Effect of Gd^{3+} on the colloidal stability of liposomes

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Lanthanide ions such as La^{3+} and Gd^{3+} are well known to have large effects on the structure of phospholipid membranes. Unilamellar vesicles of dipalmitoylphosphatidylcholine (DPPC) were prepared by sonication method and confirmed by transmission electron microscopy. The effects of concentration of gadolinium ions Gd^{3+} on DPPC unilamellar vesicles in aqueous media were studied by different techniques. As physical techniques, photon correlation spectroscopy, electrophoretic mobility, and differential scanning calorimetry were used. The theoretical predictions of the colloidal stability of liposomes were followed using the Derjaguin-Landau-Verwey-Overbeek theory. Changes in the size of liposomes and high polydispersities values were observed as Gd^{3+} concentration increases, suggesting that this cation induces the aggregation of vesicles. Electrophoretic mobility measurements on unilamellar vesicles as a function of Gd^{3+} ion concentration show that the vesicles adsorb Gd^{3+} ions. Above Gd^{3+} concentrations of 0.1 mol dm^{-3}, the ζ potential and light scattering measurements indicate the beginning of aggregation process. For comparison with similar phospholipids, the zeta potential of phosphatidylcholine interacting with Gd^{3+} was measured, showing an analogous behavior. Differential scanning calorimetry has been used to determine the effect of Gd^{3+} on the transition temperature (T_c) and on the enthalpy (ΔH_c) associated with the process.

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INTRODUCTION

Magnetic resonance imaging (MRI) has evolved into one of the most powerful techniques in diagnostic clinical medicine and biomedical research by enabling the acquisition of high-resolution three-dimensional images of the distribution of water in vivo [1]. The strong expansion of medical MRI has prompted the development of a new class of pharmacological products, called contrast agents [2]. These agents catalytically shorten the relaxation time of nearby water molecules, thereby enhancing the contrast with background tissues. In 1999, approximately 30% of all MRI scans used a contrast agent, most of which were based on gadolinium complexes [1]. In an effort to improve the design and interpretation of the protocols to use for these agents, there is a need to understand how they interact with biological molecules and assemblies. Gd^{3+} is known to induce conformational changes in some proteins [3], but the physiological effects of lanthanides are usually expected to result mainly from alterations in the structure of the membrane bilayer.

It is this expectation that has prompted us to investigate further the effect of gadolinium on the structure and surface electrical properties of liposomes. Liposomes are vesicular structures formed by a closed lipid bilayer, encompassing an aqueous core [4]. In an appropriate environment, these structures self-assemble, due to the amphiphilic character of their component molecules. Amphiphilic molecules, being composed of a hydrophobic and a hydrophilic part, in aqueous solution give rise to a variety of morphologically different structures. Among these, unilamellar or multilamellar vesicles (liposomes) are of peculiar interest as a simple model of biomembranes. Sharing with biomembranes the basic bilayer structure, they offer the unique advantage that their lipid composition can be varied in a well-defined and controlled way.

Composition, together with the characteristics of the aqueous phase, defines the physico-chemical properties of these structures, such as their stability, surface charge density, bilayer rigidity, etc., and their properties as colloidal particles, such as size, electrophoretic mobility, and interparticle interactions.

Although the self-assembling of amphiphilic lipids is mainly driven by the so-called "hydrophobic effect" [5], electrostatic interactions among the polar heads play a fundamental role in the aggregation process and in determining the properties of the bilayer. For this reason, the presence of multivalent ions in the solution can have a strong influence on the organization of the lipids within the bilayer and on structure and the dynamical properties of the liposome-water interface.

Because of their biocompatibility and of their structure, liposomes can be effectively used as drug delivery systems, since the lipid bilayer allows the entrapment of hydrophilic material within the aqueous core and hydrophobic material within the hydrocarbon chain phase [4,6].

The biological applications of Gd-labeled liposomes are focusing on the monitoring of the liposomal distribution within targeted regions, the temperature control, and the gradual administration of the gadolinium as catalytic agents to increase the contrast in the MRI [7–9].

The lanthanide ions (La^{3+}, Gd^{3+}) have effects on the structure and stability of phopholipids membranes. Several

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authors have studied the interactions of La^{3+} with the surface of negatively charged lipid layers made up of phosphatidylserine (PS) and dipalmitoyl-phosphatidyl-choline (DPPC)–phosphatidyl-inisitol (PI), with apparently contradictory results. In its interaction with PS membranes, La^{3+} induces membrane fusion of the vesicles [10–12]. On the contrary, La^{3+} does not induce fusion of DPPC-PI vesicles [13]. Averbahk and co-workers [14,15] observed that Gd^{3+} induces aggregation of di-myristoyl-phosphatidyl-serine liposomes. Studying the effects of La^{3+} and Gd^{3+} on membranes composed of phosphatidyl-choline (PC) and phosphatidyl-ethanolamine Tanaka *et al.* [16] found that chain-melting transition temperatures of PC and PE membranes increased with an increase in La^{3+} concentration indicating that the lateral compression pressure of the membrane increases with the concentration of the multivalent ions. More recently, Tanaka and Yamazaki [17] proposed a new model for the mechanism of La^{3+}-induced membrane fusion of giant unilamellar vesicles.

In the present paper, we have studied the effect of gadolinium on the stability of the liposomes and on different physical properties characterizing the bilayer. Moreover, the aggregation process of DPPC liposomes at different concentrations of Gd^{3+} have been studied by means of dynamic light scattering (DLS), and the results have been interpreted within the framework of the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory. The adsorption of Gd^{3+} ions to the membrane has been quantified by electrophoretic measurements, and differential scanning calorimetry (DSC) has been employed to investigate the effect of the Gd^{3+} on the phase transition of the membrane.

MATERIAL AND METHODS

Materials

L-α-Phosphatidylcholine Dipalmitoyl (DPPC) (No. P 0763) and the L-α-Phosphatidylcholine from egg yolk (EYPC) (No. P 3556) were purchased from Sigma and used without further purification.

The $GdCl_3$ was from Sigma Chemical and had a purity of 99.9%. Organic solvents methanol and chloroform were from Aldrich and Merck, respectively. The water was doubly distilled.

Dynamic light scattering

Dynamics light scattering measurement were performed by a standard laboratory-built DLS spectrometer equipped with a BI-9000AT digital correlator (Brookhaven Instruments) and a He-Ne laser operating at 10 mW power and 632.8 nm wavelength. Data analysis was performed using a software based on the CONTIN method [18].

Electrophoretic mobility

Zeta potentials (ζ potential) of the liposome system were measured using a Malvern Instruments Zetamaster 5002 by taking the average of five measurements at stationary level. The cell used was a 5×2 mm^2 rectangular quartz capillary. The temperature of the experiments was 25.0 ± 0.1 °C and was controlled by a Haake temperature controller. The zeta potential ζ was calculated from the electrophoretic mobility μ_E by means of the Henry correction to Smoluchowski's equation [19]:

$$\zeta = \frac{3\mu_E \eta}{2\epsilon_0 \epsilon_r} \frac{1}{f(\kappa a)}, \tag{1}$$

where ϵ_0 is the vacuum permittivity, ϵ_r is the relative permittivity of the solvent, a is the particle radius, κ^{-1} is the Debye length, and η is the solvent viscosity (water, in our case).

Differential scanning calorimetry

A Perkin-Elmer differential scanning calorimeter (Model Diamond) was used for the DSC experiments. 50 μl sealed pans of aluminum have been used as cell and reference. The former has been filled with 0.5 mg of DPPC and 40 μl of $GdCl_3$ aqueous solutions, at the different concentrations of the electrolyte, the latter has been filled with the same amount of water. The thermograms have been obtained at a cooling and heating rate of 10 °C/min. The temperature (T_c) and enthalpy (ΔH) of transition were determined using Pyris for Windows software (Perkin-Elmer Corp., Norwalk, CT). All the thermograms were measured, at least, four times with a high reproducibility.

Transmission Electron Microscopy (TEM)

The morphological examination of the liposomes was performed by transmission electron microscopy (CM-12 Philips). The samples were stained with 2% (w/v) (per weight) phosphotungstic acid and placed on copper grids with Formvart films for viewing by TEM.

Preparation of the liposomes

An appropriate amount of DPPC (20 mg) was dissolved in 2 ml of a methanol-chloroform solution (1:1 v/v) (per volume), the solution was placed in a glass vessel and allowed to form a dry film after rotary evaporation of the solvent. The film was then hydrated with 2 ml pure water at the temperature of 43 °C for 1 h. The resulting mixture was sonicated at a temperature of 43 °C for 1 h, at continuous power mode using a probe sonicator model Vibra-Cell Sonics, until the solution appeared to be optically transparent in white light. Having in mind the area per molecule, the total liposome surface is several orders of magnitude greater than the surface of the container, so the amount of Gd^{3+} adsorbed at the glass surface is expected to be, for entropic reasons, completely negligible. A homogeneous liposomal suspension of unilamellar vesicles was obtained. Lipids were usually dried under vacuum overnight.

Figures 4 and 5 confirm the existence of unilamellar liposomes with low polydispersities in the absence of gadolinium.

RESULTS AND DISCUSSION

Adsorption of Gd^{+3} to liposomes

In order to analyze the colloidal stability of liposomes in the presence of Gd^{3+} ions, we have measured the ζ potential

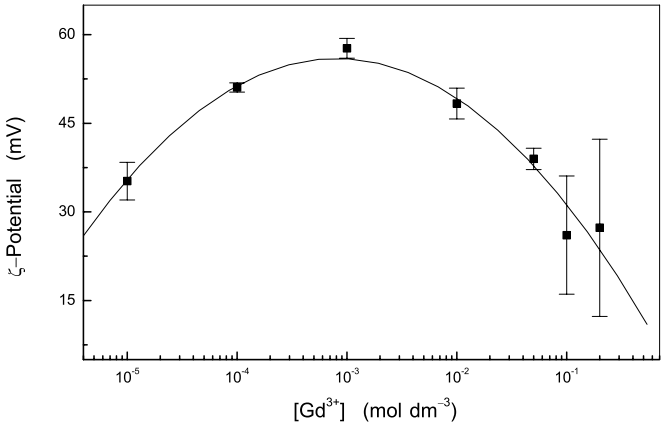

FIG. 1. ζ potential of DPPC liposomes as a function of the Gd^{+3} concentrations at 25 °C.

of DPPC liposomes as a function of concentration of Gd^{+3}. The results are shown in Fig. 1. DPPC molecules are zwitterionic, so that the liposomes bear a weak average charge, whose value depends on the *p*H of the medium.

As the Gd^{+3} ion concentration progressively increases, due to the adsorption of Gd^{+3} ions, the ζ potential increases from a value of +30 mV to a maximum of about +60 mV, which is reached at a concentration of 1 mol dm^{-3}. The subsequent decrease, observed at higher Gd^{+3} concentrations, can be justified by the increase of the ionic strength of the medium, which progressively reduces the surface charge of the liposomes. For concentrations higher than 0.1 mol dm^{-3} the uncertainties in the ζ potential values are larger, because the aggregation process begins to occur. This overall behavior was supported by other authors [20,21] who established that the dependence of the surface potential on ion concentration is governed by two different contributions: (a) screening of the surface charge by counterions, which always decreases the surface potential with increasing salt concentration, and (b) ion binding, which varies the surface potential.

In order to further study the adsorption of Gd^{3+} at the bilayer surface we have measured the ζ potential of liposomes build up with a natural zwitterionic phospholipid (EYPC), which differs from DPPC in its hydrophobic tail composition, for different gadolinium concentrations. In the absence of Gd^{3+} EYPC liposomes have a negative ζ potential of −15 mV [22,23]. Figure 2 shows the variations of the ζ potential of EYPC and DPPC liposomes depending of the Gd^{3+} concentration and different packing of the tails, having in mind that EYPC is above their phase transition temperature (−15 °C to −7 °C) and DPPC below (41 °C) [24].

In the case of the EYPC liposomes, where the ζ potential changes sign as the Gd^{3+} ion concentration is increased, we can evaluate the number of absorption sites N_1 per unit area. Plotting the ζ potential versus the logarithm of the electrolyte concentration c, and using the Ottewill and Watanabe equation [25], at the point of zero charge (pzc), we have

$$\left(\frac{d\zeta}{d\log c}\right)_{\zeta=0} = 2.303\zeta^0\left[\frac{\epsilon(1+\kappa a)\zeta^0}{azeN_1} - 1\right], \tag{2}$$

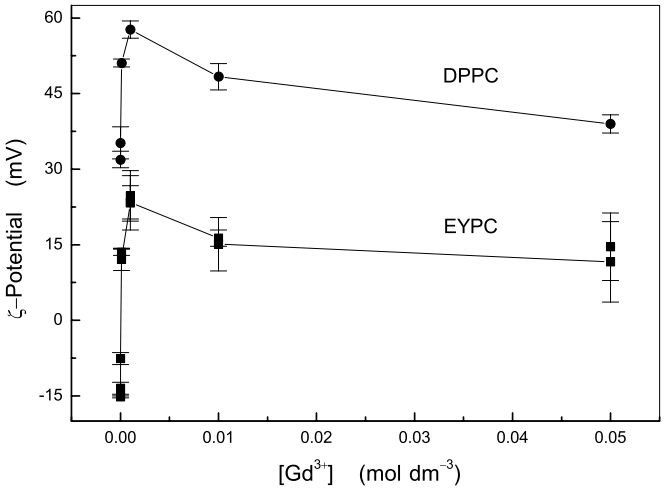

FIG. 2. ζ potential of DPPC (•) and EYPC (■) liposomes as a function of the Gd^{+3} concentrations at 25 °C.

$$\frac{1}{c_0} = k_2\left[\frac{azeN_1}{\epsilon\zeta^0(1+\kappa a)} - 1\right], \tag{3}$$

where ze is the ion charge, c_0 is the salt concentration at the pzc, ζ^0 is the ζ potential in the absence of salt, a is the liposome radius, and ε the dielectric constant of the medium. Equations (2) and (3) can be simultaneously solved using the experimental values of $(d\zeta/d\log c)_{\zeta=0}$ and c_0 to obtain values of N_1 and k_2.

The standard free energy of adsorption, ΔG^0_{ads}, can be obtained from the relationship

$$k_2 = \exp\left(-\frac{\Delta G^0_{ads}}{k_B T}\right). \tag{4}$$

The results obtained for the absorption of Gd^{3+} at the EYPC liposome surfaces are $N_1 = 5.1 \times 10^{14}$ m^{-2}, $k_2 = 8.5 \times 10^3$ M^{-1}, $\Delta G = -49.6$ kJ/g, respectively. These values are comparable with the ones obtained by other authors in the investigation of trivalent ion absorption to liposome surfaces [13,26].

Stability of Gd^{3+}-labeled liposomes

The stability of the liposomes at different concentrations of Gd^{3+} was investigated by means of photon correlation spectroscopy. Figure 3 shows the size of the DPPC liposomes at different concentrations of gadolinium. For concentrations of Gd^{3+} higher than 0.1 mol dm^{-3}, the diffusing objects start to increase in size due to the aggregation of liposomes. The formation of clusters of liposomes after this critical aggregation concentration (CAC) corresponds to the region where the ζ potential is decreasing, as shown in Figs. 1 and 2. TEM images shown in Fig. 4 confirm the existence of large clusters of aggregated liposomes, for concentration of Gd^{3+} higher than 0.1 mol dm^{-3}.

The stability of liposomes before the CAC is governed by electrostatic interactions and when the ionic strength is high enough to screen the charge of the liposomes, the aggregation process begins to occur. See Fig. 5.

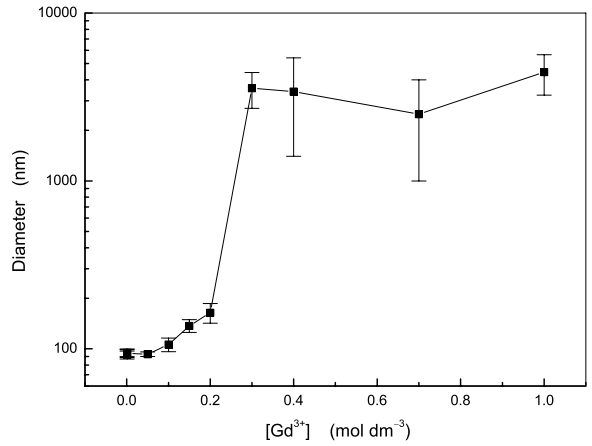

FIG. 3. Diameters of the DPPC liposomes for different Gd^{+3} concentrations measured by DLS at 25 °C.

In Fig. 6 the size of the DPPC liposomes as a function of the concentration of Gd^{3+} is reported in the range from 10^{-5} up to 0.2 mol dm^{-3}, where the aggregation begins. Data have been reported for two different lipid concentrations: 1.36×10^{-3} mol dm^{-3} and 13.6×10^{-3} mol dm^{-3}. It was observed that the CAC does not vary independently of the ratio [liposomes]/[gadolinium], confirming that the aggregation process depends of the screening of the charge of the liposomes due to the increase of the ionic strength.

To correlate these observations with the forces between particles, the data were analyzed within the DLVO theory of colloidal stability [27]. The interaction potential between particles is written as the sum of an attractive London–van der Waals potential $V_A(x)$ and a repulsive interaction potential $V_R(x)$ due to the electric charge of the particles.

$$V_{DLVO} = V_A(x) + V_R(x). \tag{5}$$

The attraction potential $V_A(x)$ [28,29] in the case of two equal vesicles of radius a and thickness of the bilayer d is given by [30]

$$V_A(x) = -\frac{Aa}{12}\left[\frac{1}{(2d+x)} + \frac{2}{d+x} + \frac{1}{x}\right] - \frac{A}{6}\ln\left[\frac{x(x+2d)}{(x+d)^2}\right], \tag{6}$$

where A is the Hamaker constant and x is the distance between the two liposomes. The expression for $V_R(x)$ per unit area has the form [31,32]

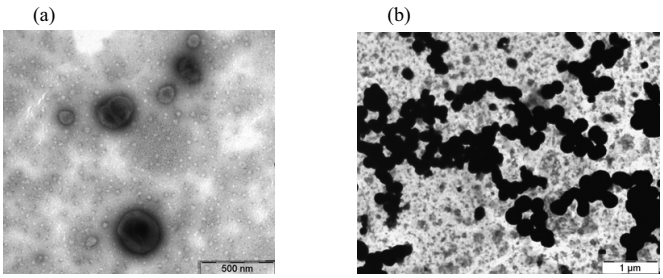

FIG. 4. Transmission electron micrograph of spontaneous DPPC liposomes at presence of (a) 0.05 mol dm^{-3} of Gd^{+3} and (b) 0.7 mol dm^{-3} Gd^{+3}, respectively.

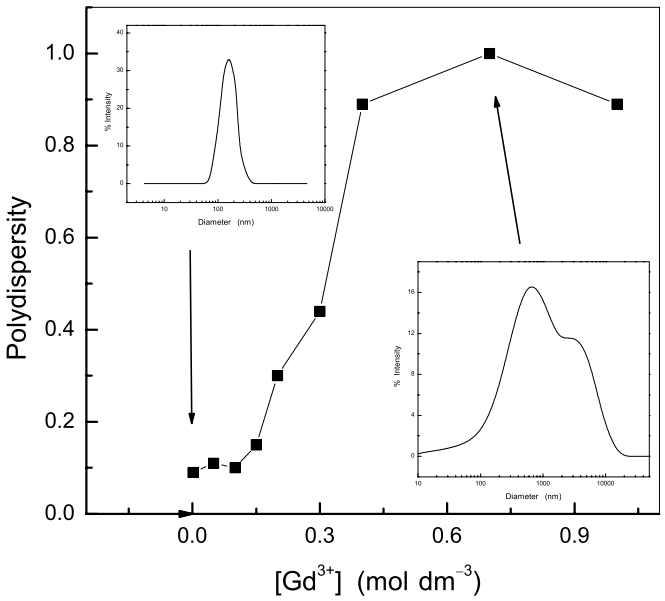

FIG. 5. Polydispersities of the DPPC liposomes for different Gd^{+3} concentrations measured by DLS at 25 °C. The inset represents the size distribution, calculated by CONTIN methods, in absence of Gd^{+3} and at 0.7 mol dm^{-3} of Gd^{+3}.

$$V_R(x) = 2\pi\epsilon_0\epsilon_r(a+\Delta)\left(\frac{4k_BT}{ze}\gamma\right)^2 \exp[-\kappa(x-2\Delta)], \tag{7}$$

where k_BT is the thermal energy and κ is the reciprocal Debye length:

$$\kappa^2 = \frac{(z_1^2 z_2 + z_2^2 z_1)c_s N_A e^2}{\epsilon_0\epsilon_r k_B T}, \tag{8}$$

where c_s is the concentration. Finally, Δ is the thickness of the Stern layer and

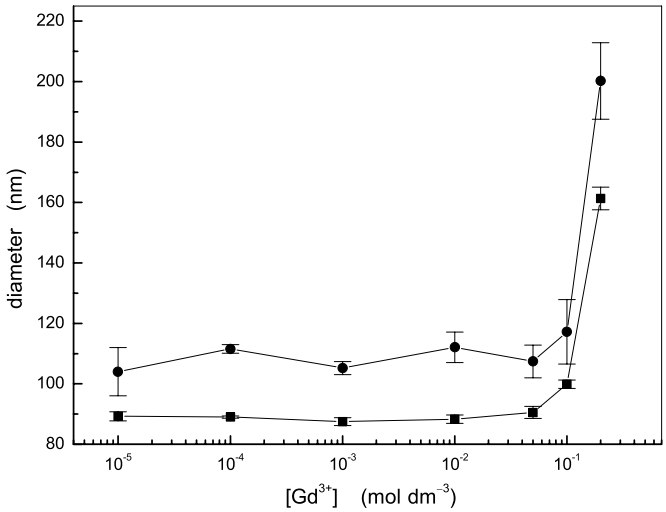

FIG. 6. Diameters of the DPPC liposomes for different phospholipid concentrations measured by DLS at 25 °C. (■) 1.36×10^{-3} mol dm^{-3} and (•) 13.6×10^{-3} mol dm^{-3}.

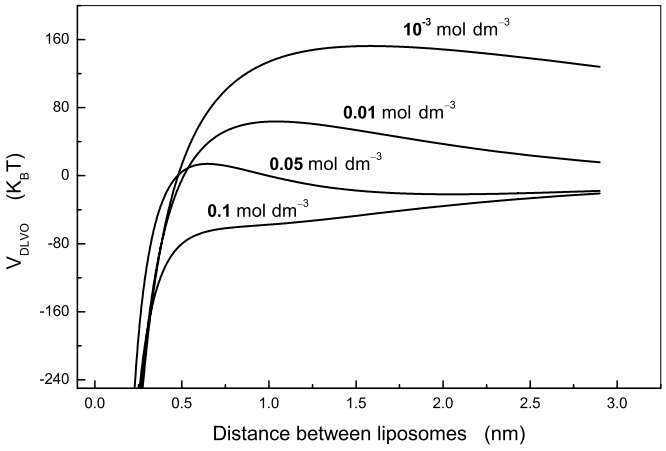

FIG. 7. DLVO potentials of EYPC liposomes as a function of the distance between two liposomes for different Gd^{3+} concentrations, 10^{-3}, 0.01, 0.05, and 0.10 mol dm^{-3}.

$$\gamma = \tan \frac{ze\psi_d}{4k_BT}, \tag{9}$$

where ψ_d is the surface potential that can be assumed as the ζ potential at low ionic strength [33].

Assuming that the aggregation process occurs when the electrostatic barrier disappear and $V_{DLVO}=k_BT$ [34,35], taking in account the value of the CAC=0.1 mol dm^{-3}, the surface potential, $\psi_d \approx +26$ mV, and the initial radius of the stable liposomes, $a=70$ nm, we can calculate the Hamaker constant using Eqs. (6) and (7). We obtained a value of the Hamaker constant of $A=11.2\times10^{-20}$ J, in reasonably good agreement with the values reported by other authors [35–38].

Figure 7 shows the calculated DLVO potential curves as a function of the distance x for several concentrations of Gd^{3+}, using the calculated value of the Hamaker constant. An increased attraction between vesicles as the salt concentration is increased is observed. As a consequence, the electrostatic barrier decrease. These observations suggest a screening of the liposome charge, close to CAC, leading to a disappearance of the barrier. However, as it is well known, the DLVO theory is inaccurate in the case of multivalent ions. For example, multivalent ions can cause net attraction between similarly charged objects, in violation of the mean-field theory. Then, other effects (other forces) non-DLVO, must be present in the system. These forces are basically due to hydration and osmosis phenomena and have been considered in the thickness of Stern layer, Δ, which is determined by the hydrated radius of the adsorbed ion.

Thermal analysis of phase transition

To proceed further in the direction of a complete analysis of the effect of the Gd^{3+} onto DPPC liposomes, DSC has been employed.

On heating, phospholipids undergo a melting process, as shown by the endothermic peaks in Fig. 8(a). They pass, in fact, from a "gel state," where the hydrocarbon chains are in the fully extended state and the polar head groups are relatively immobile, to a "liquid-crystalline state," where the head groups have increased mobility and there is a disordering of the hydrophobic chains [38].

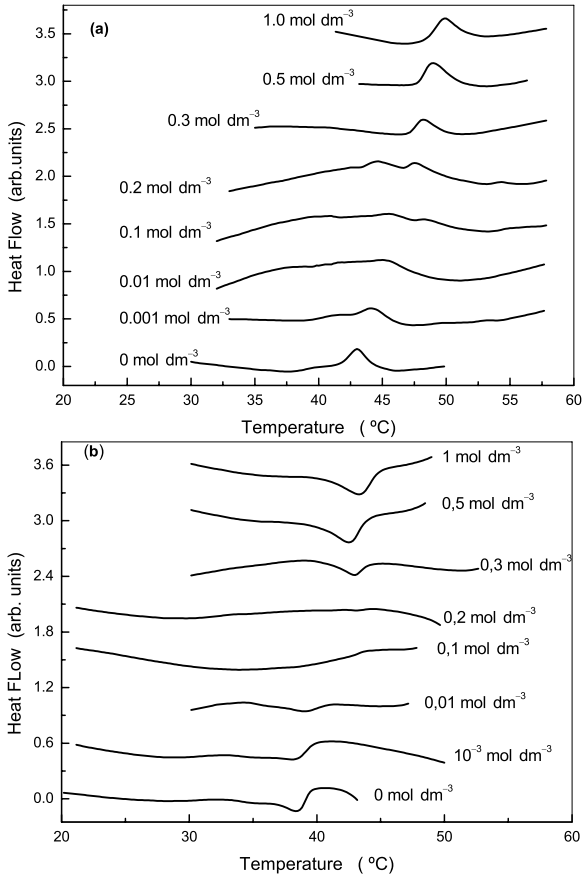

FIG. 8. The DSC endotherms of DPPC liposomes with varying amounts of Gadolinium: heating (a), cooling (b).

This "gel to liquid-crystalline" phase transition is easily detected by DSC, and characterized by a transition temperature (T_c), and an enthalpy (ΔH_c) associated to the process.

Although this phase transition is reversible, the values of T_c and ΔH_c obtained on heating the sample differ from those obtained on cooling it. This indicates that an equilibrium state is reached much faster on cooling than on heating. This effect is probably due to the fact that in the "gel phase" the phospholipids are more correlated with respect to the "liquid crystal phase," characterized by a higher lateral mobility of the phospholipids. This leads to a shorter equilibration time when the bilayer cools down from the liquid crystal phase to the gel phase [39,40].

The measurements have been performed as a function of the concentration of Gd^{3+} in the range 10^{-5}–1 mol dm^{-3}. The effect of the different concentrations of Gd^{3+} on the DPPC unilamellar liposomes is illustrated by the DSC curves in Fig. 8. In the absence of Gd^{3+} DPPC liposomes show a $T_c=40.88\pm0.08$ °C and a $\Delta H_c=9.6\pm1.3$ J/g, in reasonable agreement with literature data that, however, in the case of ΔH_c show slight discrepancies [37,39,41]. The addition of Gd^{3+}, in the range 0.4–1.0 mol dm^{-3}, to the liposomes solution results in a displacement of the transition temperature without broadening of the peak transition (ΔH_c) as shown in Fig. 9. At intermediate concentrations of Gd^{3+},

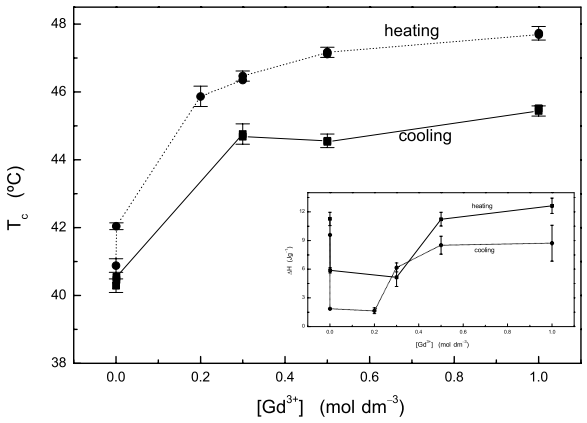

FIG. 9. Variation of the transition temperature (T_c) as a function of Gd^{3+} concentration, by heating and cooling. The inset shows the variation of the enthalpy for the gel to liquid crystalline transition (ΔH_c) of DPPC liposomes as a function of Gd^{3+} concentration, by heating and cooling.

0.1–0.3 mol dm^{-3}, the liposomes become unstable (Fig. 3) coexisting clusters of aggregated liposomes with simple liposomes. At those intermediate concentrations a displacement and broadening of the peak is present and a single transition peak is not observed anymore, as shown by the thermograms of Fig. 8.

In Fig. 9 the dependence of the transition temperature on the Gd^{3+} concentration is reported for both the heating and cooling processes. In the explored concentration range a displacement of T_c of around 6 °C is observed, in agreement with what is already found by other authors in multilamellar liposomes [16,38,42].

To explain this displacement we should remember that, at equilibrium, the attractive interfacial pressure π due to the hydrophobic interaction between the alkyl chains and water [16] balances the sum $\pi_{head}+\pi_{chain}=\pi$ of the interfacial pressure π_{head}, generated by the repulsive steric interaction between head groups, and of the chain pressure π_{chain}. Cations can specifically adsorb onto head groups of electrically neutral PC membranes and induce orientation changes of the head group, moving the N end of $P \rightarrow N$ vector perpendicular to the water phase [16,43–46]. In water (in the absence of these ions) the head group orients almost parallel to the membrane surface. Cations may bind to the phosphate of a PC head group, forming an electrostatic "salt bridge" among neighboring phospholipids. The conformational change and the formation of the salt bridge decrease π_{head} leading to a new balance of the interfacial pressure. The resulting increase in the lateral compression of the membrane, due to some rearrangement in the zwitterionic head group region in the presence of Gd^{3+}, can reasonably explain the changes on the packing of the lipid molecules in the bilayer.

As theoretical explanations for the increase in the melting temperature with ion concentration, the attraction between like-charge objects induced by correlations has been postulated. These interactions, based on mean-field theories, have been observed in a variety of systems [47]. However, no consensus exits for the precise mechanism, depending on the kind of system. A proposed mechanism [48] establishes that multivalent counterions adsorbed onto a charged membrane dramatically enhance stability through two effects: (i) a significant reduction of surface charge density and (ii) an induction of attraction between charged head groups of the membrane and thus a barrier to pore growth induced upon adding a small content of multivalent anions, the adsorbed cations are released into solution, thus reversing the membrane stabilization. Adsorption and desorption of multivalent cations provide an effective way to regulate the membrane stability. A different mechanism [49], including cooperative effects between counterions on both sides of an impermeable membrane, states that (i) the vesicle composition could remain uniform, and thus be uniformly attractive to the approaching surfaces. In this case, the vesicle should end up completely covered by particles. (ii) Alternately, binding could cause total lateral demixing of the charged and neutral surfactant in the membrane, and lead to a charged-depleted zone with no attraction to negative objects. (iii) A coexistence between high charge density and low charge density zones. An interesting experimental work evidencing the mechanism for like-charge attraction in cytoskeletal F-actin for counterion correlations has been reported recently [50].

Probably in our system, with vesicle constant composition, the mechanism proposed by Aranda-Espinoza *et al.* [49] could be of value. However, the predicted effect is too small to explain our experimental results. In this way, future studies are going to analyze this last effect with other techniques.

CONCLUSIONS

Interactions of a trivalent cation (Gd^{3+}) with phospholipid liposomes (DPPC) have been studied by different techniques: photon correlation spectroscopy, electrophoretic mobility and differential scanning calorimetry. In order to corroborate the experimental results the interaction potential between particles in the framework of DLVO theory has been calculated and used to describe the colloidal stability of liposomes.

By means of electrophoretic measurements we have characterized the adsorption of Gd^{3+} onto DPPC and EYPC liposome surface. The ζ-potential reaches a maximum at a Gd^{3+} concentration of 1×10^{-3} mol dm^{-3}; above this concentration, due to the increasing screening, the ζ potential decreases.

Dynamic light scattering measurements show a strong increase of size and polydispersity of the particles in the suspension when gadolinium ions are present at concentrations above 0.1 mol dm^{-3}. This increase is apparently due to an aggregation process of the liposomes as confirmed by the TEM images.

It was observed that the concentration of Gd^{3+}, where aggregation begins, is independent of the ratio [liposomes]/[gadolinium]. This finding suggests that the aggregation process only depends on the ionic strength which screens the electrostatic repulsion between liposomes.

Using the DLVO theory, the decrease of the electrostatic barrier with the gadolinium concentration due to the screening of the charge of the liposomes, can be calculated. The repulsive barrier disappears at concentration near the CAC.

Theoretical analysis seems to corroborate the overall picture deduced on the basis of the experimental findings.

A low Gd^{3+} concentrations is needed to commence the reduction of the steric repulsive interaction between the head groups in the phospholipid membrane. This is related to the greater charge that possesses the ion gadolinium. So that a smaller concentration of this ion is needed to induce the liposome aggregation.

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