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EXAMINATION OF THE INFLUENCE OF F6H10 FLUORINATED DIBLOCKS ON DPPC LIPOSOMES

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The interactions of unilamellar vesicles obtained by the incorporation of (1,2,3,4,5,6)-tridecafluoro-hexadecane (F6H10 diblock) to dipalmitophosphatidyl-choline (DPPC), with Gd^{3+}, Ca^{2+}, Na^{+} ions were studied by electrophoretic measurements, dynamic light scattering and differential scanning calorimetry (DSC). Electrophoretic mobility measurements on unilamellar vesicles as a function of ion concentrations show that the vesicles adsorb the different ions employed. DSC has been used to determine the effect of diblock on the transition temperature (T_c) and on the change of enthalpy (ΔH_c) associated with the process.

Keywords: *DSC, fluorinated diblocks, vesicle-ion interactions*

Introduction

Liposomes are artificial systems that are formed by the self-assembly of lipids into spherical or quasi-spherical structures. Their size is in the range from nanometers to micrometers and can be formed of a single bilayer shell, unilamellar, or multiple concentric bilayer shells, containing an aqueous central cavity. Liposomes have important applications in research and industry. Liposomes have been used as model systems in the study of membrane-bound proteins, ion transport, energy transduction, cellular functions, etc. Their ability to act as versatile containers has been used for drug delivery and targeting, for the transfer of genetic materials to cells, and for use as food and cosmetic ingredients [1–5].

To improve the stability of liposomes several strategies have been performed. A useful method has been the introduction of fluorinated or semi-fluorinated chains. These systems impart a combination of thermal, chemical and biological inertness, high gas-dissolving capacities, low surface tension, high fluidity, excellent spreading characteristic and low solubility in water to various colloidal systems of biological interest such as liposomes fluorocarbon-in-water emulsions and hydrogen-in-fluorocarbon emulsions [6].

In this work we study the behaviour of DPPC liposomes whose bilayer has been modified by the introduction of a semifluorinated chain of sixteen carbon atoms, six of which are fluorinated and ten hydrogenated (F6H10 diblock). The effects of the interaction of Gd^{3+}, Ca^{2+} and Na^{+} in these liposomes were observed through their electrokinetic behaviour, size variation and thermal response to heating and to cooling.

Experimental

Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (No. P 0763) was obtained from Sigma, of analytical grade (99%) and used without further purification. Organic solvents, methanol and chloroform, were from Aldrich and Merck respectively. All used salts [$La(NO_3)_3$, $GdCl_3$, $Ca(NO_3)_2$, NaCl] were of analytical grade with purity higher than 99%. Polycarbonate membrane filters were purchased from Millipore. F6H10 was synthesized according to reference [7] in the Institut Charles Sadron, 'Chimie des Systèmes Associatifs' group, Strasbourg, France. Their purity (>99%) was determined by TLC, NMR and elemental analysis.

Vesicle preparation

An appropriate amount of DPPC with different amounts of F6H10 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

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of 43ºC for 1 h. The resulting mixture was sonicated at a temperature up to 43ºC 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

Electrophoretic mobility

Electrophoretic mobility of the PC liposome particles was 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 rectangular quartz capillary. The temperature of the experiments was 25ºC, controlled by a Haake temperature controller. The zeta potential (ζ-potential) was calculated from the electrophoretic mobilities as previously reported [8].

Photon correlation spectroscopy (PCS)

Measurements of the size and polydispersities of the liposome systems were performed by PCS using a Spectrometer Autosizer 4800 from Malvern Instruments equipped with a Uniphase 75 mW Ar laser, operating at 488 nm with vertically polarized light at a scattering angle of 90º at 298.15±0.01 K. Time correlation was analysed by a digital autocorrelator PCS7132 from Malvern Instruments and using the CONTIN algorithm. The lipid concentration used was 1 mg mL^{-1} of DPPC (1.36 mM).

Differential scanning calorimetry (DSC)

A Perkin-Elmer differential scanning calorimeter (Model DSC-7) was used for the DSC experiments. Fifty μL sealed pans of aluminum have been used as cell and reference. DPPC concentrations used were 10 mg mL^{-1} (13.6 mM) and different amounts in molar fraction (10, 20, 40, 50%) of diblock were added. The curves were obtained at a cooling and heating rate of 10ºC 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 high reproducibility.

Results and discussion

To investigate the stability of DPPC-F6H10 vesicle the adsorption of polyvalent metal ions Gd^{3+}, Ca^{2+}, Na^{+} has been studied. We are interested in these ions to obtain a complete characterization of their interactions with liposomes, continuing our more recent works [8–10]. For this purpose, measurements of the ζ-potential of DPPC-F6H10 vesicles as a function of concentration of ions have been made. DPPC molecules are zwitterionic, so that the liposomes bear a weak average charge, whose value depends on the pH of the medium. The ion concentration ranges include in all cases the critical aggregation concentration (*cac*). Figure 1 shows ζ-potential of DPPC-F6H10 liposomes as a function of Gd^{3+}. As it can be seen, the potential increase from an initial value of +30 mV to reach a maximum, which can be identified as the point of saturation of the liposome surface with Gd^{3+} ions. After this point, a slow decrease is observed (see onset plot) that could be due to ionic strength of the medium, which progressively screens the surface charge of the liposomes [11, 12]. Figure 2 shows ζ-potential of DPPC-F6H10 liposomes as a function of Ca^{2+} and Na^{+} concentrations. In this case the potential always decreases with an increment of ion concentrations, which suggests that the screening of the surface by counterions and the consequent ζ-potential decrease is predominant *vs*. cation bindings.

To obtain deeper information regarding vesicle stabilization, photon correlation spectroscopy (PCS)

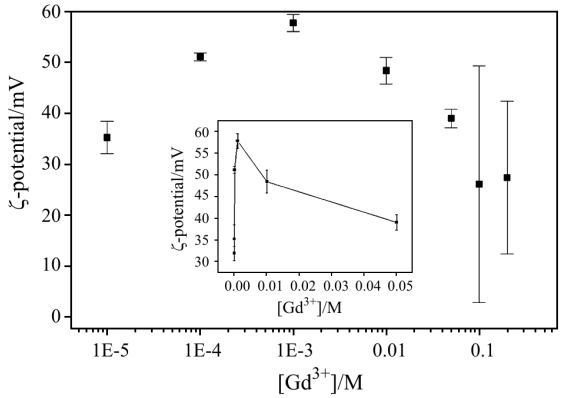

Fig. 1 ζ-Potential of DPPC-F6H10 as a function of Gd^{3+} molar concentration at 25ºC

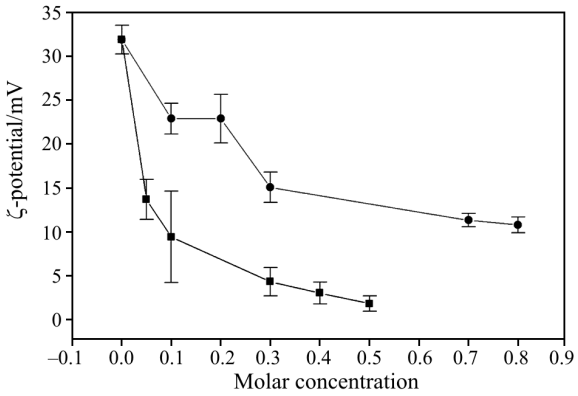

Fig 2 ζ-Potential of: ● – DPPC-F6H10 and ■ – DPPC-F6H10 as a function of Ca^{2+} and Na^{+} molar concentration, respectively, at 25ºC

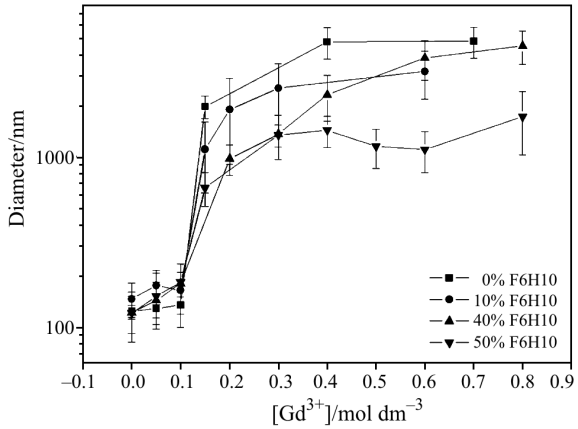

Fig. 3 Diameters of DPPC liposomes with different molar fractions of F6H10 as a function of Gd^{3+} concentration

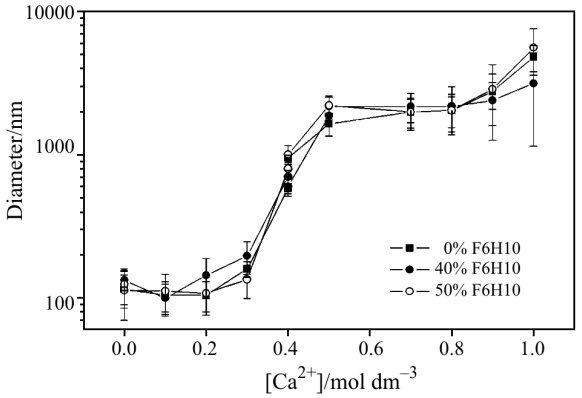

Fig. 4 Diameters of DPPC liposomes with different molar fractions of F6H10 as a function of Ca^{2+} concentration

was used. Figures 3 and 4 show size variation of DPPC-F6H10 liposomes as a function of Gd^{3+} and Ca^{2+} concentrations respectively. Liposomes in the presence of Gd^{3+} and Ca^{2+} show similarities. For concentrations >0.1 and >0.3 mol dm^{-3} respectively, the diffusing objects start to increase in size, probably due to an aggregation of liposomes. However the plot of Na^{+} (Fig. 5) offers a distinct feature. The size of liposomes does not increase with ion concentration, which suggests that the liposomes maintain stability. In all the cases, the incorporation of F6H10 to the bilayer does not affect the stability of the liposome.

The 'gel to liquid-crystalline' phase transition is easily detected by DSC, and characterized by a transition temperature (T_c), and an enthalpy change (ΔH_c), associated with the process. To proceed further in the direction of a complete analysis of the effect of the incorporation of diblock fluorinated in the membrane, DSC has been employed.

Figures 6 and 7 show cooling and heating curves, respectively, of DPPC-F6H10 liposomes. In the absence of diblock, DPPC liposomes had a gel to liquid transition temperature of $T_c \approx 42$ºC and a change of enthalpy of $\Delta H \approx 9.6$ J g^{-1}. These specific results

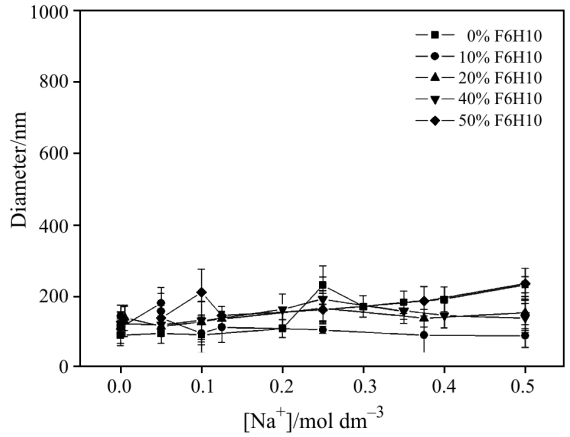

Fig. 5 Diameters of DPPC liposomes with different molar fractions of F6H10 as a function of Na^{+} concentration

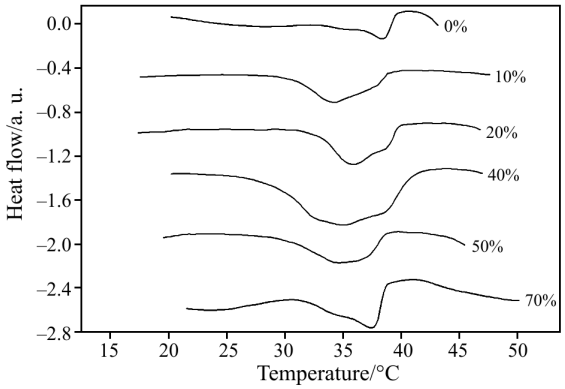

Fig. 6 Cooling curves of DPPC liposomes with different amounts of F6H10

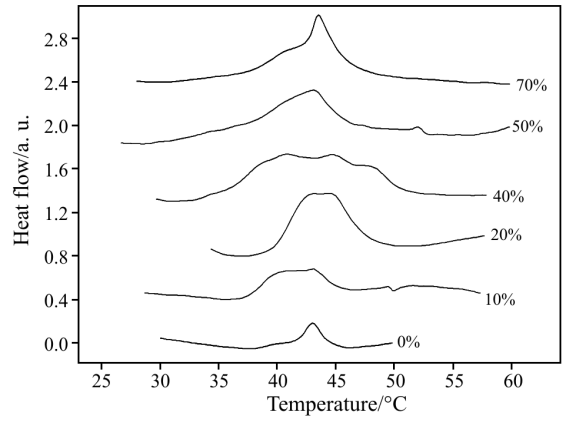

Fig. 7 Heating curves of DPPC liposomes with different amounts of F6H10

reasonable with bibliography citation [13, 14]. The addition of F6H10 to liposomes, in the range 0–15% molar fraction, results in a displacement of T_c, with broadening of the peak transition. Figure 8 shows the dependence of the transition enthalpy on diblock concentration. At intermediate concentrations of 15–50%

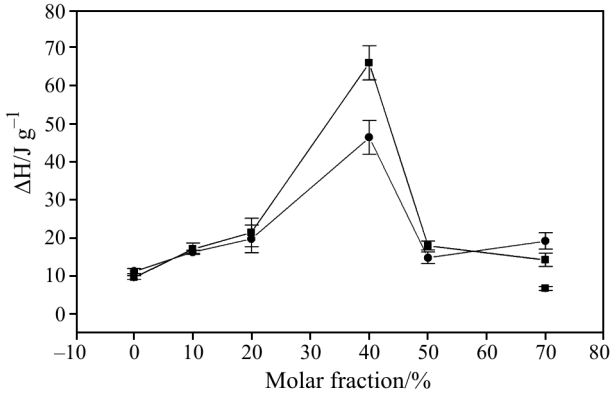

Fig. 8 Variation of enthalpy from gel to liquid crystalline transition of DPPC as a function of molar fraction of F6H10: ■ – heating, ● – cooling

molar fractions, the enthalpy change sharply increases. Beyond 50%, Δ*H* practically keeps constant.

The high hydrophobicity of diblock molecule suggests that the incorporation of the diblock into the bilayer is close to the hydrophobic tails of the lipid molecule. In previous work, anisotropic measurements show that the diblock (F6H2) is incorporated into the PC bilayer and the location of the diblock is in the middle of the bilayer [9].

Conclusions

Liposomes formed by DPPC with the semifluorinated diblock F6H10, in the presence of Gd^{3+}, Ca^{2+}, Na^{+} undergo changes in size, ζ-potential and 'gel to liquid' phase transition. The size change is enhanced more intensely in the presence of Gd^{3+}. The curves are affected by the incorporation of the fluorinated diblock. The changes in thermodynamic parameters of the phase transition are more important when the diblock concentration is around 20%.

By means of electrophoretic measurements, the adsorption of Gd^{3+}, Ca^{2+}, Na^{+} onto F6H10-DPPC liposome surface has been detected. ζ-Potential reaches a maximum, for Gd^{3+} ions, at very low salt concentrations ($\approx 10^{-3}$ M). Above this initial maximum, due to the increasing screening, ζ-potential decreases towards a constant value at higher ion concentrations.

The incorporation of the semifluorinated diblock into vesicles affects the physical properties of the bilayer. From the present study we cannot assume changes in their stability.

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