Liquidlike structures in dilute suspensions of charged liposomes

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Electrostatically stabilized aqueous dispersions of both slightly and highly charged liposomes have been analyzed by quasielastic light scattering. The obtained static structure factor $S(q)$ revealed a liquidlike ordering due to long range electrostatic interactions. The fit of $S(q)$ assuming a Derjaguin–Landau–Verwey–Overbeek interaction potential within the Debye–Hückel approximation, the Ornstein–Zernike equation and the hypernetted chain approximation, as a closure relation, allowed obtaining an effective charge value. Simultaneously, alternative charge values were estimated from electrophoretic measurements, turning out to be very close to the effective ones. This study also suggests that the charge seems to be unequally distributed over the vesicles in the case of liposomes formed by mixtures of charged and uncharged lipids. Size polydispersity was also considered. © 2003 American Institute of Physics. [DOI: 10.1063/1.1553759]

I. INTRODUCTION

Liposomes are vesicles consisting of one or more concentric lipid bilayers encapsulating aqueous volumes. Since the publication of the first papers (in the 1960s), liposomes have become a major area of research, which nowadays goes on expanding. There exist several reasons for such a great interest. On the one hand, their biocompatibility makes them ideal candidates for the delivery of drugs and other agents of therapeutic value. This widespread use of liposomes in biotechnology and medicine has been reviewed in the last decade by several authors. On the other hand, they could be used as valuable models for the study of more complex biological systems such as cellular membranes. Whatever their application, lipid vesicles can be considered colloids whose stability is theoretically described (at least partly) in terms of classical colloidal forces. We would like to emphasize that, in our opinion, there are few studies focused on the interaction between liposomes considered as colloidal particles (e.g., colloidal structures, interplay between diffusion coefficient and volume fraction). Particularly, if liposomes are charged (as a result of the dissociation of chemical groups), electrostatic forces will have a considerable effect on their behavior. This work deals with the long-ranged electrostatic interaction appearing at low ionic strengths. Any progress in the knowledge of these forces can potentially contribute to the better understanding and control of the processes in which liposomes are involved. For instance, it has been proved for other colloids (e.g., latex particles, proteins) that the long-range forces have a considerable influence on collective diffusion phenomena. Thus in the case of lipid vesicles, their usefulness as carriers could depend (to great extent) on these forces. Besides, essential biological processes (such as adsorption and transport mechanisms of charged species across the cellular membrane) are mainly established by these interactions as well.

A key parameter that determines the magnitude of the electrostatic interaction is the charge on the particle surface. The total number of ionizable groups can be estimated from well-established methods (e.g., titration). However, this number will not be quite helpful if one does not know how many of the surface groups are actually dissociated at given experimental conditions. In fact, it has been reported that the charge values obtained from liquidlike structures observed in other colloidal dispersions (usually termed effective charges) are significantly smaller than those estimated from titrations.
(see Ref. 13 and references cited therein), even if the partial dissociation of surface groups is taken into account.

Many authors have attributed this discrepancy to a controversial matter concerning the modeling of the electrostatic interaction potential, \( u(r) \). As a result of being derived under the Debye–Hückel (DH) approximation, the charge appearing in the widely known Derjaguin–Landau–Verwey–Overbeek (DLVO) expression for \( u(r) \) should be considered as a renormalized rather than an actual parameter. A recent study on polystyrene (PS) latexes suggests, however, that renormalization theories will not always predict effective charges properly if the structure of the inner part of the electric double layer is not accounted for.\(^{14} \) In other words, specific interactions could reduce the surface charge (from which the renormalized charge should be calculated). This survey also concludes that the charge obtained from electrophoresis experiments (to which we will refer as electrophoretic charge hereafter) could provide a fairly good estimate of such a reduced surface charge. This method for determining liposome charges has been used sometimes instead of titration.\(^{10,13} \) The major inconvenient of the latter technique lies in the fact that liposomes are dynamical structures and it is essential to guarantee that neither the experimental conditions modify the bilayer distribution nor the flip-flop of charged molecules between the two monolayers occurs.\(^{16} \)

In this paper, liquidlike structures were formed in dilute suspensions of liposomes to address most of the previously discussed burning issues. Being more precise, we attempted to find out the charge characterizing long-range electrostatic interactions between any pair of lipid vesicles and to confirm the results with the help of electrophoretic mobility measurements. Apart from their practical applications in biotechnology, lipid vesicles present certain advantages. From an optical viewpoint, liposomes can be considered as colloids with an effective refractive index slightly different from that of the solvent, since they consist in an aqueous volume entirely enclosed by a thin layer of lipid molecules. Therefore, concentrated but relatively transparent colloidal dispersions can be prepared. Moreover, the relative easiness to manipulate their surfaces allows synthesizing particles whose charge can be controlled, providing an interesting real system of study in colloids science that offers the possibility of discussing the different theories about size and charge polydispersity.

The rest of the paper is organized as follows: First, the method for determining effective charges from light scattering experiments is reviewed. Second, experimental details on liposome characterization and structure factor determination are given. Then, our results are presented and discussed. The obtained effective charges will be analyzed comparing to those estimated from electrokinetic data.

II. THEORETICAL BACKGROUND

Although calculations become difficult, polydispersity in size and/or charge was considered in determining effective charges by assuming an \( m \)-component discrete distribution.\(^{17} \) Species \( i \) is characterized by a number fraction \( x_i \), a diameter \( \sigma_i \) and a charge \( Z_i \) (in elementary units). The microscopic structure of an uniform multicomponent colloidal fluid like this is described by the set of total correlation functions \( h_{ij}(r) \) with \( i \leq m \) and \( j \leq i \). These can be obtained from the solutions of \( m(m+1)/2 \) coupled Ornstein–Zernike equations

\[
h_{ij}(r) = c_{ij}(r) + \rho \sum_{k=1}^{m} x_k \int h_{ik}(|r-s|)c_{kj}(s) d^3s
\]

(where \( \rho \) is the number density of macroions) plus \( m(m + 1)/2 \) approximate closure relations connecting \( h_{ij}(r) \), \( c_{ij}(r) \) and the pair interaction potential \( u_{ij}(r) \). In the present work \( m = 3 \) was taken since the numerical procedure does not become extremely difficult and/or long.

There exist many examples of closure relations. In this paper the HNC was used since it has been successfully applied to highly charged systems. Then, the time-averaged light intensity \((\text{per unit of volume})\) scattered by the colloidal dispersion can be written in terms of suitably averaged structure and form factors as\(^{18} \)

\[
I(q) = \rho f^2 \tilde{P}(q) \tilde{S}(q),
\]

where \( q \) is the modulus of the scattering vector and

\[
f^2 = \sum_{i=1}^{m} x_i f_i^2,
\]

\[
\tilde{P}(q) = \frac{1}{f^2} \sum_{i=1}^{m} x_i f_i^2 B_i^2(q),
\]

\[
\tilde{S}(q) = \frac{1}{f^2 \tilde{P}(q)} \sum_{i,j=1}^{m} f_i f_j B_i(q) B_j(q)(1 + h_{ij}(q)).
\]

The expressions for the so-called field amplitude at \( q = 0 \) \( (f_i) \) and the normalized field amplitude \((B_i(q))\) must be provided by a light scattering theory (see, for instance, Ref. 17 for further information). In this case, the Rayleigh–Gans–Debye (RGD) theory (for vesicle-like particles) was used.\(^{19} \) Functions \( h_{ij}(q) \) were obtained through a Fourier transformation from the correlation functions.

The advantage of using Eq. (2) is that the averaged structure factor can be extracted directly from experimental light intensities (as in the one-component fluid) by means of

\[
\tilde{S}(q) = \frac{\rho_0}{\rho} \frac{I(q)}{I_0(q)},
\]

where \( I_0(q) \) is the light intensity scattered by a sample of noninteracting particles with number density \( \rho_0 \).

The interaction potential deserves a brief comment as well. This work deals with the microscopic structure exhibited by dispersions of negatively charged particles at very low ionic strength. Under such conditions, the electrostatic interaction becomes so strong that other forces can be neglected. The potential used for the electrostatic contribution in this case (spherical particles with large double layers) is the widely known DLVO potential calculated by Overbeek some decades ago. In the case of moderate polydispersity, the following expression can be applied for the interaction potential between particles with radii \( a_i \) and \( a_j \)
where \( L_B \) is the Bjerrum length and \( \kappa \) is the reciprocal screening length. Expression (7) has been derived from the Debye–Hückel approximation for the distribution of small ions around the particles. However, many theoretical studies point out the possibility of using the abovementioned DH expression for the description of highly interacting systems if certain parameters (such as \( Z_i \)) are renormalized. Equation (7) was applied in this context.

### III. EXPERIMENT

In this study, liposomes prepared from egg phosphatidylcholine (PC) and phosphatidylerine (PS) were used. Egg phosphatidylcholine (PC) (Lipoid E-100) and phosphatidylerine (PS) from bovine spinal cord were purchased from Lipoid (Ludwigshafen, Germany) and Lipid Products (Nutfield, UK), respectively. Phospholipids were dissolved in chloroform in a round-bottomed flask and dried in a rotary evaporator under reduced pressure at 50 °C to form a thin film on the flask. The film was hydrated with water to give a concentration of 30 mmol/cm\(^3\). Multilamellar liposomes were formed by constant vortexing on a vortex mixer and sonication in a bath, in both cases for 4 min. Then, they were downsized to form unilamellar vesicles by extrusion\(^{20}\) through polycarbonate membranes of a nominal size of 200 nm (Poretics, Livermore, CA) in an extruder device (LiposFast, Avestin, Ottawa, Canada). Liposome A is mostly made of PC, which is a zwitterionic phospholipid. It has a choline group and a phosphate group thus it is likely to be uncharged at moderate pH values. However, this liposome also contains a small proportion (1%) of PS. As a result of the dissociation of its groups, this molecule is expected to be negatively charged (1 e\(^-\) per molecule), which would give rise to a long-range repulsion between liposomes. The vesicle mean diameter was determined from dynamic light scattering (DLS), turning out to be 103 ± 3 nm. Recently, this technique has been preferred in size measurements.\(^{20–22}\) Liposome B is made only of PS, so it is expected to be much more charged than liposome A. The diameter obtained by DLS was 112 ± 3 nm.

The set up for the light scattering experiments was a 4700C System (Malvern) with an argon laser of 75 mW and wavelength 488 nm. The scattering experiments were performed at 25 °C, from 20 to 140 deg in 2-deg steps. Average intensities were obtained from several measurements at each angle, with different cell positions to minimize the effect of scratches on the glass surface. Prior to measurements, samples were homogenized in order to avoid particle density gradients. The suspensions studied in this work were kept for at least six days over a bed of ion exchange resin (Amberlite NMR-150). During this period, the function \( I(q) \) was monitored. When no changes were observed in this function, the deionization process was supposed to be complete. The volume fraction of the stock suspensions was estimated from the lipid weight fraction (which was known) and the mean outer and inner vesicle radii (\( a \) and \( b \), respectively), considering the thickness \( \Delta = a - b \) and the density of the phospholipid shell given elsewhere (4.5 nm and 1.015 g/cm\(^3\), respectively).\(^{23}\) More experimental details about the structure formation will be given later.

Electrophoretic measurements were also performed at 25 °C with a commercial device (ZetaPals) and using potassium nitrate as 1:1 electrolyte with a concentration range from 5 × 10\(^{-5}\) to 2 × 10\(^{-3}\) M. The sample weight concentration was 0.5% and error bars were estimated from six individual measurements.

### IV. RESULTS AND DISCUSSION

#### A. Slightly charged liposome

In order to determine the structure factor, the dependence of \( I_0 \) on \( q \) (or, equivalently, on the scattering angle, \( \theta \)) for a reference sample (i.e., without spatial ordering) is required, as mentioned above. These data were obtained from a dilute sample (\( \rho = 7 \times 10^{17} \) liposome/m\(^3\) for liposome A). Some preliminary investigations proved that this particle concentration was low enough (for nondeionized samples) to neglect the spatial ordering. If reproducible and smooth \( I_0 \)-data are desired, the particle concentration of the reference sample must not be extremely low since liposomes do not scatter so much light as other colloids of similar size (e.g., polystyrene latexes). The static light scattering (SLS) results for \( I_0 \) are plotted in Fig. 1(a) (as function of the scattering angle and normalized by the value corresponding to 90°). In this figure, the prediction for the ratio \( I_0(\theta)/I_0(90) \) assuming a monodisperse size distribution whose diameter is that obtained by DLS (103 nm) is also plotted. As can be seen, the agreement between theory and experiment is not very good (particularly at low angles). If
the RGD theory and the size measured by DLS are supposed to be valid, it should be concluded that the assumption of monodispersity might be failing. Unfortunately, we have not obtained precise information about the liposome size distribution. It would be instructive, however, to assume a certain functional form for it and probe if the theoretical predictions are improved in that way. As mentioned above, discrete distributions resembling the Schulz distribution were used, as D’Aguanno et al. did in their work on structural effects of polydispersity. The reader interested in additional information about such a distribution and the procedure for its theoretical treatment is referred to the mentioned study. In Fig. 1(a) the results corresponding to a mean diameter of 103 nm and different standard deviations, \( s (s = 10\%, 15\%, 20\%, 30\% ) \), were also plotted. None of these curves managed to fit the experimental data with outstanding precision. However, the predictions for \( s = 15\% \) and 20\% seemed to improve the results obtained for the monodisperse case suggesting, therefore, that size polydispersity might be of the order of 15\%–20\%. At any rate, the agreement between theoretical predictions and experimental data is still moderate, but similar to that found for liposomes by other authors (who use a monodisperse distribution with an adjustable diameter). In relation to this, one should keep in mind that the actual size distribution was unknown. In addition, polydispersity in shape could also be possible to some extent and the RGD theory might not be so good as desired for vesicles like these (with a shell of molecular dimensions).

Next, the structure was formed for a vesicle concentration of \( \rho \sim 1.25 \times 10^{19} \) liposomes/m\(^3\). On paper, the structure factor could be calculated using Eq. (6). In practice, however, certain difficulties come out. For instance, \( \rho \) and \( \rho_0 \) are hardly known with the desired precision. For this reason, we preferred to calculate \( S(q) \) by normalizing \( I(q)/I_0(q) \) in such a way that the structure factor exhibited damped oscillations around 1 for the highest accessible scattering vectors. The result obtained for liposome A is shown in Fig. 2(a). Apparently, this procedure (which was applied successfully to latex dispersions\(^{25,26} \)) led to a reasonable structure factor. Obviously, this figure revealed the existence of liquidlike order in the vesicle suspension. The experimental structure factor was fitted using the DH-like potential and the Ornstein–Zernike equation. A size polydispersity of 20\% was assumed (according to the analysis of the reference intensity). There must also exist certain charge polydispersity if one supposes that the particle charge scales linearly with their surface area. It should be noted that this kind of charge polydispersity (associated with the nonuniform size distribution) is consistent with the assumption that the PS/PC ratio is the same (1\% in this case) for any vesicle (irrespective of its size). Given an interaction potential \( \mu(r) \), the structure factor (as well as the radial distribution function) can be calculated theoretically from three main parameters: the vesicle concentration, the charge per liposome, and \( \kappa a \) (also termed electrokinetic radius). In deionized suspensions, the latter depends strongly on \( Z \), but also on the concentration of ionic impurities. Unfortunately, the precise value of this residual ionic concentration is extremely difficult to monitor, although some estimations have been made.\(^{27} \) According to them, it was supposed that contribution to the ionic strength of these unidentified ions was of the order of \( 10^{-6} \) M. In this way, \( \kappa \) could be expressed as \( \kappa = \sqrt{\epsilon^2 (\rho Z + 10^{-2} N_A)/\epsilon_0 e^2 kT} \) (where \( N_A \) is the Avogadro number). The effective charge was determined fitting the height of the experimental scattering data.\(^{28} \) The (mean) value used for \( Z \) in this fit was 200 electrons. Concerning the fits themselves, the agreement was acceptable only for the main peak. At low \( q \)-values, the experimental structure factor was larger than predicted. In relation to this matter, it should be pointed out that Eq. (2) takes into consideration the averaged coherent intensity. However, the presence of large vesicles whose scattering material is not distributed symmetrically around the particle center would originate an incoherent scattering, whose effects would be particularly noticeable just for low \( q \)-values.\(^{28,29} \) Apart from this, the oscillations observed beyond the main peak were not so pronounced as predicted, which is not usually observed in monodisperse latex suspensions.\(^{25,26} \) This question will be discussed later.

At this point, the charge obtained fitting should be analyzed. To begin with, this value could be compared with the bare charge (i.e., the number of charged groups present in the outer leaflet of the bilayer), which was calculated taking an estimate of area per lipid into account (70 Å\(^2\))\(^{30} \) and turned out to be about 470 electrons/liposome. Perhaps, this quantity might be a bit larger since some authors have reported that the charged groups are not distributed proportionally between both leaflets if the charged/neutral aminophospholipid ratio is lower than 50\%.\(^{15,31} \) Whichever the case, the effective charge is lower than the bare one, as some authors have
found for other colloids.\textsuperscript{13,25} On the other hand, a new charge provided by electrophoretic measurements was also calculated (to which we will refer as electrokinetic charge $Z_\zeta$), which quantifies the net charge resulting from the colloidal particle and the double layer limited by the shear surface. With this aim, the electrophoretic mobility was measured as a function of the electrolyte concentration (see Fig. 3) and converted into $\zeta$-potentials applying the well-known O’Brien–White theory. Finally the following relation was used to calculate the electrokinetic charge.

$$Z_\zeta = \frac{4 \pi \varepsilon_0 e^2 k_B T \kappa \alpha^2}{e^2} \left( \sinh \left( \frac{e \zeta}{2 k_B T} \right) \right) + \frac{4}{\kappa \alpha} \tanh \left( \frac{e \zeta}{4 k_B T} \right).$$

(8)

This expression is valid for a 1:1 electrolyte and provides the electrokinetic charge to within 5% for $\kappa \alpha > 0.5$ and any $\zeta$-potential. As can be inferred from Fig. 4, the charge obeys approximately a linear dependence on $\kappa \alpha$ (this correlation between the surface charge of liposomes and electrolyte concentration has been previously reported),\textsuperscript{32} being of about 300 electrons in the limit of low ionic strength and tending to 450 electrons for $\kappa \alpha$ values around 8. It should be noted the coincidence between the latter value and the bare charge estimated previously. However, the comparison between the electrokinetic charge at low ionic strengths and the effective charge is much more interesting. At first sight, one could think merely that the former is a bit larger than the latter but it would be useful to estimate the uncertainties in both charges before drawing any conclusion.

Although the method for determining the effective charge involves a large number of steps, statistical errors might have (in our opinion) two major causes of experimental nature: the uncertainty in the height of the main peak ($S_{\text{max}}$) and the lack of precise knowledge of the ionic strength. Admitting a statistical error of $\pm 0.05$ in $S_{\text{max}}$, some calculations showed that the uncertainty in the effective charge was $\pm 20$ electron/particle ($10\%$). The result for $I_{\text{res}}$ = $10^{-6}$ M was therefore $200 \pm 20$ electron/liposome. Unfortunately, the value of $I_{\text{res}}$ was only an estimate. Considering $5 \times 10^{-6}$ M as the upper limit for this quantity, an effective charge of $230 \pm 20$ electron/liposome was obtained. Both results might be summarized as $Z_{\text{eff}} = 215 \pm 35$ electron/liposome (see Table I). Concerning the electrokinetic charge, we took its value and error as those corresponding to the independent term of the linear fit (since very low ionic strengths characterized the systems investigated). The result was $315 \pm 20$ electron/liposome. Thus the fact that the effective charge was smaller than the electrokinetic charge seemed to be confirmed.

These results allowed for size polydispersity as well as the charge polydispersity associated to it (mentioned above). Nevertheless, it might also happen that the proportion of PS fluctuates from one vesicle to another, which has been found by means of different techniques when liposomal membranes are composed of different phospholipids.\textsuperscript{33,34} In that case, the PS/PC ratio reported previously (1%) would be just a mean value determined by the synthesis method and fluctuations in charge might be larger than those caused by a nonuniform size distribution. This composition-associated charge polydispersity would also cause a loss of structure, even in the case of size monodispersity.\textsuperscript{17} Unfortunately, we had no knowledge of the extent of such kind of polydispersity for liposome A. However, its effects were illustrated assuming also the Schulz distribution and a given standard deviation, $s = 60\%$. Certainly, this value is considerably large but feasible (since the PS/PC ratio is quite small). This particular example [plotted in Fig. 2(a) as well] showed that: (i) the experimental data could be fitted with an effective charge practically identical to the electrokinetic one ($Z_{\text{eff}} = 320$ electron/liposome); (ii) the oscillations beyond the main peak were a bit more damped (for the same height of the main peak). In addition, noticeable changes came out in

![FIG. 3. Electrophoretic mobility as a function of the logarithm of the salt concentration for liposomes A (solid squares) and B (open squares).](image)

![FIG. 4. Electrokinetic charge as a function of $\kappa \alpha$ for liposomes A (solid squares) and B (open squares). Solid lines denote the best linear fits.](image)

**TABLE I.** Different values of charges (expressed in electron/liposome) obtained for liposome A and B.

<table>
<thead>
<tr>
<th>Liposome</th>
<th>Charge Value (electron/liposome)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>$Z_{\text{eff}}$ assuming $I_{\text{res}} = 10^{-6}$ M</td>
<td>200 ± 20</td>
</tr>
<tr>
<td>$Z_{\text{eff}}$ assuming $I_{\text{res}} = 5 \times 10^{-6}$ M</td>
<td>230 ± 20</td>
</tr>
<tr>
<td>$Z_{\text{eff}}$ summarizing the results shown above</td>
<td>215 ± 35</td>
</tr>
<tr>
<td>$Z_{\text{eff}}$ assuming 60% in charge polydispersity</td>
<td>320</td>
</tr>
<tr>
<td>$Z_\zeta$</td>
<td>315 ± 20</td>
</tr>
</tbody>
</table>
the low-\(q\) regime (as compared to the case of moderate size-associated charge polydispersity). As the experimental data in this regime were not so reliable, a quantitative analysis was not possible. However, certain qualitative agreement between theory and experiment seemed to exist for low angles. At any rate, the improved fit obtained considering charge polydispersity suggested that the liposomes of this study were not homogeneous, probably due to the used synthesis process. The structure factor of liposomes could be used somehow as an additional test for probing the composition heterogeneity, which is valuable information to evaluate new preparation methods of liposomes.

At any rate, it could be concluded that the electrokinetic charge is of the same order as the effective charge, perhaps a bit larger. This would be consistent with the idea of renormalization if one admits that \(Z_\zeta\) gives a reasonable estimate of the actual surface charge of liposomes.

Apart from that, it should be point out that, in this work, we have looked into a suspension whose volume fraction (close to 1%) was much larger than in the case of polymeric latexes of similar size.\(^{14}\) For volume fractions of this order or higher, some authors have reported the formation of colloidal crystals in deionized media.\(^{35}\) With the naked eye this phenomenon can be revealed by certain iridescence. However, such iridescence was not observed, which is not surprising. Colloidal crystals could be expected only if electrostatic forces are strong enough (even for volume fractions lower than 1%).\(^{11}\) This would not be our case, since Fig. 2(a) shows clearly that our dispersions are not highly structured.

### B. Highly charged liposome

In this section, the results for liposome B are presented and discussed. As this system is made of PS exclusively, the charge polydispersity associated to the composition cannot be present in its vesicles. The normalized reference intensity [plotted in Fig. 1(b)] was obtained for a sample concentration of \(\rho_0 = 4.6 \times 10^{17}\) liposomes/m\(^3\). The solid line denotes the theoretical result for a diameter of 110 nm and \(s = 0\%\). As can be seen, this plot suggests that size polydispersity was not so large as in the case of liposome A. In Fig. 2(b), the structure factor determined (for a concentration of about \(5.4 \times 10^{18}\) liposome/m\(^3\)) is shown. Among the differences that could be found comparing the structure factors of both liposomes, it should be stressed that the oscillations beyond the first peak were not so damped. This upholds the hypothesis that polydispersity does play an important role in the case of liposome A. The fit to the experimental structure factor was also tried. The result for an effective charge of 240 electron/liposome (and a residual ionic strength of \(10^{-6}\) M) is shown in Fig. 2(b) as well. As can be seen, the fit succeeded in justifying experimental data only semi-quantitatively. The main peak and the amplitude of the secondary minimum were well captured. However, the secondary maximum was not fitted properly. This failure is not common in the case of latexes,\(^{25}\) whose behavior is almost exemplary from this viewpoint. However, one should bear in mind that liposomes cannot be considered as good model systems as polymeric latexes. Lipid vesicles are not hard spheres since they could undergo certain deformations (which might be caused, for instance, by a difference of osmotic pressure between the inner and outer media).\(^{24}\)

It is worth discussing again the value of the effective charge obtained fitting (as well as its uncertainty). The results corresponding to the case \(I_{res} = 10^{-6}, 5 \times 10^{-6}\) M and the estimate based on both are also summarized in Table I. As can be concluded, the effective charge of liposome B is somewhat larger than that of liposome A (in any case), despite the fact that there is an extremely big difference between their bare charges. In order to find out if the electrokinetic properties corroborate these findings, the electrophoretic mobility was also measured. As can be seen in Fig. 3(b), this quantity is undoubtedly smaller for the slightly charged vesicles (as expected). However, the conversion into \(\zeta\)-potentials and the calculation of the electrokinetic charge provided us more information, as can be concluded from Fig. 4(b). At high \(ka\)-values, liposome B actually possessed much larger electrokinetic charges than liposome A. With decreasing the ionic strength, however, such differences became smaller. At low enough \(ka\), we only know that \(Z_\zeta\) is slightly larger for liposome B. This is just the trend found for the effective charge. One can also consider the statistical error of the electrokinetic charge (the one given by the linear fit for the independent term), obtaining that \(Z_\zeta = 300\) ± 100 electron/liposome. The accuracy of this quantity was only moderate because the slope of the fit was quite large. Within this uncertainty, anyhow, it can be concluded that the electrokinetic charge is really close to the effective charge. In other words, the electrokinetic behavior supplies valuable information about the charge characterizing the electrostatic interaction. This conclusion, which had already been established in the case of latexes,\(^{14}\) is now confirmed for liposomes. It should be emphasized that the charge is a parameter of great relevance for colloids and many researchers are nowadays trying to elucidate this kind of phenomena to connect the different concepts of charge.\(^{36}\) In the case of liposomes, for instance, the diffusion processes of these vesicles can be easily influenced by strong electrostatic forces,\(^{37}\) which allows determining another effective charge. This parameter has also turned out to be of the order of the electrokinetic charge.

### V. CONCLUSIONS

In this paper, liquidlike ordered colloidal suspensions of both slightly and highly charged liposomes have been analyzed by light scattering techniques. To the best of our knowledge, it is the first time that liquid structures involving phospholipidic vesicles have been reported and their structure factor fits have been used as a tool to determine their effective charge. This charge has been also compared to other values, i.e., electrokinetic and bare charges. Our results confirm that the electrokinetic charge is a suitable parameter to quantify the long-ranged electrostatic interaction. In the case of the highly charged liposome, the electrokinetic and effective charges agree acceptably within the estimated statistical errors, which can be considerable because of the experimental and theoretical procedures involved. In the case of the slightly charged liposome, both charges are at least of
the same order. Our results suggest the possibility of large fluctuations of charge from one vesicle to another.

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