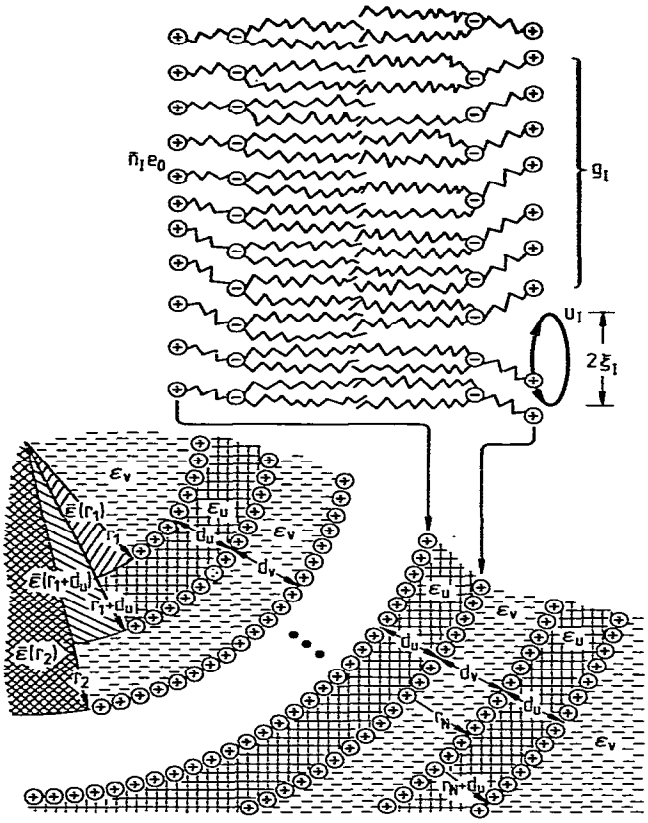

Fig. 6. Sketch of the model for the aqueous solutions of C_{16}-PN_n-lecithin aggregates and illustration of the notations for parameters.

tric of real permittivity $\epsilon_u = 2$. The diffusive motions of the cationic groups relative to the adherent anionic groups are assumed to be restricted to circular paths of radius ξ_I on the bilayer surface, as indicated in fig. 6. The cationic mobility in this motion is denoted by u_I. A cooperativity factor g_I reflects a possible correlation in the orientation of neighbouring phospholipid zwitterions. The factor g_I represents the mean number of zwitterions forming a dielectric domain.

With respect to a harmonically alternating local electric field directed tangentially to the bilayer surface the surface polarizability density of the bilayers is given by the expression

$$\alpha_I(\nu) = \alpha_I(\infty) + \Delta\alpha_I/(1 + 2i\pi\nu\tau_I) . \quad (3)$$

With respect to an radial electric field the surface polarizability is assumed to be zero. The high-frequency part $\alpha_I(\infty)$ which refers to fast polarization mechanisms of the head groups is negligible here. The relaxation time τ_I is given by

$$\tau_I = \xi_I^2/u_I kT , \quad (4)$$

and the relaxation strength $\Delta\alpha_I$ is related to the cooperativity factor g_I according to

$$\Delta\alpha_I = \frac{g_I \bar{n}_I}{2kT} (\xi_I e_0)^2 . \quad (5)$$

k denotes Boltzmann's constant in eqs. (6) and (7) and T the temperature in the Kelvin scale. $\bar{n}_I$ is the mean surface number density of zwitterions and e_0 the elementary charge.

5.2. Relaxation spectral function of the model

Derivation of the relaxation spectral function $R_m(\nu)$ for the aqueous solutions of the C_{16}-PN_n-lecithins, requires the dielectric substitute for the multilaminar vesicles to be established. In proceeding so, advantage can be taken of the presumed globular shape of the vesicles. For a dielectric sphere of radius r, which is covered by a layer of different dielectric material of thickness d and additionally by a thin polarizable layer can be exactly replaced by a homogeneous alternative sphere of radius $r+d$. Thus starting at the core of the vesicle and proceeding layer by layer to the periphery, the multilaminar aggregate can be subsequently replaced by a spherically shaped homogeneous dielectric. Some steps of this substitution procedure are indicated in fig. 6. Application of the corresponding formulae, which have been presented previously [15], results in the complex permittivity $\bar{\epsilon}(\nu) = \bar{\epsilon}(r_N + d_u)$ for the homogeneous globular substitute of the multilaminar vesicles.

The vesicles are embedded in excess solvent. Application of a mixture formula [30] to the solution of a homogeneous isotropic dielectric spheres of complex permittivity $\bar{\epsilon}(\nu)$ in solvent of complex permittivity $\epsilon_v(\nu)$ therefore yields the desired relaxation spectral function

$$R_m(\nu) = \epsilon_v(\nu) + \frac{3v_a\epsilon_v(\nu)(\bar{\epsilon}(\nu) - \epsilon_v(\nu))}{\bar{\epsilon}(\nu) + 2\epsilon_v(\nu) - v_a(\bar{\epsilon}(\nu) - \epsilon_v(\nu))} - i\frac{2\sigma_a}{\nu}, \quad (6)$$

v_a denotes the volume fraction of the water containing multilaminar aggregates, which is related to the volume fraction of the phospholipid, v, according to

$$v_a = v \frac{(r_N + d_u)^3}{\sum_{n=1}^{N}((r_n + d_u)^3 - r_n^3)} . \quad (7)$$

The additional conductivity term in eq. (6) represents conductivity contributions without Maxwell–Wagner relaxational behaviour. Such contributions may be caused, for instance, by drift of the total phospholipid aggregates, if these are electrically charged by absorption of ionic impurities.

5.3. Fixed parameters of the model

Various electron micrographs of multilaminar phospholipid vesicles [27,29,31] show the mean number of bilayers per vesicle to be $N = 6$. This value has thus been used in fitting the relaxation function $R_m(\nu)$ to the experimental permittivity spectra.

The solute bilayer thickness d_u has been estimated for normal C_{16}-lecithin according to

$$d_u(T) = 2M_u/(N_A \rho_u A(T)) , \quad (8)$$

where M_u denotes the solute molar weight N_A is Avogrado's number, $\rho_p \approx 1$ g/ml the density of the phospholipid and $A(T)$ the bilayer surface area occupied by one C_{16}-lecithin molecule. Using surface area data from ref. [32]:

$$A(T < T_t) = 48\ \text{Å}^2 , \quad A(T > T_t) = 70\ \text{Å}^2 , \quad (9)$$

the following values are obtained by eq. (8):

$$d_u(T < T_t) = 52.2\ \text{Å} , \quad d_u(T > T_t) = 36.3\ \text{Å} . \quad (10)$$

Assignment of these values to the solutions of C_{16}-lecithin analogues is justified, if the dipolar head-groups of the analogues – like the phosphorylcholine group of normal lecithin [12] – are aligned almost parallel to the bilayer surface. In this case, the d_u parameter mainly reflects structural properties of the hydrocarbon phase of bilayers. As indicated by the strikingly uniform T_t values (table 1) those properties of the various C_{16}-PN_n-lecithins appear to be very similar.

The thickness of the interlamellar layers d_v has been fixed according to the relation

$$d_v(T) = 2n_w\phi_w/(N_A A(T)) . \quad (11)$$

Herein, n_w denotes the number of water molecules, which per phospholipid molecule are contained in the interlammelar solvent region, and $\phi_w = 18$ ml/mol is the apparent molar volume of water. Following the results of deuteron magnetic resonance measurements [33], which yield $n_w = 23$ for phosphatidylcholines,

$$d_v(T < T_t) = 28.7\ \text{Å} , \quad d_v(T > T_t) = 19.7\ \text{Å} , \quad (12)$$

is obtained.

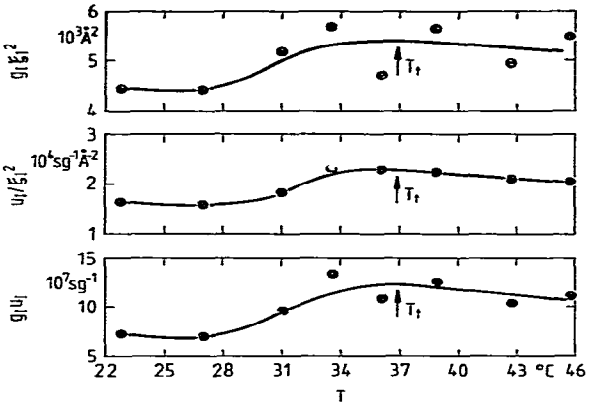

Fig. 7. The quantities $g_I\xi_I^2$, u_I/ξ_I^2, and $g_I u_I$ plotted versus temperature T for the C_{16}-PN_7-lecithin solution. g_I and u_I denote the cooperativity factor and the mobility in the diffusive motion of the trimethylammonium group, resp. ξ_I is the radius of the circular path of this motion.

5.4. Adjustable parameters of the relaxation spectral function

To find the values for the unknown parameters, the relaxation spectral function $R_m(\nu)$ has been fitted to the experimental $\epsilon(\nu)$ data with the aid of a non-linear least-squares fitting procedure. These are: the radius r_1 of the vesicle core, which according to eq. (7) is related to the volume fraction v_a of the vesicles, the relaxation strength $\Delta\alpha_I$ and the relaxation time τ_I of the surface polarizability density, and a less interesting conductivity parameter. It turned out that one of the conductivity parameters could be abandoned. The microscopic quantities r_1, $\Delta\alpha_I$, and τ_I reflect the parameters $\epsilon_D(\infty)$, $\epsilon_D(0) - \epsilon_D(\infty)$, and τ_D, respectively, of the phenomenologically introduced $R_D(\nu)$ function.

The values of the variance obtained in the least-squares fits of the model relaxation function $R_m(\nu)$ are similar to those found in the fits of the Debye relaxation function $R_D(\nu)$.

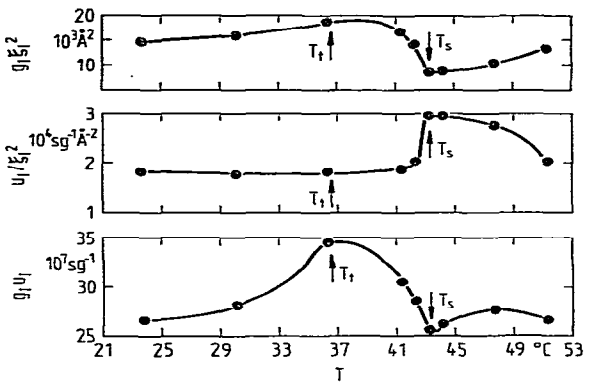

Fig. 8. The quantities $g_I\xi_I^2$, u_I/ξ_I^2, and $g_I u_I$ plotted versus temperature T for the 0.058 molar C_{16}-PN_9-lecithin solution. g_I and u_I denote the cooperativity factor and the mobility in the diffusive motion of the trimethylammonium group, resp. ξ_I is the radius of the circular path of this motion.

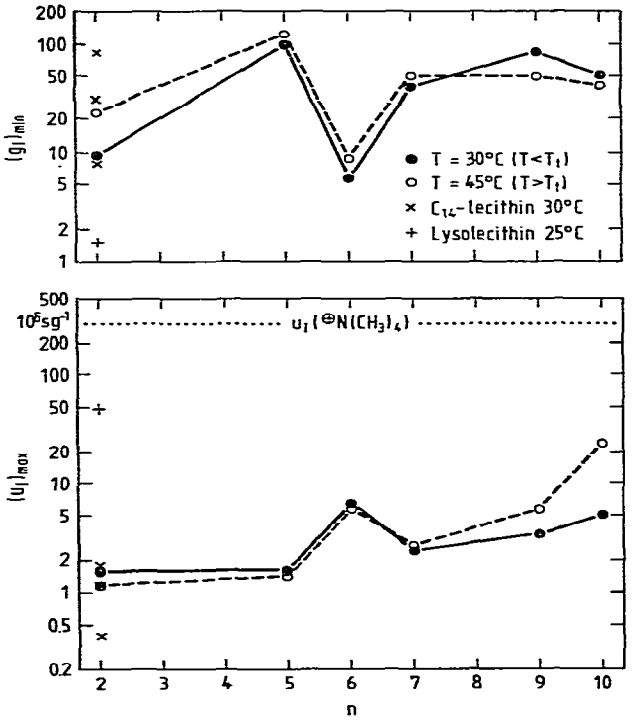

Fig. 9. Limiting values for the cooperativity factor, $(g_I)_{min}$, and the mobility, $(u_I)_{max}$, of the diffusive motion of the trimethylammonium group in aqueous solutions of C_{16}-PN_n-lecithins plotted versus n at 30°C and 45°C. Data for different C_{14}-lecithin solutions at 30°C [15] and for a lysolecithin solution at 25°C [16] are presented for comparison. u_I value for the free $^+N(CH_3)_4$ ion in H_2O as calculated from the limiting equivalent conductivity at 25°C [35] is also included.

6. Discussion

6.1. Parameters of head group microdynamics in dependence of temperature

The dependence of the quantities $g_I\xi_I^2$, u_I/ξ_I^2, and $g_I u_I$ on the temperature T is displayed in fig. 7 for the C_{16}-PN_7-lecithin solution and in fig. 8 for the 0.058 molar C_{16}-PN_9-lecithin solution. The $g_I\xi_I^2$, u_I/ξ_I^2, and $g_I u_I$ data have been calculated from the $\Delta\alpha_I$ and τ_I values according to eqs. (4) and (5).

With respect to the characteristics in the variation of solution properties with temperature, the plots shown in fig. 7 are typical examples for the C_{16}-PN_n-lecithin solutions with $n \leqslant 7$.

The quantities derived on the basis of our model show either rather smooth or even no changes at the crystalline/liquid-crystalline phase transition temperature T_t. This finding is in accordance with the behaviour of the parameter values of the phenomenologically introduced $R_D(\nu)$ function (eq. (1)).

As illustrated by the example presented in fig. 8, solutions of C_{16}-lecithin analogues with very extended zwitterionic head-groups ($n = 9$ and 10) show different properties. A distinct step-like change in the molecular quantities $g_I\xi_I^2$, u_I/ξ_I^2, and $g_I u_I$ at a temperature $T_s > T_t$ emerges with the latter solutions. The treatment of the experimental data in terms of the solution model thus supports our previous assumption (sect. 4.2) of a transition in the physical state of C_{16}-PN_9-lecithin and C_{16}-PN_{10}-lecithin bilayers at a temperature T_s well above the temperature T_t of the main transition.

6.2. Parameter head group microdynamics as a function of zwitterion length

The fact, that the unknown radius ξ_I of the circular path in the diffusive motion of the trimethylammonium group is included in the relations between parameters g_I and $\Delta\alpha_I$(eq. (5)) and u_I and τ_I (eq. (4)), respectively, prevents the cooperativity factor g_I and the mobility u_I to be explicitely calculated from the $\Delta\alpha_I$ and τ_I data. However, limiting values $(g_I)_{min}$ and $(u_I)_{max}$

can be calculated by use of the maximum radius $(\xi_I)_{max}$ for the circular path. Assuming tangential zwitterion orientation with respect to the bilayer surface and using the maximum anion-cation center distance for the zwitterionic head-group, maximum ξ_I values have been estimated according to

$$(\xi_I)_{max} = (n+2) \times 1.265\,\text{Å} . \qquad (13)$$

The resulting $(g_I)_{min}$ and $(u_I)_{max}$ values are presented in dependence of n for $T = 30°C$ and $T = 45°C$ in fig. 9. Corresponding data for some aqueous C_{14}-lecithin solutions at 30°C [15] and for a micellar lysolecithin solution at 25°C [16] are also included in the diagrams. The C_{14}-lecithin solutions with a solute concentration at around $c = 0.14$ mol/l differ from one another by the core radius r_1 of the multilaminar aggregates. In addition to the data for vesicles and micelles, the mobility u_I of the free tetramethylammonium ion at 25°C is indicated in the $(u_I)_{max}$ versus n plot.

The minimum values of the cooperativity factor for the C_{16}-PN_n-lecithins shown in fig. 9 vary between $(g_I)_{min} \approx 6$ ($n = 6$, 30°C) and $(g_I)_{min} \approx 118$ ($n = 5$, 45°C). The $(g_I)_{min}$ values of the C_{16}-PN_n-lecithin bilayer samples are thus substantially greater than that of an aqueous solution of lysolecithin micelles ($(g_I)_{min} = 1.6$ [16]) and those of other micellar aqueous solutions of zwitterionic surfactants, where always $(g_I)_{min}$ values in the order of 10^0 have been found [16,34]. Quite remarkably, however, the range of variation in the $(g_I)_{min}$ values of solutions of the various C_{16}-PN_n-lecithins nearly coincides with the range, in which minimum cooperativity values of different aqueous C_{14}-lecithin solutions are found.

The $(u_I)_{max}$ values of the bilayer forming phospholipids are substantially smaller than the $(u_I)_{max}$ value for lysolecithin which on the other hand is only one sixth of the mobility of the free tetramethylammonium ion. Above the temperature T_s, however, the mobility of the trimethylammonium group in C_{16}-PN_{10}-lecithin bilayers ($(u_I)_{max} \approx 2.3 \times 10^7$ s/g at 45°C) is only by a factor of about 2 smaller than that in lysolecithin micelles ($(u_I)_{max} = 5 \times 10^7$ s/g at 25°C [16]). The $(u_I)_{max}$ values for the solution of normal C_{16}-lecithin (1.5×10^6 s/g at 30°C, 1.2×10^6 s/g at 45°C) are within the range of data obtained for different solutions of C_{14}-lecithin ($(u_I)_{max} = 0.4 - 1.7 \times 10^6$ s/g at 30°C [15]). These maximum values for the mobility of the cationic head-group at the surface of a bilayer are of

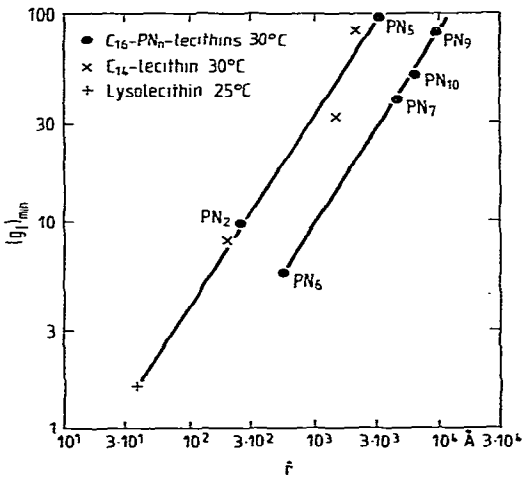

Fig. 10. Dependence of the minimum values of the correlation factor, $(g_I)_{min}$, on the size parameter $\hat{r}$ (eqs. (14), (15)) of the multimolecular phospholipid aggregates for solutions of C_{16}-PN_n-lecithins and C_{14}-lecithin [15] at 30°C and for a lysolecithin solution [16] at 25°C.

the same order of magnitude as the value [36] for the mobility of the total lipid molecule in its lateral diffusive motion.

The fact, that nearly the same range of $(g_I)_{min}$ values is obtained in different aqueous solutions of C_{14}-lecithin as in solutions of various C_{16}-PN_n-lecithins with $n = 2, 5, 6, 7, 9$, and 10 attracts attention. There seems to exist a relation between the cooperativity of the dipolar phospholipid head-groups and the curvature of the multimolecular aggregates. Therefore, $(g_I)_{min}$ values for the various phospholipid solutions, including that of lysolecithin, are plotted versus a mean radius of curvature $\hat{r}$ of the multimolecular aggregates in fig. 10. Values for this mean radius of curvature have been calculated according to

$$\hat{r} = (r_1 + r_N + d_u)/2 , \qquad (14)$$

in the case of those phospholipids, which form multilaminar aggregates, and according to

$$\hat{r} = (a + 2b)/3 , \qquad (15)$$

for the lysolecithin solution. Parameter a denotes the half-axis of revolution and b the equatorial radius of the lysolecithin micelles, which are assumed to be oblate ellipsoids of revolution in shape [16].

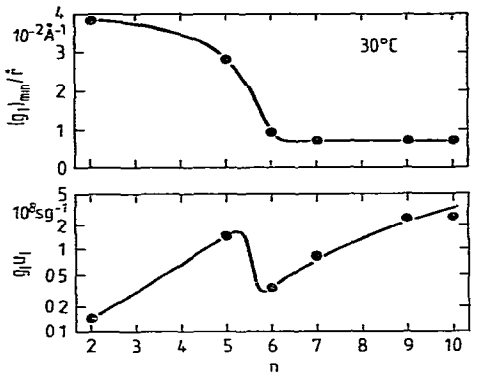

Fig. 11. The ratio $(g_I)_{min}/\tilde{r}$ and the product $g_I u_I$ as a function of the number n of methylene groups between the anionic phosphate and cationic trimethylammonium group for the C_{16}-PN_n-lecithins at 30°C.

As illustrated by the data for lysolecithin, C_{14}-lecithin, C_{16}-lecithin, and C_{16}-PN_5-lecithin,shown in the bilogarithmic plot of fig. 10, there is a marked tendency of the $(g_I)_{min}$ values to increase with increasing $\tilde{r}$ values. Obviously, rather extended dielectric domains are formed in large vesicles with only slightly curved bilayers while in small vesicles and in micelles the strong curvature limits the size of the cooperative units. As has been shown by electron spin resonance spectra of multibilayer and single-bilayer vesicles [37] similar characteristics emerge in the cooperativity of the crystalline/liquid-crystalline phospholipid phase transition.

The cooperativity factor $(g_I)_{min}$ of the C_{16}-PN_n-lecithins with $n \geqslant 6$ also increases with increasing radius of curvature $\tilde{r}$. At given $\tilde{r}$, however, the $(g_I)_{min}$ values of the latter lecithins are substantially smaller than expected from the $(g_I)_{min}$ versus $\tilde{r}$ relation for the normal lecithins and the C_{16}-PN_5-lecithin. The considerable change in the head group behaviour when going from small zwitterions ($n \leqslant 5$) to longer ones ($n \geqslant 6$) is also demonstrated by fig. 11 where the quantities $(g_I)_{min}/\tilde{r}$ and $g_I u_I$ are displayed in dependence of the number n of methylene groups between the phosphate and the trimethylammonium group. The ratio $(g_I)_{min}/\tilde{r}$ is used to compare independently of the effect of bilayer curvature on the size of the dielectric domains the cooperative behaviour of the different lecithin head groups. The product $g_I u_I$ is presented in fig. 11 in order to show a quantity, which does not explicitely depend on the parameter ξ_I. A sharp decrease in the $(g_I)_{min}/\tilde{r}$ and $g_I u_I$ data when going from $n = 5$ to $n = 6$ reflects a strong change in the head group conformations. Ring formation of the long and flexible zwitterions may be suggested. But a decrease in the orientation correlation factor may be also due to a bilayer surface structure in which to a certain degree antiparallel alignment of neighbouring dipole moments is favoured.

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