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Dielectric properties of aqueous zwitterionic liposome suspensions

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Abstract

The dielectric spectra of aqueous suspensions of unilamellar liposomial vesicles built up by zwitterionic phospholipids (dipalmitoylphosphatidyl-choline, DPPC) were measured over the frequency range extending from 1 kHz to 10 MHz, where the interfacial polarization effects, due to the highly heterogeneous properties of the system, prevail. The dielectric parameters, i.e., the permittivity $\epsilon'(\omega)$ and the electrical conductivity $\sigma(\omega)$, have been analyzed in terms of dielectric models based on the effective medium approximation theory, considering the contribution associated with the bulk ion diffusion on both sides of the aqueous interfaces. The zwitterionic character of the lipidic bilayer has been modeled by introducing an "apparent" surface charge density at both the inner and outer aqueous interface, which causes a tangential ion diffusion similar to the one occurring in charged colloidal particle suspensions. A good agreement with the experimental results has been found for all the liposomes investigated, with size ranging from 100 to 1000 nm in diameter, and the most relevant parameters have briefly discussed in the light of the effective medium approximation theory.

Keywords: Zwitterionic liposome; Dielectric spectra; Dielectric model; Dynamic light scattering; Liposome suspension

1. Introduction

Liposomes, artificial spherical vesicles formed by the self-assembly of lipids, consist of a single closed lipid bilayer which encompasses an aqueous core [1,2]. Their size ranges from 20–30 nm up to some micrometers in diameter (small and large unilamellar vesicles, respectively) and the thickness of the lipidic bilayer is about 4–5 nm. These structures, which act as appropriate containers that mimic a biological cell membrane, represent a very interesting model of a colloidal suspension and, moreover, find widespread applications as delivery systems, allowing the encapsulation in the appropriate regions of both hydrophobic and hydrophilic substances [3,4].

The dielectric spectra of these highly heterogeneous systems present a very complex and rich phenomenology, evidencing different polarization mechanisms, at a molecular level [5]. At least three different, partially overlapping, relaxation regions have been generally observed, the main of which, due to the interfacial electrical polarization, is associated with the non-homogeneous distribution, at a mesoscopic scale, of the different dielectric materials with different dielectric and conductometric properties. Moreover, a further contribution arises from the surface organization of the liposome bilayer, especially in the case of zwitterionic lipids, where a microdynamics of the dipolar headgroups favors the formation of domains with a high degree of dipole orientational correlation [6].

This latter effect, which is anyway quantitatively less significant, generally producing dielectric increments of about ten dielectric units, has been previously investigated by different authors [7,8] and in some cases overlaps the relaxation associated with the hindered reorientation motion of the water molecules bound to the interface. At lower frequencies, specially in the case of ionic interfaces, the counterion polarization dominates. This polarization mechanism is associated with a non-uniform counterion distribution at the interfaces, induced by the external electric field, that imparts to the overall particle a large dipole moment. Finally, a further contribution, which falls at higher frequencies, around 20 GHz, comes out from the orientational polarization of the water molecules.

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The deconvolution of the dielectric spectrum into its molecular components is further made more difficult by the presence of the electrode polarization effect which produces an additional ionic polarization which behaves as an artifact that, in most cases, may mask the effects to be investigated.

Despite the complexity of the dielectric response, dielectric methods are very useful tools to investigate the electrical properties of heterogeneous systems, since they are based on the response of a permanent and/or induced electrical dipole to a driving electric field and permit identification of a number of completely different mechanisms of electrical polarization. This probe, which is particularly susceptible to the electrical environment, provides a valuable knowledge about polarization mechanisms in biological cells under the influence of an electric field. When applied to biological systems, these methods can provide valuable knowledge about different biological cell structures, their functions and metabolic activities.

Here, we report on the dielectric and conductometric properties of zwitterionic liposomes built up by DPPC lipids dispersed in aqueous solution, in the low-frequency range up to 10 MHz, where the interfacial effects dominate, the other different contributions falling outside the frequency window investigated. The data in the lower frequency region have been appropriately corrected for the electrode polarization effects and the results are analyzed in the light of appropriate dielectric models for heterogeneous systems, considering the contribution of the ion diffusion in the bulk solutions, close to the interfaces. Our analysis points out that, even in the presence of an uncharged interface, owing to the zwitterionic character of the lipid employed, the ion diffusion in the bulk of the two aqueous phases plays a relevant role in the overall dielectric behavior of the system. These effects can be taken into account by solving the appropriate Poisson–Boltzmann equation together with the Nernst–Planck equation under the assumption that the ion concentration changes induced by the applied field are much smaller than their equilibrium values. The dielectric properties are described in the far-field approximation, which is accurate enough to reproduce the observed dielectric spectra with a set of parameters of reasonable values.

The introduction of an "apparent" surface charge density on both the inner and outer interface of the liposome particle leads to a significant better description of the electrical behavior of the system.

2. Experimental

2.1. Materials and Liposome preparation

Lipid α-dipalmitoylphosphatidylcholine [DPPC] was obtained from Sigma Chem. Co. [USA] and was used as received, without further purification. Lipid suspensions were prepared according to standard procedure. Briefly, an appropriate amount of DPPC was dissolved in chloroform–methanol mixture (1:1 vol/vol). Organic solvent was then removed under vacuum overnight. The resulting film was hydrated with deionized water (Millipore Super Q-system, electrical conductivity less than $\sigma = 10^{-6}$ mho/cm) and allowed to swell at a temperature of 43 °C (above the main transition temperature of the lipid [9]). Unilamellar liposome vesicles were prepared by extrusion of the lipid mixture in an extruder Lipex Biomembranes (Vancouver, Canada). The lipid mixture was extruded several times at a pressure lower than 2000 kPa through polycarbonate membranes with appropriate pore size, depending on the intended vesicle size. This technique provides almost spherical shaped vesicles with a relatively small size distribution. We employed membranes with nominal pore size of 50, 100, 200, 400, 800, 1000 nm.

2.2. Dynamic light scattering measurements

Liposome sizes were measured at a temperature of 25 °C by means of dynamic light scattering technique [10] using a Brookhaven AT9000A logarithmic correlator. The scattered light intensity, collected at a 90° angle, was analyzed using the multimodal constraint regularization algorithm CONTIN [11] which minimizes the sum of the squared differences between the experimental and calculated normalized intensity autocorrelation function. The effective size of the liposomal vesicles for the different "nominal" pore size of the extrusion membrane we have employed during the extrusion procedure is shown in Fig. 1. The data presented correspond to the intensity-weighted distribution.

2.3. Dielectric measurements

Dielectric spectra of liposome suspensions were measured in the frequency range from 1 kHz to 10 MHz by means of a Hewlett–Packard Impedance analyzer Mod. 4192A. The complex electrical impedance $Z^*(\omega)$ measured at the input of the Meter is directly related to the complex permittivity $\epsilon^*(\omega)$ through the relationship

$$Z^*(\omega) = i\omega L + \frac{1}{i\omega C_f + \frac{C_0 \epsilon^*(\omega)}{1 + K C_0 (i\omega)^{(1-\alpha)} \epsilon^*(\omega)}} \quad (1)$$

where a constant phase angle (CPA) element, i.e., an impedance of the form $Z_p^*(\omega) = K(i\omega)^{-\alpha}$, with K a constant and $0 < \alpha < 1$, has been used to describe the a.c. response of the interface between the electrodes and the solution (electrode polarization effect)

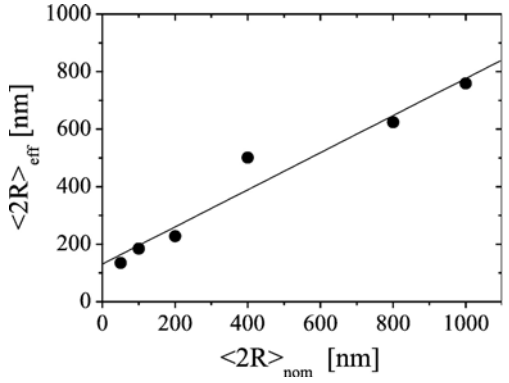

Fig. 1. The effective size $<2R>_{eff}$ of the liposomal vesicles, determined from dynamic light scattering technique, as a function of the nominal size $<2R>_{nom}$ of the membrane pore in the extrusion procedure.

[12–15]. The cell constants, i.e., the self-inductance L, the stray capacitance C_f, the cell constant C_0 and the two parameters K and α associated with the CPA element which models the electrode polarization, were determined by measuring the complex impedance of the cell filled with standard electrolyte solutions of known permittivity and electrical conductivity. The electrode polarization correction by means of an empirical fitting of the frequency dependence of a constant-phase-angle model has been widely used in impedance spectroscopy measurements of biological suspensions and has been found as an empirical way to remove this effect without influencing the plausibility of the data [16].

3. The dielectric model

According to the widely accepted dielectric models for heterogeneous systems [17], within the mean field approximation, a suspension of shelled spheroidal particles dispersed in a continuous phase should display a dielectric and conductometric behavior characterized by two contiguous, partially overlapping, dielectric dispersions, associated to the two interfaces present in the system [18]. Within this scheme, each particle is modeled by a spherical core of radius R_p and complex dielectric constant

$$\epsilon^*(\omega) = \epsilon_p + \sigma_p/(i\omega\epsilon_0) \tag{2}$$

covered by a concentric shell of thickness δ and complex dielectric constant

$$\epsilon_s^*(\omega) = \epsilon_s + \sigma_s/(i\omega\epsilon_0) \tag{3}$$

The shelled particles are uniformly distributed in a continuous aqueous medium with complex dielectric constant

$$\epsilon_m^*(\omega) = \epsilon_m + \sigma_m/(i\omega\epsilon_0) \tag{4}$$

The fractional volume of the particles is $\Phi = 4/3\pi N_0(R_p+\delta)^3$, where N_0 is their numerical concentration. Here, ϵ_j and $\sigma_j (j=p, s, m)$ are the permittivity and the electrical conductivity of each medium involved, ω is the angular frequency of the applied electric field and ϵ_0 the dielectric constant of free space. The electrical properties of each medium are characterized by two electrical parameters, ϵ_j and σ_j, both of them considered independent of frequency or, in other words, the frequency of the applied field is well below any characteristic relaxation frequency of each medium.

Within this scheme, in the case of liposome particles, where the same aqueous phase should be present in the inner and outer medium ($\epsilon_p \simeq \epsilon_m$; $\sigma_p \simeq \sigma_m$), with the further approximation $\epsilon_s \ll \epsilon_p$, ϵ_m and $\sigma_s \ll \sigma_p$, σ_m, the total dielectric $\Delta\epsilon$ increment and the total conductivity increment, to a first approximation, are given by

$$\Delta\epsilon = \epsilon_s \frac{9\Phi}{(2+\Phi)^2\delta/R_p} - \epsilon_m \frac{3\Phi}{(2+\Phi)} \tag{5}$$

$$\Delta\sigma = \sigma_m \left(\frac{3\Phi}{(2+\Phi)} - \Phi\delta/R_p \frac{\epsilon_m}{\epsilon_s} \right) \tag{6}$$

and the two dispersions are characterized by relaxation times given by

$$\tau_1 = \epsilon_0 \frac{3\epsilon_s}{(2+\Phi)\sigma_m\delta/R_p} \tag{7}$$

$$\tau_2 = \epsilon_0 \frac{\epsilon_m}{\sigma_m} \tag{8}$$

For values of σ_m of the order of 10^{-2} mho/m, as those of the aqueous phase of liposome suspensions, the "high-frequency" contribution, characterized by the relaxation time τ_2 falls outside the frequency interval investigated.

This model is based on the assumption that the shell membrane behaves as an isotropic dielectric material whose permittivity ϵ_s and conductivity σ_s are independent of frequency and moreover that the charge distributions near the interfaces have a null thickness. Although this approach is well justified in a variety of biological cell membranes [19–22], when the electrical properties of the interfaces favor different polarization mechanisms which cause additional dielectric dispersions, the above assumptions are no longer valid and the contribution of the bulk ion diffusion in the inner and outer medium must be appropriately taken into account. Owing to the diffusion of ions, a finite thickness charge distribution on both side of the shell arises, influencing the overall interfacial polarization. This results in a dielectric relaxation that prevails over the usual Maxwell–Wagner effect.

In the presence of free charges, the solution of the dielectric model requires the knowledge at every point $\vec{r}$ of the system of the electrical potential $\Psi(\vec{r})$ which obeys the Poisson equation $\nabla^2\Psi(\vec{r}) = -\rho(\vec{r})/\epsilon_0\epsilon_m$ with the appropriate boundary conditions (the continuity of the potential and the normal component of the displacement at the two interfaces at $r=R_p$ and $r=(R_p+\delta)$), where the electrical charge density $\rho(\vec{r})$ is given by the Boltzmann equation

$$\begin{aligned} \rho(\vec{r}) &\equiv \sum_i z_i e n_i(\vec{r}) \\ &= \sum_i z_i e n_{0i} \exp(-(z_i e \Psi(\vec{r}))/(K_B T)) \end{aligned} \tag{9}$$

with e the elementary charge, z_i and $n_i(\vec{r})$ the valence and the concentration of the ith ionic species and $K_B T$ the thermal energy and n_{0i} the concentration of the ionic species in the bulk solution, where the electrical neutrality prevails. These equations are linearized in the weak-field limit, assuming that the basic electrical parameters of the system have only a slight deviation from equilibrium due to the external electric field. This means that each quantity X will be expressed as

$$X(\vec{r}) = X^0(r) + \delta X(\vec{r}) \tag{10}$$

where the superscript refers to the unperturbed values. In terms of the perturbed quantities, the perturbed charge density $\delta\rho(\vec{r})$ obeys the equation [23]

$$\nabla^2\delta\rho(\vec{r}) = \gamma^2\delta\rho(\vec{r}) \tag{11}$$

with

$$\gamma^2 = \frac{i\omega}{D} + k_D^2 \tag{12}$$

where $D=(u/e)K_BT$ is the ion diffusion coefficient, $k_D^2 = \sum_1 n_{0i}z_i^2e^2/\epsilon_0\epsilon_m K_BT$ the reciprocal Debye screening length and u the ion mobility. Eq. (11) must be solved (in spherical coordinates with axial symmetry) for each of the medium involved [27], i.e., the aqueous inner phase, the lipid bilayer and the aqueous external suspending medium, and the non-trivial particular solution can be written as

$$\delta\rho_j(\vec{r}) = \left(C_j\left(\frac{\cosh(\gamma_j r)}{\gamma_j r} - \frac{\sinh(\gamma_j r)}{(\gamma_j r)^2}\right) + G_j\left(\frac{\cosh(\gamma_j r)}{(\gamma_j r)^2} - \frac{\sinh(\gamma_j r)}{(\gamma_j r)}\right)\right)\cos(\vartheta) \quad (13)$$

for ($j=p$, s, m), where the constants C_j, G_j must obey to the convergence condition for $r\to 0$ and $r\to\infty$. Consequently, solving the Poisson equation with ion density distribution given by Eq. (13) leads to

$$\Psi_j(r,\vartheta) = -A_j r\cos\vartheta + B_j\frac{\cos(\vartheta)}{r^2} - \frac{\delta\rho_j(\vec{r})}{\epsilon_0\epsilon_j\gamma_j^2} \quad (14)$$

for each of the three media ($j=p$, s, m). The constants A_j, B_j and C_j, G_j are obtained from the following boundary conditions:

i) the continuity of the potential $\Psi(r,\vartheta)$ at the interfaces $r=R_p$ and $r=(R_p+\delta)$

$$\Psi_p(r,\vartheta)|_{R_p} = \Psi_s(r,\vartheta)|_{R_p} \quad (15)$$

$$\Psi_s(r,\vartheta)|_{R_p+\delta} = \Psi_m(r,\vartheta)|_{R_p+\delta} \quad (16)$$

ii) the normal components of the displacement must be related to the charge distribution at the interfaces

$$\epsilon_p^*\frac{\partial\Psi_p(r,\vartheta)}{\partial r}|_{R_p} - \epsilon_s^*\frac{\partial\Psi_s(r,\vartheta)}{\partial r}|_{R_p} = -\sigma(R_p,\vartheta)/\epsilon_0 \quad (17)$$

$$\epsilon_s^*\frac{\partial\Psi_s(r,\vartheta)}{\partial r}|_{R_p+\delta} - \epsilon_m^*\frac{\partial\Psi_m(r,\vartheta)}{\partial r}|_{R_p+\delta} = -\sigma(R_p+\delta,\vartheta)/\epsilon_0 \quad (18)$$

iii) the normal components of the total current density mush vanish at the interfaces $r=R_p$ and $r=(R_p+\delta)$

$$\sigma_p\frac{\partial\Psi_p(r,\vartheta)}{\partial r}|_{R_p} + D_p\frac{\partial\rho_p(r,\vartheta)}{\partial r}|_{R_p} = \sigma_s\frac{\partial\Psi_s(r,\vartheta)}{\partial r}|_{R_p} + D_s\frac{\partial\rho_s(r,\vartheta)}{\partial r}|_{R_p} \quad (19)$$

$$\sigma_s\frac{\partial\Psi_s(r,\vartheta)}{\partial r}|_{R_p+\delta} + D_s\frac{\partial\rho_s(r,\vartheta)}{\partial r}|_{R_p+\delta} = \sigma_m\frac{\partial\Psi_m(r,\vartheta)}{\partial r}|_{R_p+\delta} + D_m\frac{\partial\rho_m(r,\vartheta)}{\partial r}|_{R_p+\delta} \quad (20)$$

iv) far from the particle, the potential is due to the applied external electric field E_0

$$\Psi_m(r,\vartheta)\to -E_0 r\cos\vartheta \quad r\to\infty \quad (21)$$

v) the potential $\Psi_j(r,\vartheta)$ ($j=m$) must remain finite for $r\to 0$.

vi) the "apparent" surface charges at the inner and outer interfaces are given by

$$\sigma(R,\vartheta) = \sigma_0(R) + N_1(R,\vartheta) \quad (22)$$

(with $R=R_p$ and $R=(R_p+\delta)$, respectively), where the field perturbation contribution, $N_1(R,\vartheta)$, is calculated following the derivation proposed by Schwarz [24,25]. The ion movement tangentially to the interfaces results in the expression

$$N_1(R,\vartheta) = -\frac{e\sigma_0(r)/K_BT}{1+i\omega\frac{R^2}{2uK_BT}}\cos\vartheta \quad (23)$$

Finally, the unperturbed charge densities $\sigma_0(R)$ at the two interfaces are linked by the relationship

$$\sigma_0(R_p+\delta) = \sigma_0(R_p)\frac{(R_p)^2}{(R_p+\delta)^2} \quad (24)$$

Similar conditions have been employed by Gheorghiu [26], who considers a fixed negative charge on the inner side of the shell that is balanced by positive ions in the outer medium, migrating to the external side of the shell. In the present case, we introduce an "apparent" charge distribution on both the sides of the membrane, whose perturbation, induced by the applied electric field, is described by a surface diffusion. The introduction of the bulk ion diffusion in both the inner and outer medium and, moreover, of a mechanism of tangential ion movement induced by the external field causes an apparent electric dipole moment responsible of the giant dielectric relaxation observed in these systems.

Neglecting interactions between particles, in the case of a non-conductive shell, ($\sigma_s=0$), the general solution for the dielectric properties of the whole system can be written as

$$\epsilon^*(\omega) = \epsilon_m^*\frac{1+2\Phi\frac{M^*(\omega)}{E_0(R_p+\delta)^3}}{1-\Phi\frac{M^*(\omega)}{E_0(R_p+\delta)^3}} \quad (25)$$

where the dipole coefficient $M*(\omega)/E_0(R_p+\delta)^3$ is given by

$$\frac{M^*(\omega)}{E_0(R_p+\delta)^3} = \frac{(1-Q)\epsilon_s^+ - \frac{\epsilon_m^*\sigma_m^*}{i\omega\epsilon_0\epsilon_m} + \left(\frac{R_p}{R_p+\delta}\right)^3\left((1-Q)\epsilon_s^- + \frac{\epsilon_m^*\sigma_m^*}{i\omega\epsilon_0\epsilon_m}\right)N^*(\omega)}{(1+2Q)\epsilon_s^+ + \frac{2\epsilon_m^*\sigma_m^*}{i\omega\epsilon_0\epsilon_m} + \left(\frac{R_p}{R_p+\delta}\right)^3\left((1+2Q)\epsilon_s^- - \frac{2\epsilon_m^*\sigma_m^*}{i\omega\epsilon_0\epsilon_m}\right)N^*(\omega)} \quad (26)$$

where the following relationships hold

$$\epsilon_s^+ = \epsilon_s + N_1(R_p+\delta)/\epsilon_0 \quad (27)$$

$$\epsilon_s^- = 2\epsilon_s - N_1(R_p+\delta)/\epsilon_0 \quad (28)$$

$$N^*(\omega) = \frac{-\epsilon_s(1+S) + \frac{\epsilon_p^*\sigma_p^*}{i\omega\epsilon_0\epsilon_p}}{2\epsilon_s(1+S) + \frac{\epsilon_p^*\sigma_p^*}{i\omega\epsilon_0\epsilon_p}} \quad (29)$$

$$S = \frac{\sigma_p}{i\omega\epsilon_0\epsilon_p}\frac{\gamma_p R_p - \tanh(\gamma_p R_p)}{(\gamma_p R_p)^2\tanh(\gamma_p R_p) - 2(\gamma_p R_p - \tanh(\gamma_p R_p))} \quad (30)$$

$$Q = \frac{\sigma_m}{i\omega\epsilon_0\epsilon_m}\frac{\gamma_m(R_p+\delta)}{(\gamma_m(R_p+\delta))^2 + 2\gamma_m(R_p+\delta) + 2} \quad (31)$$

Eq. (26) is analogous to the one given by Gheorghiu [26–28], derived under similar conditions. These expressions match each other in the limit $k_{Dj}^2\to 0$ ($j=p$, m). This condition, for both

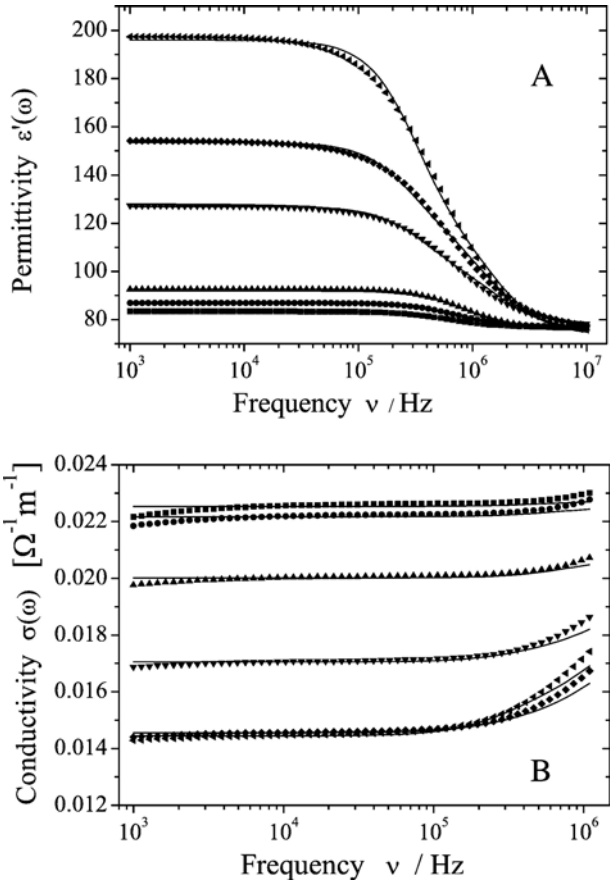

Fig. 2. Panel A: Dielectric dispersion of aqueous liposome suspensions as a function of frequency at the temperature of 25.0±0.2 °C for different values of the fractional volume Φ of the dispersed vesicles: (■):Φ=0.041; (●):Φ=0.055; (▲): Φ=0.068; (▼): Φ=0.15; (♦): Φ=0.19; (◀): Φ=0.23. Panel B: Conductometric dispersions of aqueous liposome suspensions as a function of frequency. Symbols as in panel A). Full lines are the values obtained from the fitting procedure on the basis of Eq. (25). The values of the fitting parameters are shown in Fig. 5.

the inner and outer medium, implies $\sigma_p^*(\omega)/i\omega\epsilon_0\epsilon_p \to 1$ and $\sigma_m^*(\omega)/i\omega\epsilon_0\epsilon_m \to 1$. In the absence of a bulk ion diffusion and uncharged interfaces, $\sigma(R,\vartheta)=0$, Eq. (26) reduces to the usual expression derived within the standard Maxwell–Wagner approximation [29]. In the case of liposomal particles, due to the chemical structure of the interface and the physico-chemical properties of the aqueous phase, the bulk electrical parameters of the inner and outer medium, ϵ_j and σ_j, ($j=p$, m), should assume the same value.

4. Results and discussion

Typical dielectric spectra for the permittivity $\epsilon'(\omega)$ and the electrical conductivity $\sigma(\omega)$ of different aqueous liposome suspensions measured in the frequency range from 1 kHz to 10 MHz are shown in Fig. 2. As can be seen, the system undergoes well marked dielectric and conductometric dispersions with characteristic relaxation times of the order of 10^{-7}s.

Liposomes have been built up in varying sizes between 100 and 1000 nm in diameter, starting from an aqueous lipid mixture containing the same lipid content. All our samples are prepared at a lipid concentration of 10 mg/ml.

This procedure implies that the fractional volume Φ of liposomes in each sample investigated varies from about Φ=0.04 to about Φ=0.23 according to the relationship

$$\Phi = C\frac{N_0 aR}{6M_w} \tag{32}$$

where C is the lipid weight per unit volume, N_0 the Avogadro number, a the surface of each lipid polar head in the bilayer, M_w the lipid molecular weight and R the liposome radius. The dielectric analysis of the data has been carried out on the basis of a dilute suspension, considering non-interacting particles. Although, at the maximum volume fraction investigated, the average distance between two neighboring particles is of the order of their radius, the Debye screening length is small enough to allow an almost complete electrostatic screening between particles.

In order to differentiate the effects due to the fractional volume Φ from those connected to the liposome radius R, in Fig. 3 we report the dielectric increment $\Delta\epsilon$ normalized to the fractional volume Φ as a function of the effective radius R_{eff}. We observe two distinct behaviors, corresponding to the small size region, where $\Delta\epsilon/\Phi$ decreases with the radius R_{eff}, and to the high size region, where $\Delta\epsilon/\Phi$ is independent of R_{eff}. There different dependencies suggest that the polarization strength might depend on the radius of curvature of the interface. This effect has been recently discussed [30] in the light of the formation of correlated domains at the lipid surface, whose extension depends on the local curvature of the interface.

The dielectric and conductometric data have been analyzed first on the basis of the usual heterogeneous system mixture equation (whose dielectric parameters are given by Eqs. (5) to (8)) that give the full behavior of the complex dielectric constant $\epsilon^*(\omega)$, or conversely of the complex conductivity $\sigma^*(\omega)$, depending on six different parameters involved, i.e., the permittivity ϵ and the conductivity σ of the inner medium, the shell membrane and the external medium, respectively, besides the geometrical parameters (size and membrane thickness). The volume fraction Φ deduced from the lipid concentration and the

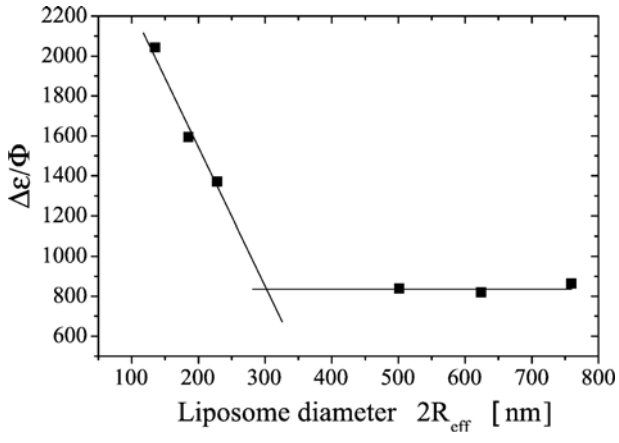

Fig. 3. The dielectric increment $\Delta\epsilon$ normalized to the fractional volume Φ of the dispersed phase as a function of the radius R_{eff} of the liposomes.

liposome size was compared with the value derived from the relationship

$$\Phi = 2\frac{\sigma_m - \sigma(\omega\rightarrow 0)}{2\sigma_m - \sigma(\omega\rightarrow 0)} \tag{33}$$

where $\sigma(\omega\rightarrow 0)$ is the limiting value of the electrical conductivity of the liposome suspension at low frequencies. These two values compare reasonably well, the differences between the two different methods being maintained within 2–3%. The best fit parameters were searched by means of a non-liner least-squares fitting procedure in order to minimize simultaneously the real and imaginary part of the complex dielectric constant.

As far as the bulk, non-diffusive, medium model is concerned, with the condition $\epsilon_m = \epsilon_p = 78.5$, corresponding to the value of the aqueous phase at the temperature of 25 °C, we obtain, for all the samples investigated, independently of the liposome radius, a membrane conductivity less than $\sigma_s = 10^{-5}$ mho/m and, for the other parameters, the values shown in Fig. 4. As can be seen, the membrane permittivity ϵ_s assumes values of the order of $\epsilon_s = 25 \div 30$, roughly independent of the liposome radius. However, these values are well above those expected for a simple lipid bilayer, where the pure hydrocarbon phase presents a permittivity of the order of $2 \div 4$. The conductivity of the external medium σ_m, of the order of 0.014 mho/m, is very close to the value measured for the suspending medium when all the inclusions are removed, whereas the conductivity σ_p of the aqueous core appears to be significantly higher. This set of parameters allows a good description of the experimental relaxation both in the permittivity $\epsilon'(\omega)$ and in the electrical conductivity $\sigma(\omega)$. However, the values of ϵ_s appear unrealistic if attributed to a simple lipid bilayer and in contrast to what expected for a liposome particle, where the inner and outer medium should present approximately the same ionic conductivity, we obtain values of σ_p larger than σ_m. Consequently, it seems more appropriate to take into account, at the lipid interface, of a non-uniform ionic distribution.

In fact, when the bulk counterion diffusion in both the inner and outer medium is considered, a somewhat different picture emerges.

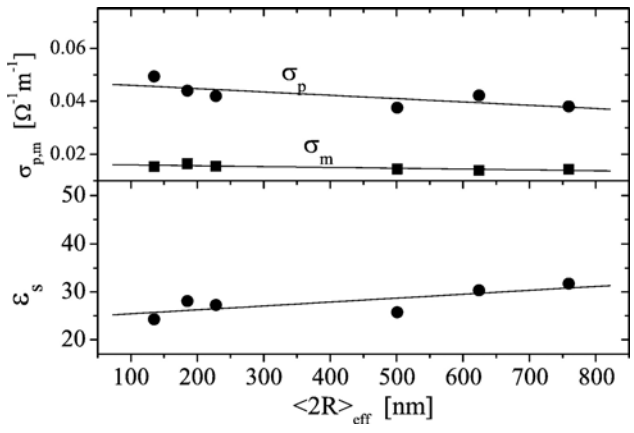

Fig. 4. The conductivity of the inner and outer medium (upper panel) and the permittivity of the lipid membrane (bottom panel) as a function of the effective radius R_{eff} of the liposome vesicles. These values are derived from the fitting procedure on the basis of heterogeneous system mixture equation, in the absence of bulk ion diffusion contribution.

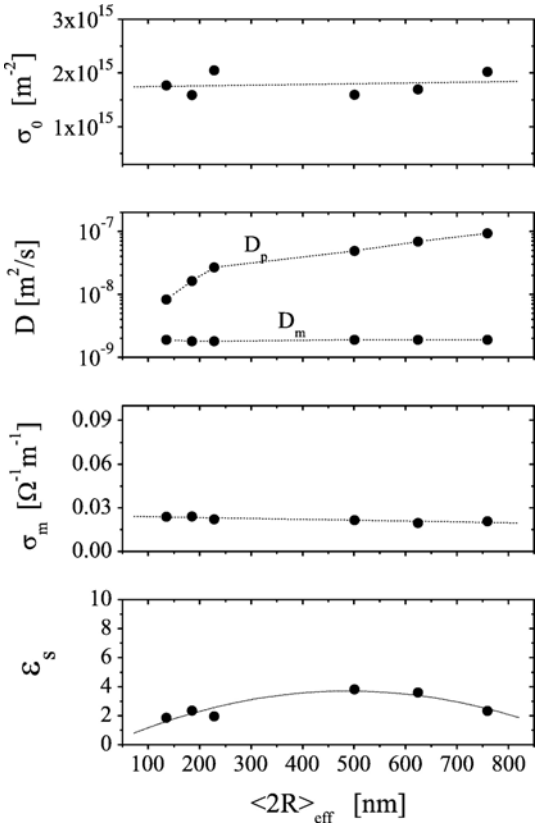

Fig. 5. The dependence of the dielectric parameters derived from Eq. (25) on the effective radius R_{eff} of the liposome particles. The values are derived from the fitting procedure on the basis of Eqs. (25) and (26). The dotted lines are to guide the eye only.

The experimental spectra have been analyzed on the basis of Eqs. (25) and (26) and the results are summarized in Fig. 5, where the main parameters are shown as a function of the effective radius R_{eff} of the liposomes. The model introduces an "apparent" surface charge density $\sigma(R)$ at both the two interfaces, enough to justify a charge non-uniform distribution in the inner and outer medium.

The net charge density at a liposome surface is a function of both the lipid molecular area and the degree of ionization. Although the degree of dissociation is zero for zwitterionic lipids, such as DPPC, it has been observed, for these liposomes, non-zero potential over a wide range of ionic strengths [31]. The presence of a strong dipole moment at the lipid-aqueous interfaces makes possible to the interface to attract counterions from the bulk solution leading to the appearance of a surface conductivity. This charge density is obviously lower that the one present at the liposome surface, in the case of ionic lipids.

As far as the apparent surface charge density is concerned, its value is about $\sigma_0 \simeq 2\ 10^{15}\ m^{-2}$, much lower than the one expected for fully ionized charged lipids in liposomes of similar size. Since each head group occupies an area of about 60 nm^2, in this latter case, at the particle surface, a charge density of about $10^{19}\ m^{-2}$ should be expected. The much lower values derived from the dielectric model employed reflect the zwitterionic character of the DPPC used, where the polar head is substantially overall uncharged.

Moreover, as can be seen in Fig. 5, the charge density σ_0 is approximately independent on the radius R_{eff}. This result further

supports that the counterion polarization at the liposome-aqueous solution interface should depend on the local character of the charge distribution at the lipid interface, driven by the electric dipole moment of the lipid molecule.

In this case, the permittivity ϵ_s of the lipid bilayer assumes a value close to 2–3 dielectric units, in very good agreement with the values attributed to the hydrocarbon phase of the inner part of the bilayer. Within the uncertainties of our fitting procedure, it is not possible to assign a dependence on the radius R_{eff} of the liposome, but its apparent constancy is highly reasonable.

The value of the bulk electrical conductivity σ_m is found to be about 0.025 mho/m, very close to the value measured directly on the aqueous phase, once the liposome component has been removed from the suspension. This finding enforces the validity of this dielectric model. The ion diffusion coefficients D_m and D_p are equal to 2 10^{-9} m^2/s and of the order of 10^{-8} m^2/s, respectively.

D_m is reasonably well in agreement with the expected value of Cl ion in a dilute aqueous solution, whereas D_p is rather higher. This reflects the analogous result for the conductivity of the inner medium in the case of absence of bulk ion distribution. We find that the conductivity σ_p of the inner medium can be much larger than the one of the suspending medium σ_m, in contrast to the previous claim that $\sigma_p \backsimeq \sigma_m$. Similar result was pointed out by Lei et al. [32] in a first-principle approach to the dielectric behavior of non-spherical cell suspensions.

5. Conclusions

We have analyzed the dielectric and conductometric properties of liposome aqueous suspensions on the basis of two different dielectric models, considering the ion distribution confined in an infinitely thin layer at the aqueous interfaces or distributed in the bulk of the inner and outer medium. Both the two models can in principle account for the dielectric behavior of heterogeneous systems characterized by a zwitterionic interface. However, whereas the absence of the bulk ion diffusion results in a permittivity higher than the one typical of a non-ionic bilayer, the addition of this ion polarization in both the inner and outer medium provides a very reasonable value for the bilayer permittivity ϵ_s and, moreover, furnishes a very good description of the observed dispersion, over the whole frequency range investigated.

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