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Interactions between liposomes and hydroxypropylmethylcellulose

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Abstract

The characteristics of the adsorption process of hydroxypropylmethylcellulose (HPMC) of molecular weight 35 400 Da and nominal viscosity 100 cps onto liposomes prepared with different egg lecithin–cholesterol molar ratios were examined. Adsorption isotherms were constructed and analysed to investigate the mechanisms implicated in the incorporation of the polymer to the interface. Only the isotherms obtained with cholesterol-free liposomes were fitted with Langmuir model. When cholesterol is present in the composition they present a sigmoidal slope. The mechanism of adsorption depends on liposome composition being the main force that drives polymer adsorption of hydrophobic nature. The apparent volumes of HPMC indicate that the conformation of the adsorbed macromolecules depends on liposome composition. Hydration enthalpy values show that adsorbed polymers do not give more hydrophilic systems after freeze-drying as expected with the hydrophilic characteristics of the HPMC.

Keywords: Liposomes; Hydroxypropylmethylcellulose; Interfacial adsorption

1. Introduction

Liposomes are one of the most potent candidates for drug carrier systems. However, the efficacy of liposomes as a drug delivery system has not yet been established. One of the reasons for this is the instability of vesicles, particularly in biological media (i.e. blood, GI tract, etc.). It has been reported that the physicochemical properties of liposomes such as stability were improved by surface modification with polymers (Dong and Rogers, 1991a; Kondo et al., 1991; Allen, 1994; Takeuchi et al., 1998). Furthermore, the surface coating should provide a potential way of adding desirable functions to the liposomes such as mucoadhesion or affinity for a specific site, with great interest in its oral, ocular or pulmonary administration (Durrani et al., 1992; Briscoe et al., 1995; Iwanaga et al., 1997). On the other hand, changes in biodistribution and pharmacokinetic behaviour

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of drugs encapsulated in liposomes were observed when phospholipids modified with polyethyleneglycols were included in the vesicle composition (Van Etten et al., 1995; Yuda et al., 1996).

Polymers under consideration for the formulation of polymer-coated liposomes as a way to improve liposomal drug delivery systems, include polysaccharides such as dextran pullulan, mannan or chitosan; glycoproteins, such as plant, bacterial or viral lectins; biodegradable and soluble polymers (Miyazaki et al., 1992; Zhou et al., 1995; Kang et al., 1997; Takeuchi et al., 1999) were investigated. The coating of vesicles can be reached in different ways, some really complicated. A very simple approach of coating liposomes with polymers is to allow a water soluble polymer to adsorb at the liposome surface, in a similar way to endogenous macromolecules interacting with colloids foreign to the body.

Even though some work has been published on quantitative aspects of the incorporation of these substances to the liposome surface (Dong and Rogers, 1991b; Fonseca et al., 1993; Mobed and Chang, 1998; Takeuchi et al., 1998), little attention has been paid to the effects of formulation factors on the process and to the mechanisms involved in the adsorption phenomena (Kang et al., 1997; Mobed and Chang, 1997).

In this paper, the results of a study are presented which focused on evaluating the physicochemical consequences of coating multilamellar liposomes of different composition with hydroxypropylmethylcellulose (HPMC). The mechanism and extension of the process are characterised, and the effects of the incorporation of the polymer on the electrical charge and on the hydrophilicity of the vesicles are evaluated.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (EPC) (Ovothin® 200, batch 170790/951, Lucas Meyer, Spain), cholesterol (batch 116H0166, Sigma, USA), and HPMC (Methocel® K100LV, nominal viscosity 100 cps, batch MM94051022, Colorcon, Spain) were used. Water of resistivity < 18.2 MΩ cm, was obtained from a reverse osmosis system (Milli-Q®, Millipore, Spain).

2.2. Characterization of the HPMC

2.2.1. Viscometric determination of the molecular weight

The mean molecular weight of the HPMC was estimated from its intrinsic viscosity. A stock polymer aqueous dispersion was obtained by the procedure outlined in USP23 and diluted to provide a series of dilute dispersions. The kinematic viscosities of these dispersions were measured in sextuplicate at 20°C in a Canon-Fenske viscometer (ref. 5354/1, Afora, Spain). These data were fitted to the model of Martin's equation (Rowe, 1982) in order to obtain the intrinsic viscosity ($[\eta]$). Then, the mean molecular weight was estimated from $[\eta]$ using the Marck–Houwink's equation (Vinogradov and Malkin, 1980) with constants k and a set to 3.39×10^{-4} and 0.88, respectively (Law and Kayes, 1983).

2.2.2. Evaluation of the molecular dimension

The conformation adopted by the polymer in water and in pH 7.4 phosphate buffer at 25° was characterized using the equation from Simha (1945)

$$[\eta] = 0.207(l/d)^{1.732} \quad (1)$$

and the equation from Kraëmer (1941)

$$l = [(6l^2 \mathrm{M}\bar{v})/(d^2 N)]^{1/3} \quad (2)$$

which related the lengths of the higher (l) and lower axis (d) of the ellipsoid of revolution described by the polymer molecules of mean molecular weight M and specific partial volume $\bar{v}$, to the corresponding intrinsic viscosity $[\eta]$. N represents Avogadro's number and $[\eta]$ was estimated under these conditions from kinematic viscosities as described above. The value of $\bar{v}$ was estimated from the slope of the straight line with equation:

$$\rho = \rho_o + (1 - \bar{v}\rho_o)c \quad (3)$$

where ρ_o and ρ are the densities of the solvent and the polymer dispersion of concentration c, respectively (Chaveau et al., 1986).

2.2.3. Determination of the hydration enthalpy

The enthalpy of hydration of the HPMC was determined at 25°C by calorimetry in a Tronac® (model 458) isoperibol calorimeter comprised of a 50-ml reaction vessel immersed in a thermostated bath. The test sample (0.09–0.11 g) was sealed in a thin walled glass ampoule and mounted on a rotating support. After thermal equilibration, the ampoule was broken by means of a spring-loaded hammer mounted below. The rise in the temperature of the system was monitored using a thermistor, and later reproduced using a heating coil in the reaction vessel. The hydration enthalpy was calculated from the applied current and voltage and the heating time.

2.3. Determination of the hydration enthalpy of the cholesterol and the lecithin

The cholesterol and lecithin enthalpy of hydration was determined as described in Section 2.2.3.

2.4. Preparation of the liposomes

Liposomes of different composition (Table 1) were elaborated using a hydration method followed by homogenization, to assess that the size and the interfacial surface available to the polymer adsorption were similar in all cases. In brief, the lipid mixture was dissolved in a small amount of chloroform in a round-bottom flask and dried in a rotary evaporator under reduced pressure to form a thin film. The film was hydrated with pH 7.4 phosphate buffer (USP23) and then the size was reduced and homogenised with a mixer (Ultraturrax®, Polytron GmbH, Kinematica AG, Spain). The liposome dispersions were left for 24 h at room temperature to complete the lipid hydration.

Table 1
Lipid composition and vesicle mean size of the liposomes employed to the adsorption studies[a]

Liposome composition	Mean size (nm)
EPC	756.97 ± 54.92
EPC/CHOL (1:0.5)	782.33 ± 65.83
EPC/CHOL (1:1)	728.60 ± 148.0

[a] Numbers in parentheses are molar ratio egg phosphatidyl choline (EPC) to cholesterol (CHOL).

2.5. Adsorption of the polymer onto the liposomes

2.5.1. Construction of the adsorption isotherms

Adsorption isotherms were obtained for a series of dispersions of liposomes (lipid concentration 0.2 mg ml^{-1}) in a solution of between 0.4 and 1.8 mg ml^{-1} of polymer. Each dispersion was equilibrated by stirring at 25°C for 24 h, then centrifuged at $100\,000 \times g$ for 2 h (Centrikon® T-1075, Kontron Instruments, Spain). Unadsorbed polymer in the decanted supernatant was determined by the method of Mildwisky (1973). The resulting adsorption data were fitted (Chattoraj and Birdi, 1984) with the Langmuir's equation

$$\Gamma = \Gamma_{max} c/[c + (1/a)] \quad (4)$$

and Freundlich's equation

$$\Gamma = kc^{1/n} \quad (5)$$

In case of liposomes containing cholesterol, their adsorption data were fitted with the following sigmoidal equation:

$$\Gamma = a/(1 + be^{-Kc}) \quad (6)$$

In these equations, Γ is the amount of polymer adsorbed per mass unit of lipid and c the equilibrium polymer concentration in the system. In Eq. (4), Γ_{max} and a are the amount of polymer per mass unit of lipid forming monolayer and the adsorption activity, and in Eq. (5), k is the capacity of adsorbent bound to the polymer and n is the affinity of the polymer to the adsorbate. In Eq. (6), a, b and K are constants.

Microcal™ Origin™ 4.10 (Microcal Software, MA) software was employed to fit the experimental data with the different equations. When statistical analysis or stepwise regressions were necessary, the Sigma Stat® and Sigma Plot® (Jandel, USA) software were used.

2.5.2. Determination of the apparent volume of the HPMC in the presence of liposomes

The density of the liposomes (ρ_o) and the polymer–liposome dispersions (ρ) were determined at

Table 2
Molecular properties of HPMC K100LV in water and pH 7.4 phosphate buffer at 25°C

Medium	$[\eta]$ (ml g^{-1})	$\bar{v}$ (ml g^{-1})	Molecular axes (nm)	
			Major (l)	Minor (d)
Water	271.04	0.648	15.38	3.57
pH 7.4 phosphate buffer	287.34	0.685	16.03	3.59

25°C by using a Gay-Lussac pycnometer and then the apparent volume of HPMC (ml g^{-1}) was estimated from Eq. (3).

2.6. Characterization of the liposomes

2.6.1. Determination of the size and the ζ potential

The mean size of the vesicles (six measurements per formulation) was determined by photonic correlation spectroscopy in a Zetasizer 3 apparatus (Malvern Instruments, Spain) equipped with a 10-mm diameter AZ10 cell.

The ζ potentials for the liposome dispersions were calculated from their electrophoretic mobilities by means of the Helmholz–Smoluchowski equation (Zetasizer® 3 Instructions Manual, Malvern Instruments, Spain). Electrophoretic mobilities were measured in triplicate by laser Doppler anemometry in a Zetasizer 3 apparatus equipped with an AZ4 4 mm diameter cell. Optimal particle concentrations were obtained by diluting the dispersions with 1 or 5 mM KCl solution. The applied field strength was 150 mV.

2.6.2. Determination of the hydration enthalpy

The hydration enthalpy of the liposomes in the absence of polymer or in the presence of 0.4 or 1.8 mg ml^{-1} of HPMC (incorporated as described in Section 2.5.1) was determined as mentioned in Section 2.2.3. The sample was concentrated by centrifugation (the pellet was resuspended in an appropriate amount of buffer) and then freeze-dried in thin glass ampoules. The congelation and sublimation of the samples were carried out at −30°C. The ampoules were withdrawn from the freeze dryer when room temperature was reached and then sealed.

2.6.3. Determination of bound water

Aliquots of free polymer liposomes dispersed in pH 7.4 phosphate were freeze-dried in differential scanning calorimeter (DSC) aluminium sample pans and kept in a dessicator until the moment of analysis. Different amounts of water were added to the samples and the pans were sealed and left at 25°C for 24 h in order to obtain hydration equilibrium. After storage (weight controls were made to assure water content of the samples), the pans and their contents were placed into the sample compartment of the DSC (Shimadzu D50, Japan), and then cooled to −35°C to promote instant freezing of any unbound water. The samples were scanned at 5°C min^{-1} to 20°C, and the enthalpy of water fusion was determined. The quantity of water taken up by lipid was calculated from the differences between the amount of water that froze in the sample and the weight of water in the pan. To obtain these values, a calibration curve was plotted with different amounts of water and the enthalpy of the fusion process. The slope of the straight line (5.87 kJ mol^{-1}) agreed well with the literature value for the melting enthalpy of ice (Offringa et al., 1987).

3. Results and discussion

The viscometric studies developed in water at 20°C indicate that the mean molecular weight of the HPMC K100LV is 35 400 Da and the results of the experiments developed to characterize the polymer in water and in pH 7.4 phosphate buffer at 25°C show that there are no significant differences between polymer molecular properties estimated in the two media (Table 2). This molecular behaviour indicates a slight sensibility of poly-

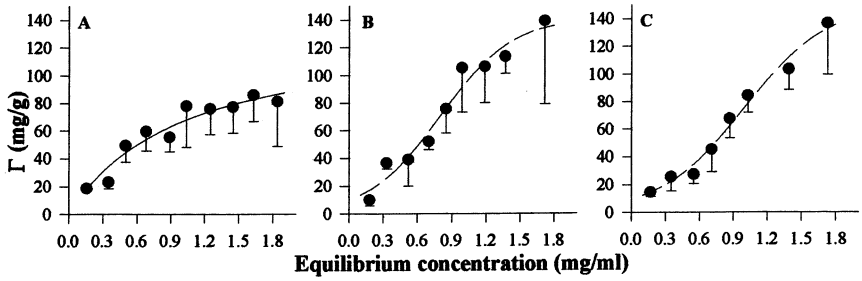

Fig. 1. Adsorption isotherms at 25°C of HPMC K100LV onto liposomes with different composition (EPC/cholesterol molar ratio): (A) EPC (1:0), (B) EPC/CHOL (1:0.5) and (C) EPC/CHOL (1:1). Filled circles are experimental data, plain line is data fitted with Langmuir equation and dotted line is data fitted with sigmoidal equation (values are the average and S.D., $n = 6$).

meric chains to the presence of ions in the medium thus must be related to the low polymer concentration and the low ionic strength of the phosphate buffer used and to the low molecular weight of HPMC. Other cellulose ethers with higher molecular weight have been shown to change their properties significantly in the presence of ionic additives (Huikari, 1990; Kublik and Müller, 1993). In order to complete HPMC characterisation and since adsorption of polymers depends on their hydrosolubility (Ross and Morrison, 1988) the hydration–dissolution enthalpy of HPMC was determined. The value obtained (-25.4 cal g^{-1}) agrees with the data of Joshi and Wilson (1993) and is not high enough to prevent polymer adsorption as occurs with most soluble cellulose ethers (Duro et al., 1998).

In order to investigate the association mechanism of the polymer onto the lipid vesicles whose size is around 750 nm, HPMC adsorption isotherms at 25°C were constructed at a HPMC concentration of 2 mg ml^{-1} (Fig. 1). The profile of the isotherm and the amount of adsorbed polymer seem to be a function of the lipidic components of the liposomes (Mobed and Chang, 1997). Furthermore, the adsorption model that the experimental data fits also depends on this factor (Fig. 1). In this sense, only the HPMC adsorption on free cholesterol liposomes fits the Langmuir model, while isotherms of liposomes with cholesterol fit better with a sigmoidal type function (Fig. 1). Langmuir type isotherms for liposomes have been reported by other authors (Kang et al., 1997; Mobed and Chang, 1998), however until the present study the polymer monolayer, typical of the model, has been not characterized. Keeping in mind the nature of the HPMC and the liposomes, the main force that provides the association of the polymer to the lipidic bilayer must be the hydrophobic interaction (Lockhead, 1992). In the case of free cholesterol liposomes, the hydrophobic interaction should take place mainly through the insertion of the hydrophobic segments of the polymer chain in the structure of the bilayer (Ohno et al., 1981; Fujiwara et al., 1997) (Fig. 2) and it will be favoured by the fluid state of the lipid membrane at the studied temperature as a consequence of the phase transition temperature of egg lecithin being below 0°C (Minetti et al., 1979; Benachir et al., 1997). As has been mentioned, when cholesterol was included as a component of the bilayer, a sigmoidal-type isotherm could be observed and at the same time, more HPMC is adsorbed (Fig. 1). This deviation from the theoretical Langmuir

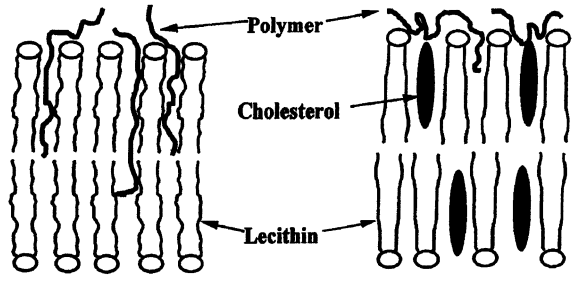

Fig. 2. Schematic drawing of different manners of adsorption of HPMC onto liposomes with or without cholesterol in the membrane composition.

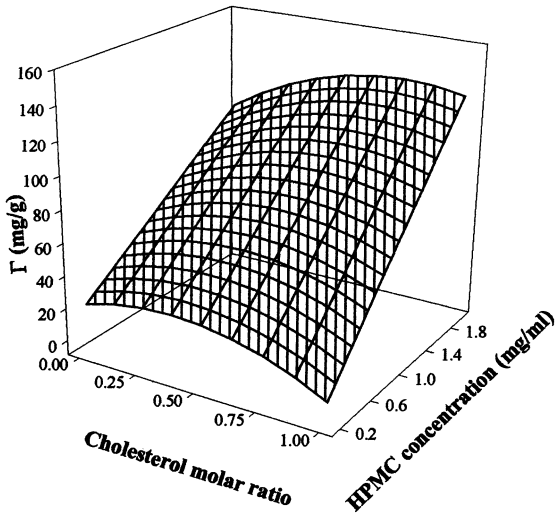

Fig. 3. Response surface of amount of HPMC adsorbed onto liposomes as a function of polymer concentration (pol) and cholesterol/phosphatidylcholine molar ratio (chol) in liposome membrane. $\Gamma = 12.8 + 43.2 \times \text{pol} + 40.1 \times \text{chol} - 62.3 \times \text{chol}^2 + 26.6 \times \text{pol} \times \text{chol}$; $R^2 = 0.705$, $F_{(4,171)} = 100$, $\alpha < 0.01$.

Table 3
Parameters of Freundlich type adsorption of HPMC onto the liposomes with different composition, at 25°C in pH 7.4 phosphate buffer

Liposomes	K (ml g^{-1})	n	R^2
EPC	64.08 ± 2.77	1.80 ± 0.24	0.895
EPC/CHOL (1:0.5)	86.61 ± 3.76	1.06 ± 0.10	0.953
EPC/CHOL (1:1)	68.02 ± 4.90	0.95 ± 0.13	0.906

isotherm could be related to the non-homogeneous surface of liposomes (Adamson, 1990) or to the existence of more than one adsorption mechanism (Chattoraj and Birdi, 1984). Certainly, cholesterol should hamper the chain polymer insertion in the bilayer; because of the tighter lipid packing when this sterol is present (Kawaguchi et al., 1992; Bakás et al., 1996). At the same time, this molecule makes the liposome surface more hydrophobic (Bernsdorff et al., 1997) allowing a hydrophobic interaction at the surface level of the vesicle. Thus when cholesterol is included in the membrane composition, the interaction between the polymer and the vesicles takes place through two mechanisms, segment insertion of polymer in the bilayer structure (probably a saturable process) at low polymer concentration and adsorption at the liposome surface, at higher polymer concentrations. This behaviour can be appreciated in Fig. 3; at low polymer concentration the amount of adsorbed polymer decreases as the bilayer cholesterol content increases, then at higher polymer concentrations the amount of adsorbed polymer increases if cholesterol is present in the membrane structure. In the latter, cholesterol makes the liposome surface more hydrophobic allowing a hydrophobic interaction at the surface level of the vesicles instead of the chain insertion in the bilayer.

Even though the experimental data fit better with the mentioned equations, the three adsorption isotherms can fit relatively well with the Freundlich model too. Therefore the n Freundlich parameter can be used to obtain comparative information on the affinity of the polymer to liposomes (Table 3). The values of this parameter show that the affinity of the HPMC for liposomes decreases as the cholesterol content in the vesicles was increased (Table 3).

The conformation of adsorbed polymer chains can greatly differ from their conformation in solution (Napper, 1989). Usually, the conformation adopted by adsorbed polymer molecules is estimated from the thickness of the adsorbed polymer monolayer (Law and Kayes, 1983; Duro et al., 1998). Nevertheless, this monolayer was not observed in either of our systems and the change of apparent volume of HPMC macromolecules in the presence of liposomes was determined from the apparent molecular volumes adopted by HPMC in the presence or absence of liposomes, as proposed by Quirion and St-Pierre (1991). The values of this parameter (Table 4) increase as the hydrophobicity of the membrane surface increases, as a consequence of the higher the cholesterol content in the liposome composition. This positive change in volume could be associated with a hydrophobic dehydration of hydrophobic segments of chain polymer due to its transfer from the pH 7.4 buffer to the liposome, allowing a better water interaction between the hydrophilic segments and water, and hence the observed polymer conformation is extended more (Quirion and Desnoyers, 1987; Quirion and St-Pierre, 1991).

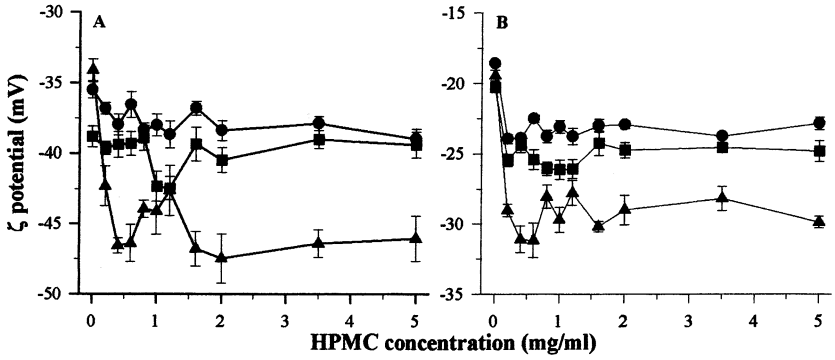

Fig. 4. Effect of HPMC on the ζ potential of liposomes (●, EPC; ■, EPC/CHOL (1:0.5) and ▲, EPC/CHOL (1:1)). Measurements were performed in 1 mM KCl (A) and 5 mM KCl (B) (values are the average and S.D., $n = 3$).

Table 4
Apparent molecular volume of HPMC (1.4 mg ml^{-1}) with or without liposomes of different compositions[a]

Liposomes	Molecular volume (ml g^{-1})
–	0.690 ± 0.12
EPC	0.694 ± 0.03
EPC/CHOL (1:0.5)	0.704 ± 0.05
EPC/CHOL (1:1)	0.884 ± 0.05

[a] Phospholipid concentration, 2 mg ml^{-1}.

The effect of the polymer on the surface electrical charge was characterised by determining the ζ potential after diluting the samples in 1 and 5 mM KCl solutions (Fig. 4). The less negative values of ζ potential observed at the higher KCl concentration is due to a shift of the shear plane to the vesicle surface. In both media, the adsorbed polymer onto either of the liposomes increased the absolute magnitude of this parameter, but the increased values were independent of the amount of adsorbed polymer. This effect could be related to the carbonyl and hydroxyl polar groups present in the hydrophilic segments of the polymer (Hsia et al., 1978) since the hydrophobic ones are associated with the liposome membrane, or to a charge intensification after the adsorption of co-ions, such as Cl^- (Li and Tiau, 1997). In this respect Huh et al. (1996) have reported that when binding penta-*O*-galloyl-a-D-glucose to egg lecithin liposomes, the vesicles can have a negative ζ potential depending on the amount of substance added.

Adsorbed HPMC onto liposomes should modify their hydrophilicity. To evaluate this effect, their hydration enthalpies were quantified. Fig. 5 shows the hydration enthalpies of the liposome formulations evaluated after freeze-drying in the presence or absence of HPMC. In spite of the higher hydrophobicity of the cholesterol (-0.79 ± 0.02 cal g^{-1}) with respect to the egg lecithin (-1.51 ± 0.25 cal g^{-1}), freeze-dried liposomes containing cholesterol presented higher values of hydration heat than freeze-dried free cholesterol liposomes and the adsorbed polymer only significantly renders hydrophilic systems to liposomes with the highest cholesterol content and when 1.8 mg ml^{-1} of HPMC was added. Finally,

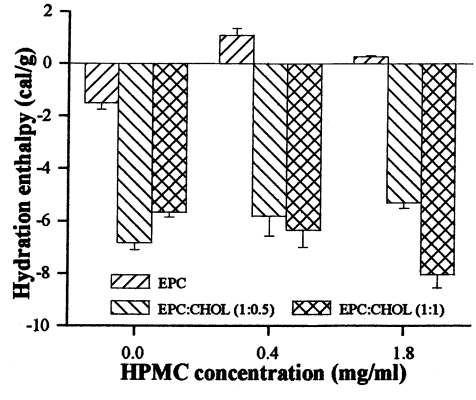

Fig. 5. Hydration enthalpy of freeze-dried liposomes after coating them with HPMC (values are the average and S.D., $n = 3$).

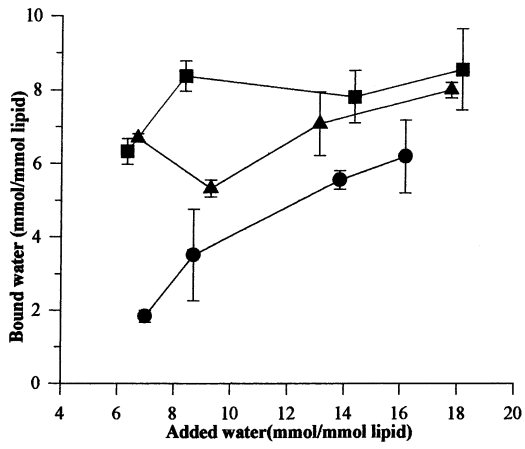

Fig. 6. Amount of bound water per mol of lipid versus ratio mol water/mol lipid for water lipid mixture liposomes (●, EPC; ■, EPC/CHOL (1:0.5) and ▲, EPC/CHOL (1:1)) (values are the average and S.D., $n = 2$).

adsorbed HPMC makes EPC liposomes slightly more hydrophobic.

These paradoxical results can be a consequence of the fact that, during dehydration in the freeze-drying process, more water could be removed from cholesterol containing liposomes than from egg lecithin liposomes because cholesterol or other related molecules may exclude water from polar head groups of phospholipids by forming H-bond bridges between opposing bilayers (Simon and McIntosh, 1989). To confirm this hypothesis, DSC studies with freeze-dried free polymer vesicles and added water were developed. This experimental work is based on the fact that only free water or bulk water can be detected from thermograms, because bound interacting water does not freeze or melt (Offringa et al., 1987; Joshi and Wilson, 1993).

The results of DSC studies of freeze-dried free polymer liposomes (thermograms not shown) agreed well with their hydration enthalpy results, because as less water is contained in the dry product the greater is its interaction with water, and hence more negative values of hydration enthalpy are observed. Fig. 6 shows that freeze-dried liposomes containing cholesterol, independent of the molar ratio uptake more water molecules per lipid molecule than freeze-dried egg lecithin liposomes.

In our study the adsorbed polymer under conditions and concentrations tested, onto liposomes with or without cholesterol in their composition, did not improve the interaction between the dry-system and water, because the values of this parameter were not reverted in the case of free cholesterol liposomes nor greatly modified in the case of liposomes containing cholesterol. Thus, it cannot be expected that under these conditions fusion and/or aggregation of vesicles is avoided after the freeze-drying process, such as when liposomes are freeze-dried with higher amounts of HPMC and no buffer was employed (Gutiérrez de Rubalcava, 1998). It is expected that greater water interaction of the dry system will improve the protective effect of HPMC on liposomes, but more studies are necessary to confirm this possibility.

In conclusion, HPMC adsorbs onto liposomes through hydrophobic interactions: by segment insertion of polymer chain in the lipid bilayer or at surface level, depending on polymer concentration and liposome composition. Nevertheless, amounts of HPMC do not significantly modify the hydrophilicity of the lipidic vesicles.

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