Probing the jellium model with colloidal dispersions of charged liposomes

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Abstract

The structure factor, $S(q)$, is measured for a set of dispersions that consist of liposome vesicles with differing numbers of ionizable surface groups. These systems allow us to probe the dependence of the local structure on the particle bare charge systematically. The height of the nearest neighbor peak in $S(q)$, which is a direct measure of the spatial order of the vesicles, does not increase monotonically with the number of ionizable groups for a given volume fraction. On the contrary, it rather decreases. This feature cannot be explained by means of the classical renormalization scheme based on the cell model. We analyze our experimental data using a renormalization model based on the jellium approximation, which predicts the weakening of the spatial order for moderate and large particle charges and we find a qualitative agreement.

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1. Introduction

In spite of the relevance that colloids play in everyday life, many colloidal properties such as structure, dynamics and rheology, and their dependence on the colloid–colloid interactions are still not entirely understood. Any progress in this field demands a better comprehension of these interactions.

A typical issue is the precise description of the electrostatic forces exerted by the particles on each other in the case of charge-stabilized dispersions. The well-known Derjaguin–Landau–Verwey–Overbeek (DLVO) theory provides an analytical expression for the interaction potential, $u(r)$, of charged spherical colloidal particles, whose functional form is similar to the one of the Yukawa-potential:

$$u(r) = \frac{(Ze^2 \exp(2\kappa a) \exp(-\kappa r))}{4\pi\varepsilon_0\varepsilon_r(1 + \kappa a)^2} r$$

here, $Z$ is the number of charges per colloidal particle, $e$ the elementary charge, $\kappa$ the Debye–Hückel screening parameter and $\varepsilon_0\varepsilon_r$ is the dielectric permittivity of the solvent. This expression was derived applying the so-called Debye–Hückel approximation and thus is strictly valid for small surface charges only. Nevertheless, a large number of theoretical work claimed that Eq. (1) could be valid if $Z$ is considered a renormalized charge, $Z_{\text{eff}}$, instead of the actual one (see [1–3] and references cited therein). Several procedures aiming to predict $Z_{\text{eff}}$ as well as $\kappa$ were proposed. In the middle 1980s Alexander et al. [4] proposed the cell model, which has been widely used since then. In this model, the colloids are assumed to occupy a fixed position within a Wigner–Seitz (WS) cell where the total charge is zero due to the electroneutrality principle. The local electrostatic potential is then calculated in one WS cell only, as that region is void from other colloids. At the same time, Beresford-Smith et al. [5] developed a renormalization scheme based on the so-called jellium approximation. For the calculation of the electrostatic potential, $\psi(r)$, around a colloidal particle this theory assumes that there are no correlations between the particles, the colloid–colloid radial distribution function is $g(r) = 1$. In contrast with the cell model, the colloidal particles are taken into account for the evaluation of $\psi(r)$ but the dispersion is supposed to be completely disordered.

Although the model developed by Beresford-Smith et al. has not been applied as extensively as the cell model has, a few recent papers have shown a resurgent interest in the jellium approximation [6–8], emphasizing some of its more peculiar and characteristic predictions. Indeed, Trizac and Levin propose...
a renormalized jellium model and argue why the effective charge obtained from it is more relevant to study colloid–colloid interactions [6–7], and Rojas et al. find in their experiments that the amplitude of the first peak of the structure factor does not increase monotonically with the volume fraction (ϕ) but shows a minimum [8]. This minimum had already been predicted by Beresford-Smith et al. [5], but it had not been corroborated experimentally before. Another interesting theoretical prediction of the model is concerned with the dependence of the structure with the particle bare charge. By increasing this parameter, Beresford-Smith et al. found that the first peak of the structure factor should exhibit a maximum. However, as far as we are aware, this maximum has not been observed experimentally to date.

The aim of this work is to investigate whether this behavior can be observed in a set of colloidal dispersions with increasing the particle surface charge. In order to characterize the evolution of static properties with the particle charge, i.e. the number of ionizable groups, we study several colloids with similar size but different composition. In particular, the systems examined are liposome dispersions. The rationale for this choice is two-fold. Firstly, these vesicles have a refractive index similar to that of the solvent, so multiple scattering is negligible, which enables us to study relatively concentrated samples. Secondly, the liposome composition can be easily varied, which enables us to make vesicles of similar size but very different numbers of ionizable groups. Besides the theoretical interest in colloidal science, the study of stability of liposome dispersions has a marked importance. Liposomes are nowadays used in pharmacy as drug delivery systems and can be used as model systems to study several biological processes, such as transport of substances across the cellular membrane or interactions with proteins or DNA.

The remainder of the paper is organized as follows. Firstly, the essential features of the jellium model are briefly reviewed. Secondly, the experimental systems and methods are described in some detail. Subsequently, the experimental results are presented and compared to theory and finally some conclusions are outlined.

2. The jellium model

In the jellium model, the functional form of colloid–colloid interaction potential is similar to the one of a Yukawa potential (see Eq. (1)) but the charge (Z) is replaced by a renormalized value (Zn) which can be computed from the electrostatic potential as follows. First, the normalized electrostatic potential, Ψ≡e/ε0kB T, is calculated by solving a Poisson–Boltzmann (PB) equation in which the presence of other colloidal particles is treated as if it was a small ion, additionally

\[ \Psi(r) = \frac{2}{\epsilon} \sum_{i} \frac{Z_i}{r_i} \exp(-\kappa r_i) \]

where \( \kappa = e^2/(4\pi\epsilon_0 n_i k_B T) \) is the Bjerrum length, \( n_i \) and \( n_s \) are the concentration of colloidal particles and monovalent salt, respectively, and \( r_i \) is the particle radius. Eq. (2) is solved together with the boundary conditions \( \Psi(\infty) = Z_n/\kappa a \) and Gauss’ law is applied to the particle surface, and \( \Psi(\infty) = 0 \). The far-field solution of Eq. (2) can be matched by:

\[ \Psi(r) = \frac{Z_n}{\epsilon} \exp(-\kappa r) \]

which is the solution of a linearized Eq. (2). This is illustrated in Fig. 1, where the solution for \( Z = 500e^-/\text{particle} \), a salt concentration of 0.005 mM and \( \phi = 0.01 \) (solid line) is compared to the asymptotic solution derived from Eq. (3) with \( Z = 323e^-/\text{particle} \) (dotted line). As can be seen, the agreement is almost perfect far from the particle surface. In any case, the value of \( \kappa \) assumes for this model:

\[ \kappa = \sqrt{4\pi n_i (2Z_n + Z_p)} \]

It should be noted that here \( \kappa \) is not renormalized, in contrast to the cell model, and it depends on the bare charge, \( Z \). This is the key point to understand our results, as discussed below.

3. Experimental

Our liposomes are composed of egg phosphatidylcholine, PC, and phosphatidylserine, PS. They are obtained according to the lipid film method where the phospholipids are 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 surface of the flask. The film is subsequently hydrated with ultrapure distilled water to give a final concentration of 30 mM/l. Multilamellar liposomes are formed by constant stirring for 4 min and sonication in a bath for 4 min. Then, they are downsized by extrusion [9] through polycarbonate membranes of a nominal size of 100 nm (Poretics, Livermore, CA) in a extruder device (Liposo-Fast, Avestin, Ottawa, Canada). This method produces nearly monodisperse, unilamellar vesicles. By varying the proportion PS/PC, five types of liposomes are prepared, with characteristics given in Table 1. Phosphatidylserine
Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>L1</th>
<th>L25</th>
<th>L50</th>
<th>L75</th>
<th>L100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (nm)</td>
<td>115±7</td>
<td>95±4</td>
<td>120±5</td>
<td>115±7</td>
<td>123±7</td>
</tr>
<tr>
<td>Polydispersity</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Composition (PS:PC) mol/mol</td>
<td>1:99</td>
<td>25:75</td>
<td>50:50</td>
<td>75:25</td>
<td>100:0</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>φ (%)</th>
<th>Z (groups PS/particle)</th>
<th>Z_{eff} (no renormalization)</th>
<th>Z_{jellium}</th>
<th>Z_{bare}</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>0.85</td>
<td>540</td>
<td>420</td>
<td>313</td>
<td>420</td>
</tr>
<tr>
<td>L25</td>
<td>1.10</td>
<td>12000</td>
<td>330</td>
<td>306</td>
<td>520</td>
</tr>
<tr>
<td>L50</td>
<td>0.82</td>
<td>29000</td>
<td>260</td>
<td>535</td>
<td>2000</td>
</tr>
<tr>
<td>L75</td>
<td>1.00</td>
<td>40000</td>
<td>165</td>
<td>547</td>
<td>2500</td>
</tr>
<tr>
<td>L100</td>
<td>0.83</td>
<td>60000</td>
<td>170</td>
<td>604</td>
<td>3200</td>
</tr>
</tbody>
</table>

has a polar head with three different groups, two anionic and one cationic. At our experimental conditions all groups are dissociated, so the molecules carry a net negative charge. On the contrary, phosphatidylcholine, which is a zwitterionic phospholipid, has a zero net charge, so by varying the ratio PS:PC we effectively can vary the bare charge of the vesicles. The total number of charged phospholipids per liposome (Table 2, column 3), is computed assuming an area per lipid molecule of 70 Å² [10].

We estimate the volume fraction of the stock suspension from the lipid weight fraction and the mean outer, $a$, and inner vesicle radii, $b$, assuming the phospholipid shell to have a thickness of $\Delta = a - b = 4.5$ nm and a density of $\rho = 1.015$ g/cm³ [11,12].

Our samples are prepared by diluting the stock solutions at the desired volume fraction of around 0.9% and subsequently deionized them with an ion exchanger (Amberlite NRM-150) in sealed quartz cells.

We measure the mean intensity scattered by the samples as a function of angle at 25°C using an ALV-5000F device with an Argon-Ion Laser (Coherent, model Innova 308) of wavelength $\lambda_0 = 514.5$ nm. Prior to measurement, the samples are homogenized in order to avoid any density gradient.

4. Results and discussion

The determination of structure factor from the dependence of the scattered intensity $I(q)$ on the magnitude of the wave-vector, $q$, requires the knowledge of the form-factor $F(q)$ of the system since,

$$S(q) \propto \frac{I(q)}{F(q)}$$

We measure the form-factor of our vesicles using a dilute sample ($\phi \sim 0.2\%$), where a certain amount of salt is added to screen the electrostatic interactions, so spatial ordering becomes negligible. Fitting our results to the form-factor of a hollow sphere [13] in the Rayleigh-Gans-Debye regime yields the diameter and polydispersity index listed in Table 1.

The structure factors of our samples exhibit a strong dependence on both, the composition and $q$, as shown in Fig. 2. All our samples display a marked nearest neighbor peak, whose height, $S_{\text{max}}$, strongly depends on the composition, which can be expressed in terms of the bare charge, $Z_{\text{bare}}$, as shown in Fig. 3. In reality, $Z_{\text{bare}}$, as appears in the graphic, is not equivalent to...
the number of charged groups per liposome but a fitting parameter obtained from the theoretical treatment of the experimental data. We find that $S(q_{\text{max}})$ decreases with $Z_{\text{bare}}$. This finding is at first sight somewhat counterintuitive as we might expect the local order should increase as the number of charged groups per particle increases. In order to gain a better insight on this matter, we have followed different approaches. First, our data are fitted assuming a Yukawa-like potential using the hypernetted chain closure relation (HNC) and the Rescaled Mean Spherical Approximation (RMSA). Both closure relations yield similar results, thus we only show the result of the HNC-closure as a dotted line in Fig. 2. In both cases, the inverse of the Debye length is calculated by using $\kappa = \sqrt{4\pi l_B (Z_{\text{bare}} + Z_{p} q_{\text{max}})}$, where $Z_{p}$ is the effective charge of the particles. In our second approach, we have assumed that most of the groups are ionized for this system. By summing all the charges, we have obtained $Z_{\text{bare}} = 420$ electrons for L1, where $Z_{p}$ corresponds to the number of PS molecules per vesicle (Table 2, Column 3); (ii) the difference between both values is more pronounced for the most charged systems. In relation to the $Z_{\text{bare}}$ values used as input parameters for the fits (Table 2, Column 6), it should be kept in mind that: (i) these do not correspond to the estimated number of charged molecules per liposome (Table 2, Column 3); (ii) the difference between both values is more pronounced for the most charged systems. For instance, we use $Z_{\text{bare}} = 420$ electrons for L1, where the number of PS molecules per vesicle is $340$; thus, we presume that most of the groups are ionized for this system. By contrast, for L100 the number of ionizable groups per vesicle is $60000$ whereas the fit provides a bare charge of $3200$ electrons. Such a low charge makes us think about a possible condensation of ions on the particle surface. Maybe, the pH in the surroundings of the vesicles is much lower than in the bulk of the solution what influences on the dissociation degree of the ionizable groups. In that case, a chemical regulation model of charge should be necessary in order to estimate the dissociation degree of ionizable groups under these conditions [14].

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![Fig. 4. Renormalized charges (left axis, solid line) and $\kappa$ values (right axis, dotted line) as a function of the bare charge provided by the Alexander and the jellium models (particle radius: 50 nm, Volume fraction: 0.0122, residual monovalent salt concentration: 0.001 mM).](image-url)
5. Conclusions

We find a reduction of the local structure in dispersions of charged liposomes with varying the surface charge. Namely, we observe the height of the nearest neighbor peak decreases as the surface charge is increased. The well-known Alexander’s model fails to describe this feature, while we find that a renormalization theory based on the jellium model describes the experimental findings at least qualitatively. Nonetheless, we believe that the jellium model might fail significantly in the case of highly structured colloidal dispersion, as it presumes a completely disordered suspension for the calculation of the electrostatic potential.

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References