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THE DIELECTRIC PERMITTIVITY SPECTRUM OF AQUEOUS COLLOIDAL PHOSPHOLIPID SOLUTIONS BETWEEN 1 kHz AND 60 GHz

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The dielectric permittivity spectrum between 1 kHz and 60 GHz of aqueous colloidal solutions of predominantly zwitterionic phospholipids is presented from results of previous and recent measurements. It shows three dispersion/loss regions around 22 GHz, 80 MHz and below 40 MHz (30°C) which are attributed to rotational diffusion of the water molecules and of the zwitterionic phosphorylcholine groups, and to limited translational diffusion of ionic lipid molecules and/or its counterions, respectively. Merely a few mole percent of ionic lipids cause comparatively large dielectric dispersion. Ignoring the fact that such impurities may be present in zwitterionic phospholipid compounds, which have not been especially purified, this has led to misinterpretation of the dielectric spectrum in the past. An approximate quantitative description of the measured spectra is given for vesicle solutions with only very small additional low-molecular-weight salt content. It reproduces the sensitive dependence of the ionic lipid-induced dielectric dispersion (step height and frequency) on various parameters: phospholipid vesicle size, ionic lipid content, as well as the self-diffusion coefficient of the ionic lipid molecules and of its counterions, moving within the phospholipid bilayers or on their surface, respectively.

1. Introduction

This article is concerned with aqueous solutions of mostly zwitterionic phospholipids of the type 1,2-diacyl- or 1,2-dialkyl-*sn*-glycero-3-phosphorylcholine $[H(CH_2)_nX]_2H(OCH)_3\text{-}H\bar{P}O_3(CH_2)_2\dot{N}(CH_3)_3$, where $n = 13$, 15, 17 and X represents –CO– (ester-lecithin) or $-CH_2-$ (ether-lecithin). The phospholipid concentrations are below 0.3 mol/l (< 23% by wt.). In these solutions the phospholipid molecules are aggregated in bilayers with the zwitterionic phosphorylcholine groups covering the bilayer surfaces. The phospholipid bilayers form nearly spherical vesicles. The vesicles contain a water core surrounded either by a single lipid bilayer or by several bilayers, each separated from the next by a layer of water [1].

In the dielectric measurements the liquid samples have been exposed to weak electric fields $\boldsymbol{E}(t)$ ($\lesssim 1$ V/cm) varying with time t either periodically ($\boldsymbol{E}(t) = \hat{\boldsymbol{E}} \exp(i2\pi\nu t)$) with frequency ν, for which many discrete values have been separately chosen between 1 kHz and 60 GHz, or by a single step pulse ($\boldsymbol{E}(t < 0; > 0) = 0; \hat{\boldsymbol{E}}$) with a continuous frequency spectrum which has been evaluated up to about 1 GHz. The dielectric polarization (dipole moment density) $\boldsymbol{P}(t)$ induced by $\boldsymbol{E}(t)$ has been indirectly recorded using several techniques. The Fourier transforms of $\boldsymbol{P}(t)$ and $\boldsymbol{E}(t)$ are connected by the frequency-dependent (relative) complex dielectric permittivity $\epsilon(\nu) = \epsilon'(\nu) - i\epsilon''_{tot}(\nu)$ according to $\boldsymbol{P}(\nu) = [\epsilon(\nu) - 1]\boldsymbol{E}(\nu)/4\pi$ if the samples are placed between metallic electrodes or within electromagnetic wave metallic transmission lines in a manner such that no macroscopic depolarizing

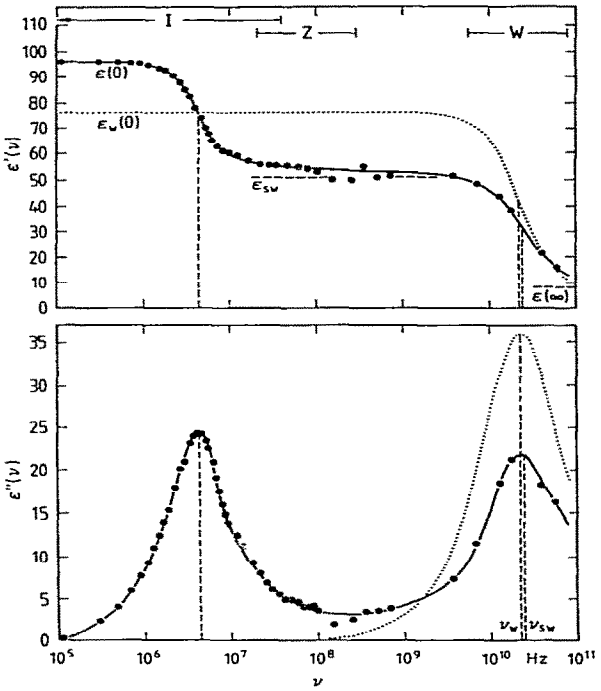

Fig. 1. Spectrum of the real part $\epsilon'(\nu)$ and of the imaginary part $\epsilon''(\nu)$ of the complex dielectric permittivity of pure water (· · · · · ·) and of an 0.139 M aqueous C_{14}-ester-lecithin solution (———) at 32°C [6].

electric fields arise. In aqueous solutions $\epsilon'(\nu)$ at frequencies of interest here, $\nu \leq 60$ GHz, is considerably larger than 2. Thus $\epsilon(\nu)$ mainly reflects dielectric polarizability enabled by thermal motion of any electric charges (bound or free) along limited paths. If, additionally free electric charges are present, diffusing around without limits, the resulting specific electric d.c. conductivity σ of the liquid samples contributes to the imginary part of the permittivity according to $\epsilon''_{tot}(\nu) = \epsilon''(\nu) + 2\sigma/\nu$ (σ in $s^{-1} \hat{=} 1.113 \times 10^{-10}\ \Omega^{-1}\ m^{-1}$).

Dielectric permittivity spectra, $\epsilon'(\nu)$ and $\epsilon''(\nu)$, of aqueous colloidal phospholipid solutions have been reported in a number of papers [2–10,12]. Two of these works [5,12] do not refer to vesicle solutions but to plain phospholipid bilayer configurations with phospholipid concentrations of considerably more than 23% by weight. The widest spectrum published so far is presented in ref. 6 and is reproduced here in fig. 1. In order to treat the peculiarities of this spectrum it is divided into three regions, 'W', 'Z' and 'I', which will be explained as being attributable to the water, zwitterions and ions in the solutions, respectively.

2. Spectral region W (6 GHz $\leq \nu \leq$ 80 GHz)

The W region covers the frequency range between about 6 and 80 GHz (frequencies at which $\epsilon'' \approx \frac{1}{2}\epsilon''_{max}$ at 30°C) within which pure water shows large dispersion ($d\epsilon'/d\nu < 0$) and high loss (peak of ϵ'') as shown by the dotted $\epsilon'(\nu)$ and $\epsilon''(\nu)$ curves in fig. 1. This behavior is due to the diffuse thermal rotational motion of the water molecules around axes perpendicular to the molecular permanent electric dipole moment. The water in the phospholipid solutions also causes dispersion and loss in the W region as shown, for example, by the $\epsilon'(\nu)$ and $\epsilon''(\nu)$ curves of the 0.139 M C_{14}-lecithin solution in fig. 1. However, the asymptotic low-frequency ϵ' value ϵ_{sw} is considerably smaller than that of pure water, $\epsilon_w(0) \equiv \epsilon_{w0}$, and the relaxation frequency ν_{sw} (at which $d\epsilon''/d\nu = 0$) is slightly larger than that of pure water, ν_w.

The difference $\epsilon_{w0} - \epsilon_{sw}$ is not only due to dilution of the water by the phospholipid but is also especially a consequence of depolarizing electric fields which are produced by dielectric polari-

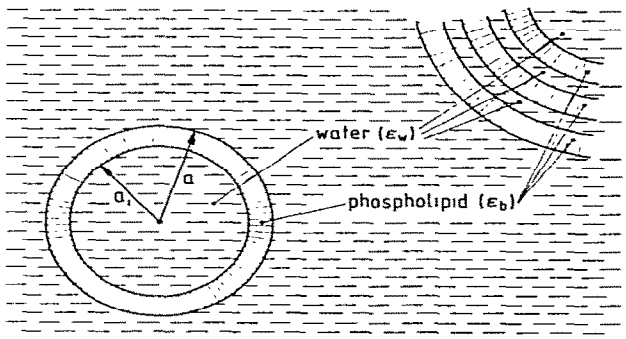

Fig. 2. Model of aqueous colloidal phospholipid solutions for the W spectral region. The water and phospholipid regions are substituted by continuous dielectrics with the permittivity ϵ_w of pure water and the high-frequency permittivity ϵ_b of dry phospholipid, respectively.

zation within the core and the interlamellar water of the phospholipid vesicles. The model of the phospholipid solutions shown in fig. 2 can fully account for the experimental $\epsilon_{w0} - \epsilon_{sw}$ values by reasonable choice of the vesicle dimensions. No influence of specific phospholipid-water interactions has to be introduced nor can it be significantly extracted from the evaluation procedure. In the case of single bilayer vesicles, $\epsilon_{w0} - \epsilon_{sw}$ is a reliable measure of the vesicle size. The Wagner mixture formula for the dielectric permittivity of heterogeneous media [18], corrected by us for large vesicle volume fractions according to the Bruggeman mixture formula [18], yields

$$\frac{\epsilon_{w0} - \epsilon_{sw}}{\epsilon_{w0}} = 3v_v(\epsilon_{w0} - \epsilon_{2\infty})\frac{(2\epsilon_{w0} + \epsilon_{2\infty}) - v_v(\epsilon_{w0} - \epsilon_{2\infty})}{(2\epsilon_{w0} + \epsilon_{2\infty})^2 - v_v(\epsilon_{w0} - \epsilon_{2\infty})^2} \tag{1}$$

with the vesicle substitute homogeneous sphere permittivity

$$\epsilon_{2\infty} = \epsilon_b \frac{2\left[1-\left(\frac{a_i}{a}\right)^3\right]\epsilon_b + \left[1+2\left(\frac{a_i}{a}\right)^3\right]\epsilon_{w0}}{\left[2+\left(\frac{a_i}{a}\right)^3\right]\epsilon_b + \left[1-\left(\frac{a_i}{a}\right)^3\right]\epsilon_{w0}} \tag{2}$$

where ϵ_{w0} is the static permittivity of pure water, ϵ_{sw} the asymptotic low-frequency permittivity as extrapolated from the W spectral region, v_v the volume fraction of the vesicles, ϵ_b the phospholipid bilayer substitute permittivity due to hydrocarbon electronic polarizability and zwitterion libration polarizability ($\epsilon_b \approx 6$), and a and a_i the outer and inner vesicle radius, respectively.

The difference $\nu_{sw} - \nu_w$ is expected to contain a distinct positive contribution also due to the influence of depolarizing electric fields on the intravesicle water. This had indeed been found in previous studies [4,6,10] in the case of multi-bilayer vesicles. However, in the recently studied case of single-bilayer vesicles [23], this contribution seems to be partly compensated by an additional negative contribution due to specific phospholipid-water interactions.

3. Spectral region Z (20 MHz $\leq \nu \leq$ 300 MHz)

The Z region covers the frequency range between about 20 and 300 MHz (frequencies at

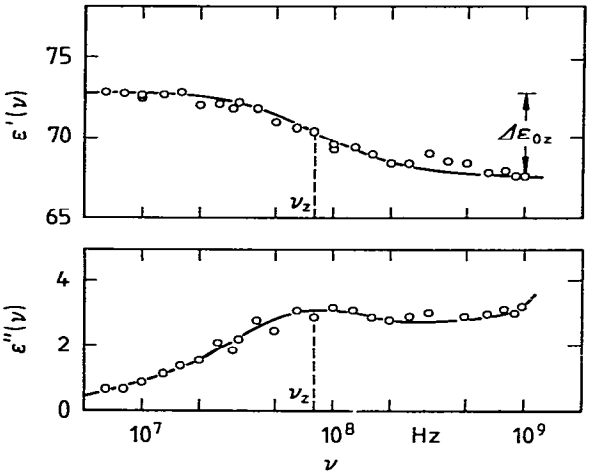

Fig. 3. Z region spectrum of the real part $\epsilon'(\nu)$ and of the imaginary part $\epsilon''(\nu)$ of the complex dielectric permittivity of a 0.09 M aqueous solution of C_{14}-ether-lecithin at 30°C [11]. ϵ'' at $\nu > \nu_z$ contains a contribution from the W spectrum.

which $\epsilon'' \approx \frac{1}{2}\epsilon''_{max}$ at 30°C). It seems that from dielectric spectroscopy on aqueous phospholipid single-walled vesicle solutions in this frequency range a discrete dispersion step has been resolved firstly by Redwood et al. [3]. The $\epsilon'(\nu)$ data from ref. 6 (fig. 1 of the present paper) show weak dispersion but the data scattering above 100 MHz prevents a quantitative evaluation. Recent more precise measurements reported in refs. 11 and 23 clearly show a discrete weak dispersion/loss region with peaks of $-d\epsilon'/d\nu$ and ϵ'' at about 80 MHz (30°C) (fig. 3). There are strong indications that this dispersion/loss peak can be attributed, deviating from the interpretation in ref. 3, to the diffuse thermal rotational motion of the zwitterionic phosphorylcholine groups around axes perpendicular to their permanent electric dipole moment. The Z relaxation frequency ν_z in the C_{14}-lecithin vesicle solutions lies between that in lysolecithin micelle solutions (200 MHz, 30°C [11]) and that in fully hydrated plain C_{16}-lecithin multi-bilayer stacks (20 MHz, 35°C [12]).

The rotational diffusion of the zwitterionic phosphorylcholine groups at the phospholipid bilayer/water interfaces imparts a fluctuating electric dipole moment to the vesicles. The dipole

moment due to the inner zwitterions is kept small by depolarizing electric fields and will thus be neglected in the following approximate analytical description of the Z dielectric incremental spectrum. Transforming the vesicle surface polarizability due to zwitterion rotational diffusion into a volume polarizability of a vesicle substitute homogeneous sphere and applying the Wagner mixture formula [18] to the suspension of these spheres in water yields

$$\Delta\epsilon_z(\nu) = \frac{\Delta\epsilon_{0z}}{1 + i2\pi\nu\tau_z} \quad (\nu \ll \nu_w) \tag{3}$$

with (in the case of negligible low-molecular-weight salt content)

$$\Delta\epsilon_{0z} = \frac{9v_2\Delta\epsilon_{2z}}{(2+v_2)^2\left(1+\frac{1-v_2}{2+v_2}\cdot\frac{\epsilon_{2\infty}}{\epsilon_{w0}}\right)\left[1+\frac{1-v_2}{2+v_2}\left(\frac{\epsilon_{2\infty}+\Delta\epsilon_{2z}}{\epsilon_{w0}}\right)\right]} \tag{4}$$

$$\Delta\epsilon_{2z} = \frac{4\pi n_z e_0^2 \xi^2 g}{akT} \tag{5}$$

$$\tau_z = \frac{1}{2\pi\nu_z} = \frac{1+\frac{1-v_2}{2+v_2}\cdot\frac{\epsilon_{2\infty}}{\epsilon_{w0}}}{1+\frac{1-v_2}{2+v_2}\left(\frac{\epsilon_{2\infty}+\Delta\epsilon_{2z}}{\epsilon_{w0}}\right)}\tau_{2z} \tag{6}$$

$$\tau_{2z} = \frac{1}{D_z} \tag{7}$$

where $\epsilon_{2\infty}$ is the same term as that in eq. 2 in the case of single-bilayer vesicles; n_z the number of zwitterions per vesicle surface unit area, e_0 the elementary electric charge, ξ the distance between charge centers in the zwitterion, g the zwitterion orientation correlation and/or local field correction factor; k Boltzmann's constant, T the absolute temperature; and D_z the zwitterion rotational diffusion coefficient. For C_{14}-lecithin vesicle solutions as well as for lysolecithin micelle solutions $g = 2$ has been derived from the experimental $\Delta\epsilon_{0z}$ values with $\xi = 5$ Å [11]. The local field correction factor must be greater than unity but is not known exactly for the quasi-two-dimensional arrangement of the zwitterions (two-dimensional electrostatics yields ≈ 2 [25]). So a possible contribution to g from zwitterion orientation correlation cannot be extracted but can be stated to be of unsubstantial magnitude.

4. Spectral region I ($\nu < 40$ MHz)

4.1. Results of measurements

The I region covers the frequency range below about 40 MHz (30°C). Large dielectric dispersion and loss at those frequencies have been reported previously in refs. 2, 3, 5–10 and 12. With respect to the spectra presented in refs. 6–10, recent investigations in this laboratory have shown that in applying the time-domain spectroscopy technique truncation errors and lack of sensitivity at long times might have distorted the shape of the low-frequency section of some of the spectra ($\nu < 3$ MHz in fig. 1) so that $d\epsilon'/d\nu \approx 0$ for $\nu < 1$ MHz might be questionable.

In refs. 6–10 the dispersion/loss region below about 40 MHz had been attributed to the diffuse thermal rotational motion of the zwitterionic phosphorylcholine groups ascribing to it long-range spatial orientation correlation. This interpretation has to be corrected because of two new findings: the Z dispersion/loss region around 80 MHz mentioned in section 3, and the sensitive dependence of the I spectrum parameters on the presence of certain ionic admixtures within the phospholipid solutions [13] as will be described below.

These papers [6–10] refer to synthetic zwitterionic phospholipid samples which had not been especially purified with respect to ionic impurities. All phosphatidylcholine samples originated from Koch-Light Laboratories Ltd. (Colnbrook, U.K.) and were of purity grade 'pure' (95% by wt.; batch no. 65508). In recent work [13] phospholipid samples from several sources have been investigated, and no dispersion in the $\epsilon'(\nu)$ spectra has been found below 20 MHz down to 5 kHz (30°C) with ether-lecithin samples if these are especially purified in methanolic solution by an ion exchanger. Also, Redwood et al. [3], with a very carefully prepared phosphatidylcholine vesicle suspension, measured no dielectric dispersion below about 10 MHz down to 0.1 MHz (17°C). However, in both investigations dielectric dispersion at those frequencies did appear after a few mole percent of fatty acid or of its salts had been admixed to the phospholipids. An example of such results is reproduced in fig. 4. For an ester-lecithin sample in

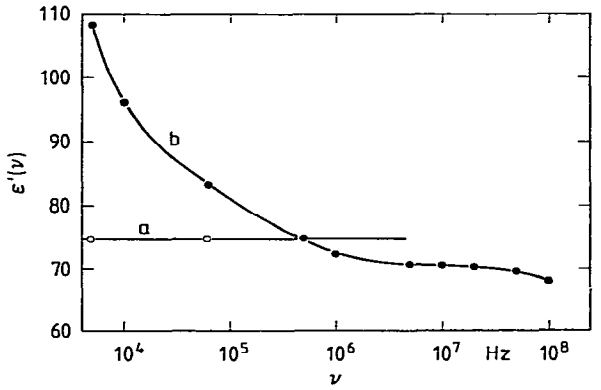

Fig. 4. I region spectra of the real part $\epsilon'(\nu)$ of the complex dielectric permittivity of 0.1 M aqueous solutions of especially purified C_{14}-ether-lecithin (a) without and (b) with admixed 1 mol% potassium salt of myristic acid at 30°C [13].

ref. 13 dielectric dispersion is reported, despite purification. Here, small amounts of fatty acid could have been formed by hydrolysis of ester bonds during purification in the ion exchanger and/or during dispersing the lecithin by sonication.

The presence of fatty acid or its salts and of salt of low molecular weight (e.g., KCl, NaCl) within phospholipid samples favors dissolving of the phospholipid and induces irreversible growth of the phospholipid aggregates if the temperature, after being high during the preparation procedure, is reduced below the liquid-crystalline/crystalline phase transition temperature [14]. This growth of the phospholipid aggregates (by coagulation or fusion of vesicles) causes $|d\epsilon'/d\nu|$ to increase strongly as shown by an example in fig. 5. At the lowest frequencies used in ref. 13 ϵ' attains values up to several hundreds after the phospholipid aggregate conversion, even in dilute solutions (e.g., 10^{-3} M). Addition of salts of low molecular weight shifts the region of dielectric dispersion towards higher frequencies. In all cases of spectra with distinct dielectric dispersion below 10 MHz the measurements in ref. 13 never gave $d\epsilon'/d\nu \cong 0$ at the lowest frequencies used (often 5 kHz, once 0.1 kHz). Therefore, only upper limits of mean relaxation frequencies and lower limits of dispersion step heights can be estimated from these spectra. The

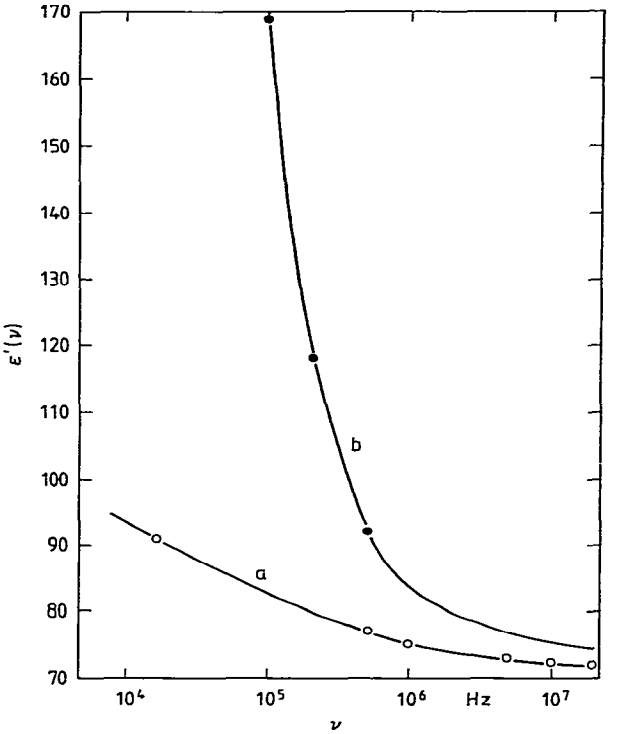

Fig. 5. I region spectra of the real part $\epsilon'(\nu)$ of the complex dielectric permittivity of 0.1 M aqueous solutions of especially purified C_{14}-ether-lecithin with admixed 1 mol% potassium salt of myristic acid and 0.5 mol% potassium chloride at 30°C (a) before and (b) after aggregate conversion [13].

former are found to lie below 0.1 MHz (25–30°C) in all cases in which the measurements have been performed down to lower freuquencies (solutions with negligible content of low-molecular-weight salt).

4.2. *Interpretation of the I spectra*

Distinct dispersion in the I spectral region is clearly caused by the presence of relatively few molecules of fatty acid or its salts (more general: ionic lipids). Most of the fatty acid molecules are integrated in the phospholipid bilayers with the anionic head group being located at the bilayer/water interface. The corresponding countercations are more or less distributed within the solvent.

Thus, the dielectric dispersion must be due to diffusion of fatty acid anions (more general: charged lipid molecules) or/and their counterions along limited paths. Like phospholipid molecules, the fatty acid molecules perform mostly lateral diffusion in the bilayers [15] so that diffusion of the anionic head groups is restricted to the spherically closed bilayer surfaces of the phospholipid aggregates.

Whether the diffusion paths of the counterions are also limited depends on the extent of the counterion atmospheres of the phospholipid aggregates. The decisive quantity in this respect is the counterionic thermal energy/potential energy ratio

$$\frac{\epsilon_{w0} kT}{4\pi n_- e_0^2 a}$$

if the solutions contain distinctly less low-molecular-weight salt than ionic lipid (a = outer aggregate radius, n_- = number of outer singly charged anionic groups per aggregate surface unit area). If

$$\frac{\epsilon_{w0} kT}{4\pi n_- e_0^2 a} \gtrapprox 1 \tag{8}$$

the counterion atmospheres are spread out widely, and consequently the atmospheres of different aggregates overlap. The counterion diffusion paths are then unlimited. This is expected to be the case in fresh (sonicated) phospholipid solutions with small content of ionic lipid ($\lesssim 1$ mol% monovalent) which have been prevented from aggregate conversion by avoiding temperatures below the liquid-crystalline/crystalline phase transition temperature T_t so that predominantly small vesicles are present (e.g., $a \approx 130$ Å).

If

$$\frac{\epsilon_{w0} kT}{4\pi n_- e_0^2 a} \ll 1 \tag{9}$$

the counterion atmosphere is condensed into a thin layer of approximate mean radius

$$a_- = a\left(1 - \frac{\epsilon_{w0} kT}{4\pi n_- e_0^2 a}\right)^{-1} \tag{10}$$

on the aggregate surface. The counterion diffusion is thus restricted to that spherically closed layer. This is expected to be the case in phospholipid solutions of small ionic lipid content (≈ 1 mol% monovalent) which have undergone irreversible aggregate conversion by cooling below T_t so that large vesicles (e.g., $a \approx 600$ Å) or multi-bilayer aggregates are present, or also in solutions with moderate ionic lipid content ($\gtrsim 7$ mol% monovalent) if only small vesicles are present.

4.2.1. Solutions of small vesicles with small content of ionic lipid

In fresh (sonicated) phospholipid solutions with a small content of fatty acid salt or other ionic lipid the solute aggregates are predominantly small single-bilayer vesicles if the temperature is always kept above T_t. For C_{14}-ester-lecithin, e.g., the vesicle has a typical outer radius value of $a \approx 130$ Å and contains about 3000 lecithin molecules [16]. With admixed 1 mol% fatty acid salt, for instance, the number of its outer anionic groups is $4\pi n_- a^2 \approx 20$. Those values of a and n_- together with $T = 303$ K and $\epsilon_{w0} = 76.6$ yield $\epsilon_{w0} kT/4\pi n_- e_0^2 a \approx 0.9$ which indicates wide counterion distribution and thus a lack of dielectric polarizability due to restricted counterion diffusion. The counterions give rise to the specific electric conductivity σ_l of the solvent only.

The diffusion of the fatty acid anionic head groups along the phospholipid bilayer/water interfaces imparts a fluctuating electric dipole moment to the vesicles. The dipole moment due to the inner anionic groups is kept small by depolarizing electric fields and will thus be neglected in the following approximate consideration.

A derivation similar to that of Schwarz [17] yields the following dielectric incremental spectrum due to the outer anionic group diffusion:

$$\Delta\epsilon_-(\nu) = \frac{\Delta\epsilon_{0-}}{1 + i2\pi\nu\tau_-} \quad (\nu \ll \nu_z) \tag{11}$$

with (in the case of negligible low-molecular-weight salt content)

$$\Delta\epsilon_{0-} = \frac{9 v_v \Delta\epsilon_{2-}}{(2+v_v)^2\left(1 + \frac{1-v_v}{2+v_v}\cdot\frac{\bar{\epsilon}_{2\infty}}{\epsilon_{w0}}\right)\left[1 + \frac{1-v_v}{2+v_v}\left(\frac{\bar{\epsilon}_{2\infty} + \Delta\epsilon_{2-}}{\epsilon_{w0}}\right)\right]} \tag{12}$$

$$\Delta\epsilon_{2-} = \frac{4\pi n_- e_0^2 a}{kT} \tag{13}$$

$$\tau_- = \frac{1+\frac{1-v_v}{2+v_v}\cdot\frac{\bar{\epsilon}_{2\infty}}{\epsilon_{w0}}}{1+\frac{1-v_v}{2+v_v}\left(\frac{\bar{\epsilon}_{2\infty}+\Delta\epsilon_{2-}}{\epsilon_{w0}}\right)}\tau_{2-} \tag{14}$$

$$\tau_{2-} = \frac{a^2}{2D_-} \tag{15}$$

$$\bar{\epsilon}_{2\infty} = \epsilon_h \frac{2\left[1-\left(\frac{a_i}{a}\right)^3\right]\epsilon_h + \left[1+2\left(\frac{a_i}{a}\right)^3\right]\left(\epsilon_{w0}+\frac{a}{a_i}\Delta\epsilon_{2z}\right)}{\left[2+\left(\frac{a_i}{a}\right)^3\right]\epsilon_h + \left[1-\left(\frac{a_i}{a}\right)^3\right]\left(\epsilon_{w0}+\frac{a}{a_i}\Delta\epsilon_{2z}\right)} + \Delta\epsilon_{2z} \tag{16}$$

where $\Delta\epsilon_{2z}$ is as given in eq. 5, and D_- is the lateral self-diffusion coefficient of the fatty acid anions within the phospholipid bilayer.

The formulae for $\Delta\epsilon_{2-}$, τ_{2-} and $\bar{\epsilon}_{2\infty}$ transform the relevant molecular properties of the vesicle into the substitute homogeneous sphere permittivity

$$\epsilon_{2-}(\nu) = \frac{\Delta\epsilon_{2-}}{1+i2\pi\nu\tau_{2-}} + \bar{\epsilon}_{2\infty} \quad (\nu \ll \nu_z) \tag{17}$$

with the first term representing the anionic group contribution and $\bar{\epsilon}_{2\infty}$ denoting the contributions from the water core (ϵ_{w0}) and the phospholipid molecules (ϵ_h, $\Delta\epsilon_{2z}$). The formulae for $\Delta\epsilon_-$, $\Delta\epsilon_{0-}$ and τ_- follow from the Wagner mixture formula for the apparent permittivity of a suspension of well separated homogeneous spheres with permittivity ϵ_{2-} in a homogeneous medium with permittivity ϵ_1 ($=\epsilon_{w0}$ here) [18]. The formulae for $\Delta\epsilon_{0-}$ and τ_- refer to the case of such a small concentration of low-molecular-weight ions in the solvent (e.g., N_1 monovalent ions per solvent unit volume) that the Debye screening layer thickness, $\sqrt{\epsilon_{w0}kT/4\pi N_1 e_0^2}$, is larger than the clear distance between neighboring vesicles. This means that excess charge accumulation by those ions at the vesicle surfaces is prevented by the ion thermal diffusion and therefore that Maxwell-Wagner interfacial polarization can scarcely build up [18]. The separation of neighboring vesicles implied in the Wagner mixture formula is warranted by coulombic repulsion being effective due to the lack of screening action of the counterions.

With typical data for 0.1 M C_{14}-lecithin solutions with admixed 1 mol% fatty acid salt (potassium myristate) at 30°C ($a \approx 1.3\times10^{-6}$ cm, $a_i \approx 10^{-6}$ cm, $v_v = 0.184$, $n_- \approx 9.4\times10^{11}$ cm^{-2}, $D_- \approx 10^{-7}$ cm^2 s^{-1} [15], $\epsilon_{w0} = 76.6$; $\epsilon_h \approx 6$, $n_z \approx 9.4\times10^{13}$ cm^{-2}, $\xi \approx 5\times10^{-8}$ cm, $g = 2$ [11]), the calculated values of the dielectric increment and the relaxation frequency due to the outer $-CO_2^-$ group diffusion amount to $\Delta\epsilon_{0-} \approx 15$ and $\nu_- = (2\pi\tau_-)^{-1} \approx 2.5\times10^4$ Hz, respectively. The corresponding experimental values from the measurements [13] are $\Delta\epsilon_{0-} \gtrsim 8$–$20$ and $\bar{\nu}_- \lesssim (2$–$5)\times10^4$ Hz ($\bar{\nu}_-$ = mean relaxation frequency). The fairly good agreement between the measured and calculated values justifies confidence in the obviously simplified description presented of the I spectrum in the case of small phospholipid vesicles in solutions with small fatty acid salt and still smaller low-molecular-weight salt content. As there is a distribution of vesicle sizes one finds a broadened I spectrum from the measurements instead of the Debye-type spectrum presented above.

4.2.2. Solutions of large vesicles with small content of ionic lipid

In phospholipid solutions with a small content of fatty acid salt or other ionic lipids, if only once cooled down below T_t, the phospholipid aggregates are large sized and remain so also after heating up above T_t [14]. In the case in which still single-bilayer vesicles are predominantly present, the experimental outer radius values amount to $(5$–$7)\times10^{-6}$ cm [14]. With admixed 1 mol% fatty acid salt the number of outer $-CO_2^-$ groups per unit surface area is $n_- \approx 1.43\times10^{12}$ cm^{-2} at $T > T_t$. This value together with $a \approx 6\times10^{-6}$ cm, $T = 303$ K and $\epsilon_{w0} = 76.6$ yields $\epsilon_{w0}kT/4\pi n_- e_0^2 a \approx 0.13$ which indicates counterion condensation at the approximate mean radius $a_+ \approx 1.15a$. The counterion diffusion, thus being mostly confined to relatively thin layers near the phospholipid bilayer surfaces, predominantly due to its lateral component imparts fluctuating electric dipole moments to the vesicles. The dipole moments due to the inner counterions are kept small by depolarizing electric fields and will therefore be neglected in the following approximate consideration.

In order to obtain an estimate of the dielectric incremental spectrum

$$\Delta\epsilon_+(\nu) = \frac{\Delta\epsilon_{0+}}{1+i2\pi\nu\tau_+} \quad (\nu \ll \nu_z) \tag{18}$$

due to the outer counterion limited diffusion, as mentioned above, for solutions with distinctly less low-molecular-weight salt than fatty acid salt content, we assume ideally that these counterions are totally condensed immediately at the vesicle surface. Then the formulae in section 4.2 should apply with the subscript + instead of − and with n_+ being the number of outer counterions per vesicle surface unit area ($=n_-$) and D_+ the self-diffusion coefficient of the counterions. However, in the case of large vesicles the vesicle volume fraction, v_v, and the vesicle substitute homogeneous sphere permittivity at zero frequency, $\epsilon_2(0)$, may be so large that the Wagner mixture formula used so far for calculating $\Delta\epsilon_-(\nu)$ (cf. section 4.2.1) may not be sufficient here, as its validity is limited to small v_v and moderate $|\epsilon_2/\epsilon_1|$ values [18]. So one has to use one of the appropriately modified versions of the Wagner formula. These formulae exhibit a considerably more complicated analytical structure than that of the simple Wagner formula. In order to simplify the matter for the purpose of obtaining fair estimates, we use the modified version of the Wagner formula according to the Günther-Heinrich theory as presented by Dukhin [18] and take the correction for the limiting case $0 < |3\epsilon_{w0}/(2\epsilon_{w0} + \epsilon_2)| \ll 1$. This correction is performed by furnishing each v_v in the formulae in section 4.2.1 with the factor $(3.38 - 2.88v_v)/[1 + (1.19 - 1.44v_v)v_v]$ except the v_v in the numerator of $\Delta\epsilon_0$ which has to be furnished by $[1 + (1.19 - 1.44v_v)v_v]^{-1}$.

Insertion of typical data for 0.1 M C_{14}-lecithin solutions with admixed 1 mol% fatty acid salt (potassium myristate) at 30°C ($a \approx 6.\times 10^{-6}$ cm, $a_1 \approx 5.7 \times 10^{-6}$ cm, $v_v = 0.46$, $n_+ \approx 1.43 \times 10^{12}$ cm^{-2}, $D_+ = 2.2 \times 10^{-5}$ cm^2 s^{-1} (for K^+ in H_2O), $\epsilon_{w0} = 76.6$, $\epsilon_h \approx 6$, $n_v \approx 1.43 \times 10^{14}$ cm^{-2}, $\xi \approx 5 \times 10^{-8}$ cm, $g \approx 2$ [11]) yields the dielectric increment $\Delta\epsilon_{0+} \approx 141$ and the relaxation frequency $\nu_+ = (2\pi\tau_+)^{-1} \approx 3.2 \times 10^5$ Hz.

If the formulae for $\Delta\epsilon_{0+}$ and τ_+ are modified for the case of the counterion diffusion being totally confined to the extremely thin spherical shell with radius a_+, then with the numerical data used above and with $a_+ \approx 1.15a$ the dielectric increment and the relaxation frequency amount to $\Delta\epsilon_{0+} \approx 243$ and $\nu_+ = (2\pi\tau_+)^{-1} \approx 2.0 \times 10^5$ Hz, respectively. These calculated $\Delta\epsilon_{0+}$ and τ_+ values have to be taken as upper limits within the frame of the present model as the volume fraction of the substitute homogeneous (ϵ_2) dielectric spheres with radius a_+, $v_+ = (a_+/a)^3 v_v = 0.7$, already approaches the dense packing limit 0.74 in the case of equally sized spheres.

The corresponding experimental values from the measurements [13] are $\Delta\epsilon_{0+} \gtrsim 140$–$220$ being in fair agreement with the calculated ones, and $\bar{\nu}_+ \lesssim 2 \times 10^4$–$10^5$ Hz, being smaller. In connexion with this comparison one has to remember that in contrast to the basis of the calculation the counterions are not only distributed between the radii a and a_+ but also somewhat beyond a_+. Thus, with the large v_+ found above, overlapping of the counterion atmospheres of the vesicles has to be expected. Consequently, the model used so far in which counterion diffusion is confined to individual vesicles cannot be appropriate. In particular, it does not account for experimental values of $\Delta\epsilon_{0+} \gtrsim 750$ and $\bar{\nu}_+ \lesssim 7 \times 10^4$ Hz as found by the measurements [13] for a 0.05 M C_{14}-lecithin solution.

Overlapping of the counterion atmospheres of several vesicles may already arise at vesicle volume fractions distinctly smaller ($\lesssim 0.33$) than the dense packing limit. The reason for this is the tendency towards formation of clusters (e.g., chains) of vesicles being inherent even in a pure statistical spatial distribution [19]. This tendency, obviously growing with increasing volume fraction v_v, may be favored by intervesicle attraction due to London dispersion forces and due to the fluctuating dipole moments imparted by the fatty acid anion and counterion limited diffusion [20]. If there are well separated chain-like clusters of vesicles without overlapping of the counterion atmospheres of neighboring clusters then long, limited counterion diffusion paths and consequently large $\Delta\epsilon_+$ and τ_+ values have to be expected.

In order to obtain a rough estimate of the dielectric incremental spectrum due to outer counterion limited diffusion in the case of chain-like clusters of vesicles, we assume the existence of straight pieces of vesicle chains of length L and of randomly distributed orientations, to which the counterion diffusion is confined. A derivation similar to that of Wyllie and Oosawa [21] yields

$$\Delta\epsilon_+(\nu) \approx \frac{\Delta\epsilon_{0+}}{1+i2\pi\nu\tau_+} \quad (\nu \ll \nu_z) \tag{19}$$

with (in the case of negligible low-molecular-weight salt content)

$$\Delta\epsilon_{0+} \approx \frac{16}{\pi^3}\cdot\frac{N_+(Le_0)^2}{kT}F \tag{20}$$

$$\tau_+ \approx \frac{L^2}{\pi^2 D_+}F \tag{21}$$

where N_+ is the number of condensed outer counterions per solution unit volume, and $F \lesssim 1$ takes into account the interaction between a counterion and the charge distribution along the chain piece to which it belongs. Insertion of experimental $\Delta\epsilon_{0+}$ and $\bar{\nu}_+ = (2\pi\bar{\tau}_+)^{-1}$ values from ref. 13 for 0.05 and 0.1 M C_{14}-lecithin solutions with small fatty acid salt content (30°C; $D_+ = 2.2\times10^{-5}$ cm^2 s^{-1}) yields mean $\overline{L\sqrt{F}}$ values corresponding to straight chain pieces of at least two to four vesicles ($a \approx 6\times10^{-6}$ cm), and reasonable $2N_+$ values in the range 0.1–4 mol% relative to phospholipid. As there has to be expected a distribution of L values and a multi-mode counterion distribution along the straight chain pieces [21], one finds a broadened I spectrum from the measurements instead of the Debye-type spectrum presented above which has been derived under simplifying conditions.

In solutions of large vesicles with small content of ionic lipid, besides the counterions, the fatty acid salt anions (or other charged lipid molecules) with their negatively charged head groups at the vesicle surface, due to their limited diffusion are also expected to contribute an increment

$$\Delta\epsilon_-(\nu) = \frac{\Delta\epsilon_{0-}}{1+i2\pi\nu\tau_-} \quad (\nu \ll \nu_+) \tag{22}$$

to the I spectrum. For $\Delta\epsilon_{0-}$ and τ_- the formulae of section 4.2.1 (modified for large v_v and ϵ_2) hold if, in eq. 16 for $\bar{\epsilon}_{2\infty}$ to $(a/a_i)\Delta\epsilon_{2z}$ is added $(a_i/a)\Delta\epsilon_{2+}$ and to $\Delta\epsilon_{2z}$ is added $\Delta\epsilon_{2+} = 4\pi n_+ e_0^2 a/kT$. Insertion of typical data, as already used above in this section, and of D_- from section 4.2.1, for 0.1 M C_{14}-lecithin solutions with admixed 1 mol% fatty acid salt (potassium myristate), yields the dielectric increment $\Delta\epsilon_{0-} \approx 59$ and the relaxation frequency $\nu_- = (2\pi\tau_-)^{-1} \approx 1.2\times10^3$ Hz. The $\Delta\epsilon_{0-}$ value is relatively small as a result of dielectric screening by the counterions. Comparison of these calculated $\Delta\epsilon_{0-}$ and ν_- values with experimental values from ref. 13 is not possible as the small value of ν_- lies at or below the lower end of the frequency range of those measurements.

4.2.3. Solutions with moderate content of ionic lipid

In sections 4.2.1 and 4.2.2 phospholipid solutions were considered with small ionic lipid content of about 1 mol% relative to phospholipid. Then counterions are not condensed in the case of small vesicles ($a \approx 1.3\times10^{-6}$ cm), whereas most are condensed in the case of large vesicles ($a = 6\times10^{-6}$ cm). In solutions containing a moderate quantity of ionic lipid the counterions of small vesicles may be also condensed at the vesicle surface. This follows for a monovalent ionic lipid content of, e.g., 7 mol% ($n_- = 6.6\times10^{12}$ cm^{-2}), $a = 1.3\times10^{-6}$ cm, $T = 303$ K and $\epsilon_{w0} = 76.6$ from $\epsilon_{w0}kT/4\pi n_- e_0^2 a \approx 0.13$. In phospholipid solutions investigated previously [6–10] ionic lipid contents, yielding 7–2 mol% (of total lipid) diffusable monovalent counterions, and vesicle radii in the $(1.3–4)\times10^{-6}$ cm range, might have been present so that $\epsilon_{w0}kT/4\pi n_- e_0^2 a \ll 1$ held. With these assumptions the experimental values $\Delta\epsilon_{0+} = 15–100$, $\bar{\nu}_+ \approx (8–1)\times10^6$ Hz for 0.1–0.14 M C_{14}- and C_{16}-lecithin solutions at 30 or 45°C, respectively, are reproducible by the formulae used in section 4.2.2. Relatively small $\Delta\epsilon_{0+}$ values may appear together with not only large $\bar{\nu}_+$ values (corresponding to small vesicles) but also smaller ones (larger vesicles) if the number of condensed counterions is reduced ($n_+ < n_-$) due to partial overlapping of neighbor vesicle counterion atmospheres, or if the vesicle volume fraction v_v is reduced due to the existence of vesicles consisting of more than one phospholipid bilayer shell.

Especially small $\Delta\epsilon_{0+}$ values together with large $\bar{\nu}_+$ values had been found with C_{14}-lecithin solutions with greater than approx. 8 mol% cholesterol admixed, despite large vesicle size as indicated by $\epsilon_{w0} - \epsilon_{sw}$ [8]. Perhaps cholesterol induces phase separation within the phospholipid/cholesterol/fatty acid anion bilayer [22] so that regions enriched with fatty acid anions are separated by regions emptied of fatty acid anions, whereby the condensed counterion diffusion path length is diminished.

Particularly large $\Delta\epsilon_{0+}$ values together with unexpectedly high $\bar{\nu}_+$ values had been found with solutions of C_{16}-lecithin analogues (C_{16}-PN_n-lecithins) containing $n = 9$ or 10 $-CH_2-$ groups between the phosphate group and the trimethylammonium group, e.g., $\Delta\epsilon_{0+} \approx 480$ and $\bar{\nu}_+ \approx 2.2 \times 10^6$ Hz in the case of 0.106 mol/l C_{16}-PN_9-lecithin at 40°C [7]. Reproduction of these values by the formulae used in section 4.2.2 (modified for large $v_\times$ and ϵ_2) requires a content of charged lipid components with about 10 mol% monovalent counterions, vesicle radius $a \approx 7 \times 10^{-6}$ cm ($a_1 \approx 6.61 \times 10^{-6}$ cm, $v_\times \approx 0.55$), and a counterion self-diffusion coefficient of approx. $2D_+(K^+)$. This remarkably high condensed counterion content could have been due to about 5 mol% phosphatidic acid admixed to the C_{16}-PN_n-lecithins from the synthesis of these lecithins (Eibl, H., personal communication). This yields 10 mol% protons as diffusable counterions because the $-PO_4H_2$ group of the phosphatidic acid surrounded by the $-(CH_2)_n\overset{+}{N}(CH_3)_3$ groups of the lecithin is fully deprotonated at bulk pH ≈ 7. The latter groups might also be responsible for the self-diffusion coefficient of the H^+ counterions being reduced from $4.4D_+(K^+)$ to approx. $2D_+(K^+)$. It could thus have been possible that different experimental $\Delta\epsilon_{0+}$ and $\bar{\nu}_+$ values for C_{16}-PN_n-lecithins with different n values (2, 5, 6, 7, 9, 10) predominantly reflected different phosphatidic acid contents and/or vesicle sizes.

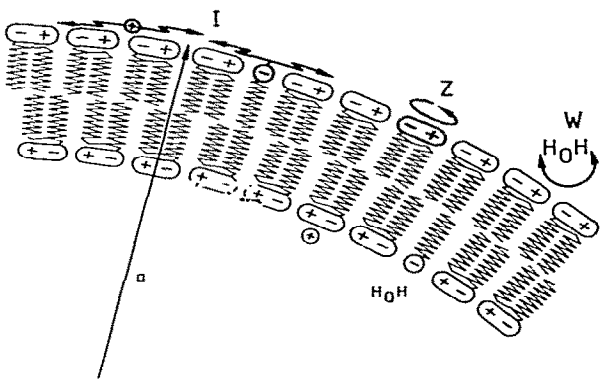

Fig. 6. Illustration of the molecular mechanisms as reflected by the W, Z and I dielectric spectra of phospholipid vesicle aqueous solutions: rotational diffusion of the water molecules, rotational diffusion of the phospholipid zwitterions, and limited translational diffusion of lipid ions and its condensed counterions, respectively.

5. Conclusion

The dielectric permittivity spectrum $\epsilon(\nu)$ at frequency $\nu < 60$ GHz of aqueous colloidal solutions of predominantly zwitterionic phospholipid vesicles shows dielectric dispersion around $\nu_{sw} \approx 22$ GHz (30°C) due to rotational diffusion of the water molecules (W) and around $\nu_z \approx 80$ MHz (30°C) due to rotational diffusion of the zwitterionic head groups (Z) of the phospholipid molecules (fig. 6). The water dispersion step $\epsilon_{sw} - \epsilon(\infty)$ (fig. 1) decreases with increasing vesicle size, i.e., with increasing amount of water trapped within the vesicle interior where it is strongly influenced by depolarizing electric fields. Both the zwitterion dispersion step $\Delta\epsilon_{0z}$ and frequency ν_z (fig. 3) are scarcely sensitive to the liquid-crystalline/crystalline phase transition of the phospholipid bilayer [11]. Towards lower frequencies the dielectric spectrum of exclusively zwitterionic phospholipid solutions exhibits no additional dispersion step [23].

If to the zwitterionic phospholipids is admixed only a low amount (1–10 mol%) of ionic lipids then the dielectric spectrum shows dispersion around frequencies $\nu_\pm$ between about 10 MHz and a few kilohertz (30°C). Both the dispersion step $\Delta\epsilon_\pm$ and frequency $\nu_\pm$ depend sensitively on the vesicle size (fig. 5), the ionic lipid content (fig. 4) and on the content of additionally admixed cholesterol.

Aqueous solutions of synthetic, thin-layer chromatographically pure zwitterionic phospholipids usually contain such small amounts of ionic lipids from synthesis and/or from molecular decomposition during preparation (sonication) of the solutions, which may not be easily detectable by thin-layer chromatography [24]. Disregarding this fact was the reason for misinterpretation of the dielectric spectrum below 40 MHz of aqueous zwitterionic phospholipid solutions in the previous works [6–10].

We now have attributed the dielectric dispersion in that spectral region to limited translational diffusion of the ionic lipid counterions, condensed to the vesicle surface, and/or of the ionic lipid molecules themselves, integrated in the phospholipid bilayers (I, fig. 6). In order to specify this attribution quantitatively we have selected from the variety of possible states of phospholipid solutions, being determined by numerous not all easily regulable parameters, a few limiting cases (single bilayer vesicles of two typical sizes, small ionic lipid content and still smaller content of low-molecular-weight salt, temperature above the crystalline/liquid-crystalline phase transition temperature). For these cases we have described the phospholipid solutions by simplifying models with respect to the essential molecular mechanisms of dielectric polarizability (transformation of molecular into continuum polarizabilities). For these models we have derived dielectric permittivity spectral functions of necessarily limited validity with respect to the real solutions in order to point out the essence of the relation between the measured dielectric spectra and the underlying molecular processes rather than to present a complete theoretical description. Therefore, the numerical results, dielectric dispersion step heights and dispersion frequencies, calculated from those spectral functions may not be overemphasized. However, the comparison with the corresponding experimental data allows the conclusion to be made that the ionic lipid-induced dielectric spectra can be sufficiently explained by limited ion diffusion.

The present paper shows the dielectric spectroscopy in the ion dispersion frequency region to be a remarkably sensitive detector for ionic lipids within zwitterionic phospholipid vesicles, and to be a useful tool for studying the size of vesicles or perhaps also of clusters (chains) of vesicles and the ionic particle diffusion within and at the surface of the phospholipid bilayers.

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References

1 E. Sackmann, Ber. Bunsenges. Phys. Chem. 78 (1974) 929.
2 H.P. Schwan, S. Takashima, V.K. Miyamoto and W. Stoeckenius, Biophys. J. 10 (1970) 1102.
3 W.R. Redwood, S. Takashima, H.P. Schwan and T.E. Thompson, Biochim. Biophys. Acta 255 (1972) 557.
4 U. Kaatze, R. Henze, A. Seegers and R. Pottel, Ber. Bunsenges. Phys. Chem. 79 (1975) 42.
5 J.C.W. Shepherd and G. Büldt, Biochim. Biophys. Acta 514 (1978) 83.
6 U. Kaatze, R. Henze and R. Pottel, Chem. Phys. Lipids 25 (1979) 149.
7 U. Kaatze, R. Henze and H. Eibl, Biophys. Chem. 10 (1979) 351.
8 R. Henze, Chem. Phys. Lipids 27 (1980) 165.
9 U. Kaatze and R. Henze, Ber. Bunsenges. Phys. Chem. 84 (1980) 1102.
10 U. Kaatze, Prog. Coll. Polym. Sci. 67 (1980) 117.
11 K.-D. Göpel, D. Thesis, Math.-Nat. Fachber., University of Göttingen (1982).
12 J.C.W. Shepherd and G. Büldt, Biochim. Biophys. Acta 558 (1979) 41.
13 V. Uhlendorf, D. Thesis, Math.-Nat. Fachber., University of Göttingen (1982).
14 J.H. Prestegard and B. Fellmeth, Biochemistry 13 (1974) 1122; H.L. Kantor and J.H. Prestegard, Biochemistry 14 (1975) 1790; H.L. Kantor, S. Mabrey, J.H. Prestegard and J.H. Sturtevant, Biochim. Biophys. Acta 466 (1977) 402; J.M.H. Kremer and P.H. Wiersema, Biochim. Biophys. Acta 471 (1977) 348; A.L. Larrabee, Biochemistry 18 (1979) 332; S. Massari, P. Arslan, A. Nicolussi and R. Colonna, Biochim. Biophys. Acta 599 (1980) 118.
15 E. Sackman, Ber. Bunsenges. Phys. Chem. 82 (1978) 891.
16 A. Watts, D. Marsh and P. Knowles, Biochemistry 17 (1978) 1792.
17 G. Schwarz, J. Phys. Chem. 66 (1962) 2636.
18 L.K.H. van Beek, Prog. Dielectr. 7 (1967) 69; S.S. Dukhin and V.N. Shilov, Dielectric phenomena and the double layer in disperse systems and polyelectrolytes (Halsted/Wiley, New York, 1974) ch. II, VII.
19 D. Stauffer, Phys. Rev. B1. 36 (1980) 124; D.M. Grannan, J.C. Garland and D.B. Tanner, Phys. Rev. Lett. 46 (1981) 375; D.J. Bergman and Y. Imry, Phys. Rev. Lett. 39 (1977) 1222; D. Stroud and D.J. Bergman, Phys. Rev. B25 (1982) 2061; Y. Gefen, A. Aharony and S. Alexander, Phys. Rev. Lett. 50 (1983) 77; R. Schiller, Á. Vértes and L. Nyikos, J. Chem. Phys. 76 (1982) 678.
20 J. Mahanty and E.W. Ninham, Dispersion forces (Academic

Press. London. 1976); F. Oosawa, Polyelectrolytes (Dekker. New York. 1971) ch. 9.IV.
21 G. Wyllie. Dielectr. Relat. Mol. Processes 1 (1972) ch. 2.3; F. Oosawa. Polyelectrolytes (Dekker. New York. 1971) ch. 5. IV.
22 D.J. Recktenwald and H.M. McConnell, Biochemistry 20 (1981) 4505.
23 U. Kaatze, A. Dittrich. K.-D. Göpel and R. Pottel (1983) Chem. Phys. Lipids. submitted for publication.
24 K. Tajima and N.L. Gershfeld, J. Colloid Interface Sci. 81 (1981) 283.
25 E.R. Smith. Mol. Phys. 45 (1982) 915.