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INTERACTION OF GADOLINIUM WITH PHOSPHOLIPIDS BILAYER MEMBRANES
Photon correlation spectroscopy and DSC study

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The effects of concentration of gadolinium ions Gd^{3+} on dipalmitoyl L-α-phosphatidylcholine (DPPC) unilamellar vesicles in aqueous media were studied by photon correlation spectroscopy (PCS) and differential scanning calorimeter. The theoretical predictions of the colloidal stability of liposomes were followed using the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory. Changes in the size of liposomes were observed as Gd^{3+} concentration increases, suggesting that this cation induces the aggregation of vesicles. To determine the effect of Gd^{3+} on the transition temperature (T_c) and on the enthalpy (ΔH_c) associated with the process differential scanning calorimetry (DSC) has been used. The addition of the metal ion provided DSC curves with different behavior to DPPC bilayer.

Keywords: *colloidal stability, DPPC vesicles, DSC, gadolinium*

Introduction

The expansion of medical magnetic resonance imaging (MRI) has prompted the development of a new class of pharmacological products, called contrast agents [1]. These agents catalytically shorten the relaxation time of nearby water molecules, thereby enhancing the contrast with background tissues. Most of these agents are based on gadolinium complexes [2]. 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 inter-particle 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.

The lanthanide ions (La^{3+}, Gd^{3+}) have effects on the structure and stability of phopholipids membranes. Several authors have studied the interactions of La^{3+} with the surface of negatively charged lipid layers made up of phosphatidylserine (PS) and dipalmitoylphosphatidylcholine (DPPC)–phosphatidyl-inisitol (PI), with apparently contradictory results. In its interaction with PS membranes, La^{3+} induces membrane fusion of the vesicles [6–8]. On the contrary, La^{3+} does not induce fusion of DPPC–PI vesicles [9].

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Averbahk *et al*. [10, 11] observed that Gd^{3+} induces aggregation of di-myristoyl-phosphatidyl-serine (DMPS) liposomes. Studying the effects of La^{3+} and Gd^{3+} on membranes composed of phosphatidyl-choline (PC) and phosphatidyl-ethanolamine Tanaka *et al*. [12] 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.

Consequently, the purpose of this work was to study the effect of the 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. Differential scanning calorimetry (DSC) has been employed to investigate the effect of the Gd^{3+} on the phase transition of the membrane.

Experimental

Materials

Dipalmitoyl *L*-α-phosphatidylcholine (DPPC) (No. P 0763) 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.

Preparation of the Gd^{3+}-labelled liposomes

An appropriate amount of DPPC (20 mg) was dissolved in 2 mL of a methanol-chloroform solution (1:1 *v*/*v*), 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. A homogeneous liposomal suspension of unilamellar vesicles was obtained.

Methods

Dynamic light scattering

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

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 curves have been obtained at a cooling and heating rate of 10°C min^{-1}. The temperature (T_c) and enthalpy (ΔH) of transition were determined using Pyris for Windows software (Perkin-Elmer Corp., Norwalk, CT). All the curves were measured, at least, four times with a high reproducibility.

Results and discussion

To analyze the colloidal stability of liposomes in the presence of Gd^{3+} ions, it was used photon correlation spectroscopy (PCS). Figure 1 shows the size of the DPPC liposomes at different concentrations of gadolinium. For concentrations of Gd^{3+} higher than 0.1 M, the diffusing objects start to increase in size due to the aggregation of liposomes.

The stability of liposomes before the critical aggregation concentration (*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 [13].

In Fig. 2 the size of the DPPC liposomes as a function of the concentration of Gd^{3+} is reported in

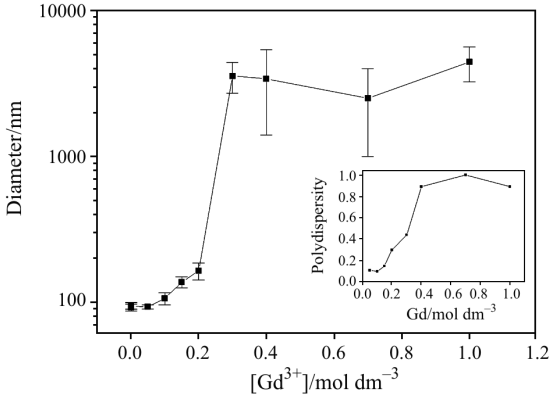

Fig. 1 Diameters of the DPPC liposomes for different Gd^{3+} concentrations measured by DLS at 25°C. Inset polydispersities of the DPPC liposomes for different Gd^{3+} concentrations measured by DLS at 25°C

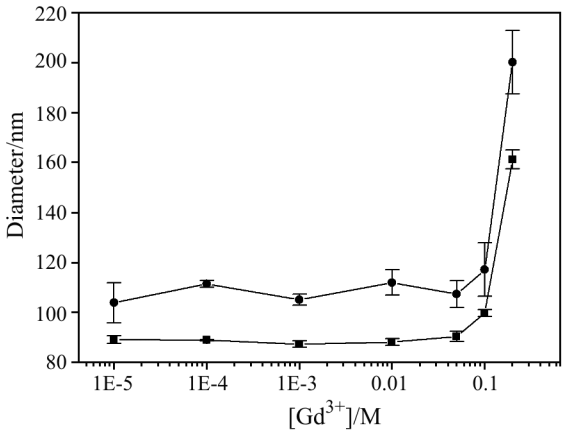

Fig. 2 Diameters of the DPPC liposomes for different phospholipid concentrations measured by DLS at 25°C. ■ – 1 and ● – 10 mg mL^{-1}

the range from 10^{-5} up to 0.2 M, where the aggregation begins. Data have been reported for two different lipid concentrations: 1 and 10 mg mL^{-1}. 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 [14]. 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.

$$V_{DLVO}=V_A(x)+V_R(x) \tag{1}$$

The attraction potential $V_A(x)$ [15, 16] in the case of two equal vesicles of radius a and thickness of the bilayer d is given by [17]:

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

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 [18, 19]

$$V_R(x) = \\ = 2\pi\varepsilon_0\varepsilon_r(a+\Delta)\left(\frac{4k_BT}{ze}\gamma\right)^2\exp[-\kappa(x-2\Delta)] \tag{3}$$

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

$$\kappa^2 = \frac{(z_1^2z_2+z_2^2z_1)c_sN_Ae^2}{\varepsilon_0\varepsilon_r k_BT} \tag{4}$$

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

$$\gamma = \tan\frac{ze\psi_d}{4k_BT} \tag{5}$$

where ψ_d is the surface potential that can be assumed as the ζ-potential at low ionic strength [13, 20].

Assuming that the aggregation process occurs when the electrostatic barrier disappear and $V_{DLVO}=k_BT$ [21, 22] taking in account the value of the *cac*=0.1 M, the surface potential, $\psi_d\approx+26$ mV [13].

The initial radius of the stable liposomes, a=70 nm, we can calculate the Hamaker constant using Eqs (2) and (3). We obtained a value of the Hamaker constant of $A=11.2\cdot10^{-20}$, in reasonably good agreement with the values reported by other authors [22–25].

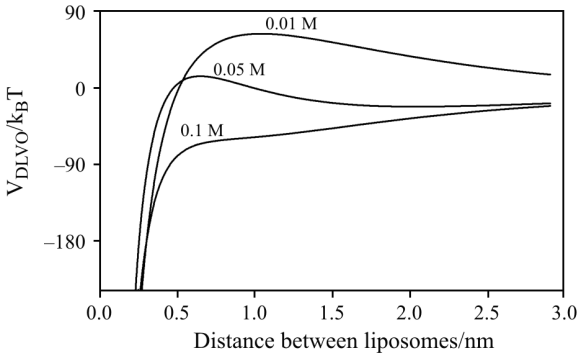

Fig. 3 DLVO potentials of PC liposomes as a function of the distance between two liposomes for different Gd^{3+} concentrations. 0.01, 0.05 and 0.10 M

Figure 3 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. The observed decrease of the electrostatic barrier with increasing Gd^{3+} concentration is due to the screening of the liposome charge which, close to the *cac*, makes the repulsive barrier to disappear.

To advance 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. 4. 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 [24]. This 'gel to liquid-crystalline' phase transition is easily detected by DSC, and char-

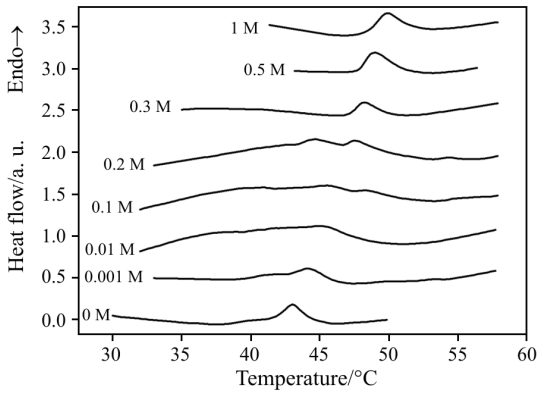

Fig. 4 DSC curves of DPPC liposomes with varying amounts of gadolinium (heating)

acterized by a transition temperature (T_c), and an enthalpy (ΔH_c) associated to the process.

The measurements have been performed as a function of the concentration of Gd^{3+} in the range 10^{-5}–1 M. The effect of the different concentrations of Gd^{3+} on the DPPC unilamellar liposomes is illustrated by the DSC curves in Fig. 4. In the absence of Gd^{3+} DPPC liposomes show a T_c=40.9±0.1°C and a ΔH_c=9.6±1.3 J g^{-1}, in reasonable agreement with literature data that, however, in the case of ΔH_c show slight discrepancies [24, 26–30]. The addition of Gd^{3+}, in the range 0.4–1.0 M, to the liposomes solution results in a displacement of the transition temperature without broadening of the peak transition (ΔH_c) as shown in Table 1. At intermediate concentrations of Gd^{3+}, 0.1–0.3 M, the liposomes become unstable (Fig. 1), 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 curves of Fig. 4.

In Table 1 the dependence of the transition temperature on the Gd^{3+} concentration is reported for both the heating processes. In the explored concentration range a displacement of T_c of around 6°C is observed, in agreement with what already found by other authors in multilamellar liposomes [12, 24, 31].

To explain this displacement we should remind that, at equilibrium, the attractive interfacial pressure

Table 1 Transition temperature (T_c) and the enthalpy for the gel to liquid crystalline transition (ΔH_c) of DPPC liposomes by heating

$[Gd^{3+}]$/M	T_c/°C	ΔH_m/J g^{-1}
1E-3	42.04±0.10	1.86±0.17
0.2	45.87±0.30	1.65±0.21
0.3	46.35±0.15	6.18±0.47
0.5	47.17±0.15	8.52±0.94
1.0	47.73±0.20	8.72±0.87

π due to the hydrophobic interaction between the alkyl chains and water [32] 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→N vector perpendicular to the water phase [12, 33–35]. 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 neighbouring 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.

Considering that the liposomes initiate the aggregation at 0.1 M of Gd^{3+}, it was assumed that the aggregation of liposomes and the interaction between neighbour liposomes also affects the packing of phospholipids within the bilayer [32] with a consequent shift of the transition temperature as shown by our results.

Conclusions

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 up to 0.1 M.

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.

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. 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 smaller Gd^{3+} concentration 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 pos-

sesses the ion gadolinium. So that a smaller concentration of this ion is needed to induce the liposome aggregation.

Finally DSC measurements have been done to better understand the effect of Gd^{3+} on DPPC liposomes. Two main processes are observed: (a) the rearrangement of the bilayers due to the adsorption of the Gd^{3+} ions to the liposomes surfaces which changes the orientation of the headgroup of the lipid molecules and translates into a displacement of the transition temperature T_c; (b) the beginning of the aggregation process due to the reduction of the charge of the liposomes. The consequent interaction between liposomes and clusters of liposomes affects the packing of phospholipids within the bilayer and influences the phase transition translating into a displacement and broadening of the transition peak.

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