Interplay between hydrodynamic and direct interactions using liposomes

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This work deals with the short-time effective diffusion coefficient of charged and uncharged liposomes, measured (as a function of the volume fraction) using fiber optic dynamic light scattering. Particularly, we are interested in the interplay between electrostatic and hydrodynamic interactions on the diffusion of these lipid vesicles. Regarding the charged liposome, it has been found that the effective diffusion coefficient can be theoretically justified for volume fractions not exceeding certain critical value. In applying the theoretical approach, a surface charge has been obtained which is consistent with the electrokinetic characterization of the liposome. Regarding the uncharged liposome, the hard-sphere model seems to account for reasonably well the self-diffusion data. In addition, comparing the measurements of the short-time self-diffusion coefficient for both liposomes, we conclude that strong electrostatic forces (direct interactions) slow down diffusion processes. © 2003 American Institute of Physics. [DOI: 10.1063/1.1578628]

I. INTRODUCTION

It is well known that diffusion processes of colloidal particles depend on their concentration. Generally speaking, this is a consequence of direct interparticle interactions (DIs), electrostatic or hard-sphere repulsion, and solvent-mediated many-body hydrodynamic interactions (HIs), due to a coupling of the particle motion via the fluid. When both become important, the particle dynamics is a fascinating subject in which the interplay between HIs and DIs remains an open question. In this paper, we focus on the relationship between hydrodynamic and electrostatic interactions via the measure of the short-time diffusion coefficient of colloidal particles, using light scattering methods. Reviewing the scientific literature, one readily concludes that finding out if their combined effect helps charged colloidal particles to diffuse faster than the uncharged ones is controversial.1–6 For instance, Overbeek et al.1 work with polystyrene particles of 135 nm in radius and they use dynamic-light-scattering (DLS) cross-correlation techniques. From measurements of the short-time translational self-diffusion coefficient \( D_{\text{self}} \), as a function of volume fraction \( \phi \), they obtain that \( D_{\text{self}} \) is higher for charged spheres as compared to theoretical hard spheres at similar volume fractions. Bergenholtz et al.2 using fiber optic quasielastic light scattering (FOQELS), have also measured \( D_{\text{self}} \) for polystyrene particles with diameters ranging from 120 to 310 nm. They conclude, however, that the self-diffusion coefficient is unaffected by changes in particle size and ionic strength—i.e., changes in the direct interactions. Few years ago, van Veluwen et al.7 obtained similar results with dynamic light scattering technique using charged and sterically stabilized silica dispersions of 800 nm in diameter. Other authors obtain that an increase in DIs slows down the particle diffusive process. This is what Riese et al.3 report using a combination of dynamic x-ray scattering (DXS), cross-correlated dynamic light scattering (CCDLS), small-angle x-ray scattering (SAXS), and colloidal silica spheres of 110 nm in diameter. In the case of colloidal monolayers, Zahn et al.4 use paramagnetic polystyrene particles and, measuring the self-diffusion at intermediate and long times, they find that HIs enhance the self-diffusion coefficient of colloidal particles.

In this work, we also address this burning issue, but using a different colloidal system: liposomes in water. Lipids form vesicles that can be considered as colloidal particles. In biotechnology, they are extensively used as vehicles for the
delivery of drugs. In colloid science, their potential value relies upon the relative easiness with which their surfaces can be manipulated, either by choice of the bilayer lipid composition or by chemical modification to incorporate different molecules; i.e., we can obtain both charged and uncharged spheres of similar sizes. In this way, we have particles different from those customarily used (silica, latex, PMMA) for diffusion studies. Apart from that, it is well known that a crucial feature in light scattering experiments is to avoid the effects of multiple scattering. In this sense, liposomes present certain advantage, since they consist of an aqueous volume entirely enclosed by a thin layer of lipid molecules. Thus, from an optical viewpoint, liposomes can be considered as colloids with an effective refractive index slightly different from that of the solvent and concentrated, but relatively transparent colloidal dispersions can be prepared. In our case, as we will see below, liposomes allow us to explore moderate particle volume fractions (up to 10%).

With reference to experimental aspects, we have probed the behavior of the effective translational diffusion coefficient of liposomes (as a function of the volume fraction) by dynamic light scattering experiments (at a fixed angle). One should recall that there exists a characteristic length in a colloidal suspension, \( q_m^{-1} \): i.e., the reciprocal of the modulus of the scattering vector value at which the main peak of structure factor appears. If measurements are performed at \( q \) values (where \( q \) stands for the modulus of the scattering vector) larger than \( q_m \), we deal with \( D_{\text{self}} \), whereas if \( q \ll q_m \) the collective short-time diffusion coefficient \( D_q = D_{\text{eff}}(q \rightarrow 0) \) will be measured. In the scientific literature, we can find measurements both at \( q \ll q_m \) and \( q \gg q_m \). However, at \( q \sim q_m \) these data are rather scarce, which has also been pointed out by other authors.\(^8,9\) The paper also deals with the effective diffusion coefficient precisely in the vicinity of \( q_m \).

As mentioned above, we are interested in the effect of the electrostatic interactions on the diffusion of these lipid vesicles, since their usefulness as carriers (in biotechnology) could depend certainly on these forces. Our results will be analyzed with the help of some theories related to diffusion processes which take into account the structure factor as well as the hydrodynamic function\(^10\) (which had already been applied to latex particles). In the case of charged liposomes, being more precise, we will also examine to what extent the charge obtained through an additional technique of colloidal characterization (electrophoresis) can justify our experimental diffusion data.

The remainder of this work is organized as follows: First, the theoretical background concerning these topics is briefly outlined. Then, in Sec. III, some details about the synthesis and characterization of the liposomes used in this work are given. The experimental determination of the effective diffusion coefficient is also included. In Sec. IV, the results are presented and discussed. Finally, several conclusions are highlighted.

II. THEORETICAL BACKGROUND

To begin with, we should point out that liposomes are not usually so monodisperse as other colloids (e.g., polymer latexes). In spite of this, we will restrict ourselves to theoretical treatments of identical spheres, since allowing for polydispersity requires the precise knowledge of the size distribution, which in this case is not so easy to obtain. In this sense, this simple analysis should be considered as a starting point for more sophisticated studies.

In DLS, it is well known that the fluctuations of the scattered light intensity can be characterized by means of the normalized electric field autocorrelation function, whose short-time behavior can be written as

\[
g^{(1)}(q, \tau) = \exp(-q^2 D_{\text{eff}} \tau).
\]

Here \( \tau \) stands for time and \( D_{\text{eff}} \) is an effective diffusion coefficient, which is given by\(^11\)

\[
\frac{D_{\text{eff}}(q)}{D_0} = \frac{H(q)}{S(q)}.
\]

where \( D_0 \) is the single-particle diffusion coefficient, \( H(q) \) is the hydrodynamic function, and \( S(q) \) the structure factor of the dispersion. \( H(q) \) contains the configuration-averaged effect of hydrodynamic interactions on the dynamics of particles. For a colloidal dispersion in which the separation between colloidal particles are higher than the particle radius \( a \), Nagéle and Baur showed\(^10\)

\[
H(z) = 1 - 15\phi \frac{J_3(z)}{z} + 18\phi \int_1^\infty y \{ g(y) - 1 \} \frac{J_1(zy)}{zy} \frac{J_1(zy)}{6y^2} dy.
\]

In this equation, \( z = 2qa, \ y = r/2a, \ J_n(z) \) is the spherical Bessel function of order \( n \), and \( g(y) \) is the radial distribution function. With regard to \( S(q) \), this function includes the same information about the spatial ordering as the radial distribution function \( g(r) \). Given a suspension of particles interacting via a known potential, \( g(r) \) can be calculated through different avenues.\(^12\) For instance, it can be obtained solving the Ornstein–Zernike equation together with a suitable (approximate) closure. In this work, the structure factor of the charged liposome was calculated using the rescaled mean spherical approximation (RMSA).\(^13\)

Concerning the self-diffusion (i.e., \( q \rightarrow \infty \)) coefficient of uncharged hard spheres, there exists extensive literature.\(^14–18\) At small particle volume fractions, the well-established result is

\[
\frac{D_{\text{self}}}{D_0} = 1 - 1.831\phi + 0.71\phi^2 + O(\phi^3),
\]

where \(-1.831\) incorporates two-body HIs (Refs. 16–18) and 0.71 accounts for three-body HIs (Ref. 14). However, Beenakker and Mazur\(^14\) obtain \(-1.73\) instead of \(-1.831\) in considering only two-body HIs in the calculation to the \( \phi \) term.
III. EXPERIMENT

A. Characterization of the liposomes

In this work we employ liposomes with different sizes and surface charges. The substances used in their preparation are egg phosphatidylcholine (PC) and phosphatidylserine (PS). Egg PC (Lipoid E-100) was purchased from Lipoid (Ludwigshafen, Germany), and PS from bovine spinal cord was from Lipid Products (Nutfield, UK). 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 μmol/ml. Multilamellar liposomes were formed by constant vortexing for 4 min on a vortex mirror and sonication in a bath for 4 min. Then they were downsized to form unilamellar vesicles by extrusion through polycarbonate membranes of a nominal size of 200 nm (Poretics, Livermore, CA) in an extruder device (LiposFast, Avestin, Ottawa, Canada). This method produced unimodal distribution of vesicles. Two types of liposomes were prepared: Sample L1 (125±7 nm in diameter) is formed solely by PS. Thus it carries a residual charge, due to the presence of PS. This molecule has a negatively charged group, which gives rise to a long-range repulsion between liposomes. Sample L2 (of about 102±4 nm in diameter) is made of PC, which is a zwitterionic phospholipid, having a choline group and a phosphate group, so, at moderate pH’s, it is expected to be uncharged. It has been proven that the extrusion method produces unilamellar vesicles. In addition, transmission electron microscopy measurements show that vesicles are roughly spherical. The vesicle size was determined by photon correlation spectroscopy (PCS) with a commercial light scattering setup (4700C, Malvern Instruments, Malvern, UK). Electrophoretic measurements were also performed with a commercial device (BI-ZetaPALS, Brookhaven Instrument Corp., NY) and using potassium bromide as 1:1 electrolyte. Error bars were estimated from several individual measurements.

B. Estimation of the volume fraction

Before presenting our main results, we would like to comment one of the major difficulties with micellelike systems; i.e., the determination of volume fractions. Particularly, the conversion from weight fraction to volume fraction can be problematic. The difficulty lies in determining both size and density of the phospholipid shell particle. For these types of liposomes, the thickness of the phospholipid shell Δ is close to 4.5 nm. As the phospholipid weight fraction used in each synthesis (x) is known, the volume fraction could be estimated via the expression

$$\phi = \frac{x}{4 \pi a^3} \frac{4 \pi a^3}{3} \rho',$$

where a is the outer liposome radius, $R_1=a-\Delta$ is the inner radius, and $\rho'$ is the relative density of the phospholipid shell on colloidal particle (1.015). The errors in this computation lie in the uncertainty at Δ and $\rho'$. Suitably of the values used in this work for these parameters was checked as follows. In an ordered colloidal dispersion, it is well known that $q_m$ is related with volume fraction through Bragg’s law

$$q_m = (6 \pi^2 \phi)^{1/3}/\alpha.$$

In Fig. 1, we show the light intensity (in arbitrary units) scattered by a sample of liposome L1 formed for x = 0.0076. The scattering experiment was performed at 25 °C, from 20° to 120° in 2° steps (4700C, Malvern Instruments, Malvern, UK). Average intensities were obtained from three individual measurements at each angle, with different cell positions to minimize the effects of scratches on the glass surface. Prior to measurements, samples were homogenized in order to avoid gradients in particle density. The suspension was kept for at least two days over a bed of ions exchange resins in cylindrical quartz glass cuvettes with outer diameters of 25 mm. As can be observed, the first peak appears at a scattering vector of 0.0216 nm$^{-1}$. According to Eqs. (5) and (6), $q_m$ should be 0.0208 nm$^{-1}$, which slightly smaller (only 3.7%) than the experimental one. In our opinion, this clearly means that Eq. (5) is a valid assumption (as well as the value of Δ) and volume fractions can be estimated within certain accuracy. Also, this confirms that our liposomes are unilamellar.

C. Determination of the effective diffusion coefficient

The short-time effective diffusion coefficient was measured with a commercial setup, known as ALV-NIBS7HPPS, which works using fiber optic and detecting light in a backscattering mode. It is designed to avoid multiple scattering in concentrated colloidal dispersions. The wavelength is 632.8 nm, and the modulus of the scattering vector turns out to be $q=0.026$ nm$^{-1}$. Experiments were carried out at 25 °C. The samples were handled in a similar way to other colloidal systems. The liposomes were kept in square cuvettes of 12.5 mm for (at least) 2 days over a bed of ions exchange resins. Then, using the ALV-NIBS7HPPS setup, we measured the normalized intensity correlation function $g^{(2)}(q, \tau) = 1 + c|g^{(1)}(q, \tau)|^2$ where c is an experimental constant smaller than one. Figure 2 shows some measured intermediate scat-
When the short-time dynamics of colloidal particles are studied, a requirement concerns the temporal scale that characterizes the particle dynamics. We have plotted \( \sqrt{g^{(2)}(q, \tau) - 1} \) against \( \tau \) instead of \( g^{(1)}(q, \tau) \) itself. The single-particle diffusion coefficient was measured by DLS using diluted samples of liposomes and extrapolating to \( \phi \rightarrow 0 \) (measurements were performed at several angles). The results for samples L1 and L2 were found to be \( D_0 = (3.97 \pm 0.20) \times 10^{-12} \text{m}^2/\text{s} \) and \( D_0 = (4.86 \pm 0.10) \times 10^{-12} \text{m}^2/\text{s} \), respectively. In the inset of Fig. 2 we show the behavior at intermediate and long delay times. As can be seen, interactions have a marked effect: \( \sqrt{g^{(2)}(q, \tau) - 1} \) departs strongly from a simple dependence on \( \tau \) and its decay cannot be described by a single-exponential law. But we are interested in the short-time behavior. In Fig. 2, we observe that the decay of \( g^{(1)}(q, \tau) \) is linear for \( \tau \rightarrow 0 \), so the short-time effective-diffusion coefficient can be measured from their slope \(-D_{\text{eff}}q^2\). We determined the field autocorrelation function (ACF) of the samples by averaging ten ALV500/E correlator runs lasting 40 s each. To obtain \( D_{\text{eff}} \), a cumulant expansion of second order was fitted to the autocorrelation function. By averaging the first cumulant obtained fitting, the mean diffusion coefficient and its standard deviation were calculated.

When the short-time dynamics of colloidal particles is studied, a requirement concerns the temporal scale that characterizes the particle dynamics. Here \( \tau_B \) being the Brownian relaxation time, \( \tau \) the correlation delay time, and \( \tau_a \) the time needed by a particle in a dilute suspension to diffuse over a distance comparable to its radius \( a \), \( \tau_a = a^2/D_0 \), the short-time regime is fulfilled if \( \tau_B \ll \tau \approx \tau_a \). The ALV correlator sample time was 200 ms and the \( \tau_a \) values are of the order of 543 \( \mu \)s (sample L2). Additionally, other conditions must be satisfied. It deals with the decay time \( \tau_q \) of the field ACF, \( \tau_q = (D_{\text{eff}}^2)^{-1} \). For an accurate measure of the ACF decay, this fluctuation time of the light intensity should be smaller than \( \tau_a \). As can be seen in Fig. 2, all requirements are fulfilled using \( q = 0.026 \text{ nm}^{-1} \).

IV. RESULTS AND DISCUSSION

A. Dependence on the volume fraction: Charged liposome

As mentioned in the Introduction of this paper, one of our purposes is studying the interplay between DIs and HIs and, more specifically, finding out if strong electrostatic forces can slow down diffusion processes. This matter has been customarily addressed analyzing the behavior of the diffusion coefficient with varying the volume fraction (or, equivalently, the particle number density). In the same spirit, we have measured the diffusion coefficient as a function of \( \phi \) (for a fixed and above-mentioned \( q \)). The results are plotted in Fig. 3(a) for the case of the charged liposome. As can be observed, the diffusion coefficient decreases markedly for moderate volume fractions.

In a first analysis of these data, one could recall the \( D_{\text{self}} - \phi \) plots reported by other authors. In order to compare results and reach well-founded conclusions, we must stress that our experimental technique provides an effective diffusion coefficient that also depends on \( q \), \( D_{\text{eff}}(q, \phi) \). Strictly speaking, this value could be interpreted as a self-diffusion coefficient if \( q \rightarrow \infty \). In practice, however, Segre et al. showed in their study about short-time dynamics of hard spheres that are values of \( D_{\text{eff}} \) for \( q > q_m \) can be identified with those of self-diffusion to within 10% (providing that \( \phi < 0.35 \)). Taking \( q = 0.026 \text{ nm}^{-1} \), it can be readily inferred from Eq. (6) that the condition \( q > q_m \) is satisfied (for this liposome) if \( \phi < 0.06 \). The diffusion data below this threshold volume fraction can therefore be compared with the behavior predicted for the self-diffusion coefficient of (uncharged) hard spheres [Eq. (4)], which is also shown in Fig. 3(a), concluding that our results support the fact that DIs slow down the diffusive processes (as suggested by other authors).
Since we are dealing with suspensions of extremely low ionic strength, dispersion forces can be neglected. In relation to the electrostatic interaction, the Debye–Hückel (DH) potential was assumed to be valid:

\[ u(r) = \frac{Z^2e^2}{4\pi\varepsilon_0\varepsilon_r} \left( \frac{\exp(\kappa a)}{1 + \kappa a} \right)^2 \frac{\exp(-\kappa r)}{r}, \]

where \( e \) is the elemental charge, \( \varepsilon_0 \) and \( \varepsilon_r \) are the dielectric constant of vacuum and the relative constant of the solvent, and

\[ \kappa = \sqrt{\frac{e^2}{\varepsilon_0 \varepsilon_r kT} \sum_i \rho_i z_i^2} \]

is the reciprocal Debye screening length (here \( k \) is Boltzmann’s constant and \( T \) the absolute temperature). The sum is taken over the counterions and electrolyte ions with bulk number density \( \rho_i \) and valence \( z_i \). Expression (7) has been derived using the DH approximation for the distribution of small ions around the particles. However, many theoretical studies point out the possibility of using the above-mentioned DH expression in the description of highly interacting systems if certain parameters (such as the charge \( Z \)) are renormalized (see references cited by Quesada-Pérez et al.25). We will apply Eq. (7), in this context, using \( Z \) as a phenomenological (effective) parameter in the attempt to fit (roughly) the experimental data shown in Fig. 3(a). Its physical meaning will be discussed later. Another unknown parameter is \( \kappa \), which is not easy to control or monitor in deionized suspensions. Some studies suggest, however, that the ionic strength due to residual ionic impurities (\( I_{\text{res}} \)) is of the order of \( 10^{-6} \) M.26 In this work, we have used \( I_{\text{res}} = 10^{-6} \) and \( 10^{-5} \) M as two limiting values to check the effect of this parameter. After trying out several \( Z \) values, we concluded that the theory based on Eqs. (2) and (3) and the RMSA could not fit successfully the behavior of \( D_{\text{eff}}/D_0 \) on the whole range of volume fractions. However, a charge of about 200e/liposome was able to justify the experimental results below a certain volume fraction (of about 0.06), within practically the same accuracy for \( I_{\text{res}} = 10^{-6} \) or \( 10^{-5} \) mol [see the theoretical predictions corresponding to these values also plotted in Fig. 3(a)]. As can be seen, above the mentioned \( \phi \) value, the predicted diffusion coefficient increases rapidly with the volume fraction. On the contrary, the experimental one goes on decreasing (although a bit more slowly). The reasons for the differences found for \( \phi > 0.06 \) are not clear. In principle, one has to bear in mind that Eq. (3) is just an approximation, which may fail for suspensions of near particles. At any rate, we would like to call the reader’s attention to the following somehow striking fact: This volume fraction coincides with value limiting the self-diffusion regime (\( q > q_m \)). In other words, the failure of the theoretical approach used in this work seems to be related with the regime \( q < q_m \) (which might be called collective regime). Although the reason for this remains an open question, it should be pointed out that other works have also found some discrepancies in this regime for suspensions at low ionic strengths.27–29 Particularly, it has also been widely reported for deionized latex suspensions that \( S(q) \) is larger than expected for \( q \rightarrow 0.25 \). This has been found in similar experiments with liposomes as well.29 According to Eq. (2), this disagreement in the structure factor for \( q < q_m \) between theory and experiment could be related with the fact that \( D_{\text{eff}} \) is smaller than predicted.

At this point, the discussion about the value obtained for \( Z \) is still pending. Certainly, 200e/liposome seems to be quite small, specially as compared to the total number of charged groups on the (outer) surface. This number can be estimated from the molecular area of PS [about 70 Å² per molecule (Ref. 30)] and exceeds 70000e/liposome. Nevertheless, similar findings have been reported for deionized suspensions of other highly charged colloids (see references cited in Ref. 25). Charge renormalization [due to the failure of Eq. (7)] might explain partly such a considerable difference, but additionally, some authors have proposed diverse experimental techniques to determine (at least, approximately) the actual surface charge. For instance, Palberg et al. put forward the shear modulus titration,31 whereas Quesada-Pérez et al. suggested that electrophoresis experiments could provide a reasonable estimate of this quantity.32 In fact, the charge obtained from this technique (to which we will refer as electrokinetic charge) turned out to be useful to justify the effective charges found through static light scattering. Accordingly, we carried out measurements of electrophoretic mobility as a function of \( \kappa a \), plotted in Fig. 4. The electrokinetic charge (also shown in this figure) \( Z_\zeta \) was estimated by the following expression:

\[ Z_\zeta = \frac{4\pi a^2 \varepsilon_0 \varepsilon_r \kappa T \kappa}{e^2} \left[ 2 \sinh \left( \frac{e \zeta}{2kT} \right) + \frac{4}{\kappa a} \tanh \left( \frac{e \zeta}{4kT} \right) \right]. \]

where \( \zeta \) is the zeta potential, which was calculated by means of the O’Brien–White theory from mobility. As can be observed, this electrokinetic property is large for high \( \kappa a \) values. With decreasing this parameter, however, \( Z_\zeta \) undergoes a significant reduction. At low ionic strengths corresponding to deionized suspensions it is quite difficult to measure electrophoretic mobilities, but the charge obtained from \( D_{\text{eff}} \) under such conditions seems to be consistent with the decreasing trend revealed by the electrokinetic data. This
forces. In other words, there exists experimental evidence of an effective screening of HIs due to an increase in the electrostatic repulsion between colloidal particles. This result agrees with other measurements at intermediate and long-time\(^4\) and short-time collective diffusion.\(^3\) In relation to the charged liposome, the \(\phi\) dependence of the effective diffusion coefficient can be explained for volume fractions not exceeding a critical value (for which \(q\) is that corresponding to the main peak of the structure factor; i.e., we are dealing again with the self-diffusion regime). The surface charge obtained from diffusion data is quite small (as compared to the total number of charges per lipid vesicle). However, such a reduced quantity agrees with the charges obtained from electrophoresis. Regarding the uncharged liposome, the hard-sphere model seems to justify for reasonably well the measured diffusion coefficients.

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