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Characterization of a new ocular delivery system based on a dispersion of liposomes in a thermosensitive gel

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Abstract

This paper describes a novel approach for designing an ocular delivery system based on the dispersion of liposomes into a thermosensitive gel made of a copolymer of ethylene oxide and propylene oxide (poloxamer 407). At high copolymer concentrations (20–30%), and from a temperature of approximately 20°C, poloxamer 407 passes from a solution to a gel. In order to stabilize liposomes in the gel, PEG2000-DSPE was introduced in their composition. Adsorption studies investigated by size and ζ-potential measurements have shown that the adsorption was higher for positively charged or neutral non-sterically stabilized liposomes. Poloxamer 407 adsorbed to a lower extent with negatively charged or PEG-DSPE containing liposomes. Furthermore, using a fluorescent aqueous marker, it was shown that liposome permeability was dramatically reduced in the presence of poloxamer 407 when PEG-DSPE was incorporated into the liposomes. This data suggests that poloxamer 407 could adsorb, at different extents, to all types of vesicles but that bilayer destabilization by the copolymer was reduced when liposomes were sterically stabilized. This was explained by the poor accessibility of the poloxamer to the phospholipidic which is the possible consequence of the steric repulsion effect induced by polyethylene glycol. Finally, it was shown that the thermosensitivity of poloxamer 407 was maintained after introducing the liposomes into the gel. In conclusion, a new system based on a dispersion of peggylated liposomes into thermosensitive poloxamer 407 is proposed, offering new potentialities for delivery of drugs.

Keywords: Liposomes; Poloxamer 407; Rheology; Adsorption; Stability

1. Introduction

The anatomical structure and the protective physiological process of the eye exert a strong defense against ocular drug delivery. This is the

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reason why conventional ocular dosage forms exhibit extremely low bioavailability. Limited absorption of the drug through the lipophilic corneal barrier is mainly due to short precorneal residence time related to the tear turn-over, rapid nasolacrimal drainage of instilled drugs from the tear fluid and non-productive absorption through the conjunctiva. Only a small proportion (1–3%) of the applied drug penetrates the cornea and reaches intraocular tissues (Mezei and Meisner, 1993). For these reasons, new ocular delivery systems urgently need to be developed.

Different strategies can be carried out to improve the precorneal residence time and/or penetration ability of the active ingredient. Among them, one approach consists in using colloidal drug delivery systems such as nanoparticles (Wood et al., 1985; Fitzgerald et al., 1987), microparticles (Beal et al., 1984) or liposomes (Singh and Mezei, 1983; Stratford et al., 1983; Guo et al., 1988). Several studies have shown that liposomes used as ocular delivery systems can provide increased efficacy (Smolin et al., 1981; Schaeffer and Krohn, 1982; Pleyer et al., 1993; Meisner et al., 1989), reduced toxicity (Tremblay et al., 1985) and prolonged activity (McCalden and Levy, 1990). However, due to the low viscosity of colloidal suspensions that does not allow sufficient retention time of the dosage form, this approach needs to be improved. One other strategy used to delay the precorneal drainage rate of liposomes consists in coating the vesicles with polymers. Davies et al. (1992) have used γ-scintigraphy to evaluate the precorneal clearance of uncoated, Carbopol 934P and Carbopol 1342-coated liposomes in rabbit eye. The coated liposomes at pH 5 remained for a longer time on the corneal surface compared to those formulated at pH 7.4. In the same way, Durrani and Davies (1992), have demonstrated that Carbopol 1342-coated liposomal formulations increased the bioavailability of pilocarpine in the rabbit eye compared with the uncoated preparation.

On the other hand, poloxamer 407 (a non-toxic copolymer of polyoxyethylene-polyoxypropylene) gels have been studied extensively as drug delivery systems (Schmolka, 1972; BASF Wyandotte, OS-30121 (765) BASF, 1973). One main characteristic of this copolymer is its ability to undergo a reverse thermal gelation. Concentrated solutions (20–30% w/v) of the polymer are fluid at 4–5°C, but turn to soft gels at the body temperature. In addition, low toxicity, mucomimetic properties and optical clarity make poloxamer 407 particularly suitable for ophthalmic formulation (BASF Wyandotte, OS-796; Waring and Harris, 1979). Another advantage of this system is that it can be applied to many drugs for controlled delivery. Indeed, poloxamer gels containing various medications have been used with success in clinics for patients suffering from a variety of ocular diseases, including red eye, corneal edema, dry eye syndrome (Henry and Schmolka, 1989) and glaucoma (Miller and Donovan, 1982).

In this paper, a new ocular drug delivery system based on the dispersion of liposomes (acting as a drug reservoir) into thermosensitive poloxamer 407 gels is proposed. This formulation is supposed to be useful in prolonging the precorneal residence time of the encapsulated drugs, in controlling their release and subsequently, in improving corneal bioavailability. Since poloxamer 407 is a non-ionic surfactant, special attention is paid in this study on the interactions between the copolymer at high concentrations and the gel-dispersed liposomes. Conditions for successful preparation of this formulation are described so that ophthalmologists could use it for in vivo evaluation.

2. Materials and methods

2.1. Materials

Poloxamer 407 (Lutrol F127®) was a gift from BASF. Soybean Phospholipid was provided by Lipoid (KG, Ludwishafen, Germany). 1,2-Distearoyl-sn-glycero-3 phosphatidylethanolamine-*N*-(poly(ethyleneglycol)-2000) (2000PEG-DSPE) was purchased from Avanti Polar Lipids (Birmingham, AL). Cholesterol (CHOL), stearylamine (SA), phosphatidylglycerol (PG) and calcein were purchased from Sigma (St. Louis, MO). All reagents were 99% pure.

2.2. Poloxamer preparation and characterization

Poloxamer 407 gels were prepared by the cold process described by Schmolka (1972): an appropriate amount of Poloxamer 407 was slowly added to Hepes/NaCl buffer 10/145 mM, pH 7.4 at 4°C under constant stirring after which the dispersion was stored at 4°C. After an overnight period, a clear viscous solution was obtained.

2.3. Preparation of the liposomes

Liposomes were composed either of soybean phosphatidylcholine (SPC) 100 mol%, or SPC:CHOL (molar ratio: 70:30), or SPC:CHOL:SA (molar ratio: 60:30:10), or SPC:CHOL:PG (molar ratio: 60:30:10), or SPC:CHOL:2000PEG-DSPE (molar ratio: 64:30:6). Liposomes were prepared by the method described by Bangham et al. (1965). Practically, lipid mixtures were dissolved in chloroform and evaporated to dryness in a round-bottomed flask (100-ml volume). Liposomes were formed by rehydrating the dried lipid film with Hepes buffered saline (10 mM Hepes, 145 mM NaCl, pH 7.4) to yield a final lipid concentration of 20 mM. The resultant multilamellar vesicles were extruded ten times through a 0.4-μm polycarbonate membrane and ten times through a 0.2-μm filter with an extruder (Lipex Vancouver, Canada). The final size of liposomes was around 200 nm. Liposomes containing calcein (concentration of 40 mM) were prepared in the same conditions. Free calcein was separated from calcein, containing liposomes by exclusion-diffusion gel chromatography on a 2.5×40 cm Sephadex G-50 column. The calcein encapsulation efficiency in liposomes was ranged from 0.6 to 1.5%.

2.4. Dispersion of liposomes into poloxamer solutions

The dispersion of liposomes into poloxamer 407 solutions was achieved by mixing the liposome aqueous suspensions with poloxamer solutions under continuous magnetic stirring in ice. Final lipid concentration was 2 mM and final poloxamer concentrations were respectively 0.9, 9.0, 13.5, 18.0 and 27% w/v in the different preparations.

2.5. ζ-Potential

The ζ-potential of the single aqueous suspension of liposomes (PC:CHOL; PC:CHOL:PG; PC:CHOL:SA and PC:CHOL:PEG-DSPE) and the ζ-potential of the same formulations dispersed in poloxamer preparations at 27% were measured using a Zetasizer 4 (Malvern, France). The determination of the ζ-potential was realized after 5 h of incubation of the liposomes together with the poloxamer 407 at 4°C. Prior to measurements, small aliquots of the single aqueous suspension of liposomes (40 μl) or liposomes dispersed in poloxamer (200 μl), were diluted in 6 ml of buffer solution (10 mM Hepes, 145 mM NaCl, pH 7.4). The diluted solutions were considered to be Newtonian.

2.6. Size measurements

Liposome size measurements were achieved by dynamic light scattering using a nanosizer N4MD (Coultronics, France). Measures were carried out at 90° angle and at a temperature of 20°C. The liposomes studied were PC:CHOL; PC:CHOL:PG; PC:CHOL:SA and PC:CHOL:PEG-DSPE. The size measurements of single aqueous suspensions of liposomes and liposomes dispersed in various concentrations of poloxamer 407 (0.9–27%) were investigated after incubation at 4°C during 5 h. To evaluate the variation of liposome diameters in time, we have determinated the size of single aqueous suspensions of liposomes and the size of liposomes dispersed in poloxamer 407 at 27% after 5 min, 5 h and 45 days of incubation at 4°C. Prior to measurements, small aliquots of the single aqueous suspension of liposomes (40 μl) or liposomes dispersed in poloxamer (200 μl), were diluted in 6 ml of buffer solution (10 mM Hepes, 145 mM NaCl, pH 7.4). The diluted solutions were considered to be Newtonian.

2.7. Viscosity measurements

A Carri-Med CSL 100 rheometer (Rheo, Champlan, France) was chosen for viscosimetric measurements. A cone-and-plate geometry was selected for all the measurements. The cone diameter was 4 cm and the cone angle was 2°. Viscosity measurements were performed with two liposome formulations (PC:CHOL and PC:CHOL:PEG-DSPE). Liposomes dispersed into poloxamer 407 preparations were incubated during 24 h at 4°C before measurements. The final poloxamer concentration were 27% and the final lipid concentrations in the mixtures were either 2 or 12 mM. Measurements were realized at 10 and 37°C. At 10°C the rheograms were composed of three phases:

(1) the shear stress was continuously increased, for a period of 2 min until a value of 40 N/m^2 was attained,

(2) the sample was subjected to a constant shear stress for a period of 2 min,

(3) the shear stress was continuously decreased for a period of 4 min, from its maximal value until it reached zero.

At 37°C, the rheograms were composed of three phases:

(1) the shear stress was continuously increased, for a period of 4 min until a value of 400 N/m^2 was attained,

(2) in the second part, the sample was subjected to a constant shear stress for a period of 2 min,

(3) in the third part, the shear stress was continuously decreased for a period of 4 min, from its maximal value until it reached zero.

At 37°C, the rheograms have been fitted with the help of the Herschel-Bulkley equation, Eq. (1); which described a plastic behavior.

$$\tau = \tau_0 + K\dot{\gamma}^n \quad (1)$$

τ and τ_0 represent the shear stress and the yield value, respectively; $\dot{\gamma}$corresponds to the shear rate, K corresponds to the consistency index and n represents the power law exponent.

Table 1
ζ-Potential of liposomes dispersed in buffer (−) or in an aqueous solution of poloxamer 407 at 27% (+) ($n = 3$)

Liposome composition	Poloxamer	ζ-Potential (mV ± S.D.)
PC:CHOL:PG	−	−25.0 ± 0.5
	+	−15.2 ± 0.1
PC:CHOL:PEG-DSPE	−	−8.6 ± 1.9
	+	−12.9 ± 0.4
PC:CHOL	−	−9.5 ± 2.1
	+	−12.4 ± 0.8
PC:CHOL:SA	−	+14.6 ± 0.4
	+	−8.2 ± 1.7

2.8. Calcein leakage

Leakage of calcein from liposomes (PC:CHOL and PC:CHOL:PEG-DSPE) dispersed in poloxamer gels at 27% was measured after keeping the formulations at 4°C. After different time intervals (30 min, 1 h, 24 h, 7 days and 1 month), fluorescence was measured in the absence and in the presence of Triton X-100 (1% w/v final concentration) using a fluorescent spectrometer JY 3D ISA (Jobin Yvon, France). Excitation and emission wavelenghts were of 489–519 nm, respectively. Samples were diluted in buffer (10 mM Hepes, 145 mM NaCl, pH 7.4) before measurements. Efflux of calcein from liposomes was calculated by the following equation:

$$\% \text{ leakage} = (F_x - F_0)/(F_t - F_0) \times 100$$

where F_x was the fluorescence of the liposomes in poloxamer 407 at time x, F_0 the fluorescence intensity of the sample of liposomes at time zero and F_t the total fluorescence intensity of the liposomes in poloxamer 407 after addition of Triton X-100 at time x.

3. Results

3.1. ζ-Potential and size measurements

ζ-Potential measurements have shown that poloxamer 407 interacted strongly with and ad-

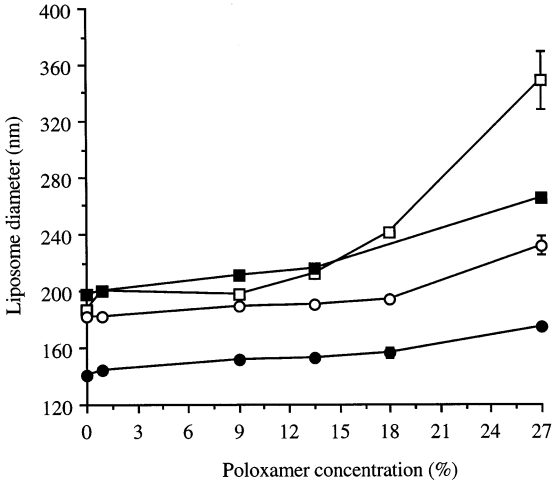

Fig. 1. Diameter of liposomes (nm) composed of (□) PC:CHOL SA; (■) PC:CHOL; (○) PC:CHOL:PG and (●) PC:CHOL:PEG-DSPE in the presence of various poloxamer concentrations (5 h of incubation) ($n = 3$).

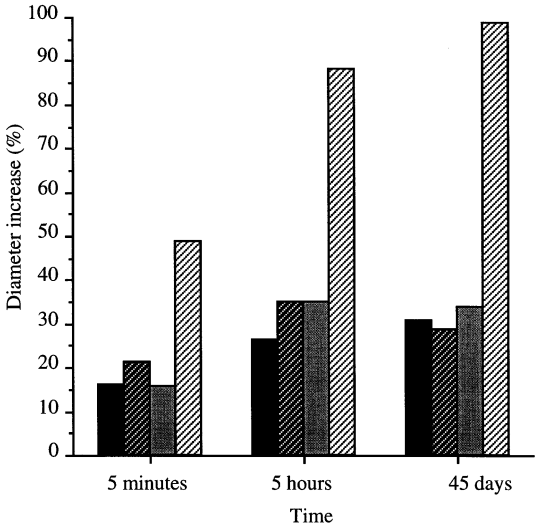

Fig. 2. Diameter increase (%) composed of (▨) PC:CHOL:SA; (▩) PC:CHOL; (▨) PC:CHOL:PG or (■) PC:CHOL:PEG-DSPE incubated in poloxamer 407 (27%; 4°C) after 5 min, 5 h and 45 days ($n = 3$).

sorbed onto both negatively (PC:CHOL:PG) and or positively (PC:CHOL:SA) charged liposomes (Table 1). In both cases, the surface charges of the liposomes were shielded by the presence of poloxamer 407. This was attributed to the formation of a polymer layer onto the surface of the liposomes. The measurements of the ζ-potential of PC:CHOL and PC:CHOL:PEG-DSPE showed on the contrary, no important modification of ζ-potential, after incubation with poloxamer 407. This became obvious since these liposomes were very close to the neutrality. To investigate more in details the interactions of poloxamer molecules with the liposomes, ζ-potential measurements were complemented by size measurements in the presence of increasing concentrations of poloxamer (Fig. 1). The size of the liposomes increased as a function of the poloxamer concentration. The thickness of the layer was dependent on the composition of the liposomes and of the polymer concentration (Fig. 1). In general, liposomes coating increased with increasing poloxamer concentration. PC:CHOL:SA composition exhibited a maximum interaction with poloxamer 407 since the diameter of those liposomes increased by 85%. The incubation of poloxamer 407 with negatively charged or PEG-DSPE containing liposomes resulted in a lower diameter increase. The kinetics of adsorption of poloxamer 407 (27%) onto liposomes revealed in Fig. 2 that the adsorption was very quick and occurred almost after 5 min of incubation.

3.2. Leakage of calcein from liposomes

Efflux of calcein from liposomes was monitored by measuring fluorescence intensity increase, as calcein became dequenched upon release from the liposomes in the incubation medium. The kinetics

Table 2
Release of calcein from PC:CHOL:PEG-DSPE- or PC:CHOL liposomes in poloxamer gels at 27%

Time (h)	Leakage (%) at 4°C	
	PC:CHOL:PEG-DSPE	PC:CHOL
0	0	0
0.5	0	0
1	0	18.3 ± 1.4
24	0	50.7 ± 3.93
7×24	0	52
30×24	54.9 ± 2.9	100

Table 3
Viscosity measurements of poloxamer gels (27%) at 10°C in the presence of various liposome concentrations in gels ($n = 3$)

Formulations	Viscosity (mPa.s)
Poloxamer	43.7 ± 0.30
Poloxamer + PC:CHOL 2 mM	43.7 ± 0.05
Poloxamer + PC:CHOL 12 mM	43.2 ± 0.20
Poloxamer + PC:CHOL:PEG-DSPE 2 mM	43.3 ± 0.05
Poloxamer + PC:CHOL:PEG-DSPE 12 mM	41.9 ± 0.00

of calcein release from liposomes dispersed into poloxamer gels are shown in Table 2. Calcein was released more quickly from PC:CHOL liposomes than from PEG-DSPE containing liposomes for which no calcein was released after 24 h, whereas, from non-sterically stabilized liposomes 50% of encapsulated calcein was liberated.

3.3. Viscosity measurements

At 10°C (Table 3), the preparations were in a 'sol' state and displayed a Newtonian behavior with a linear relationship existing between shear rate and shear stress (Fig. 3). As the temperature was increased to 37°C, the preparations are in a

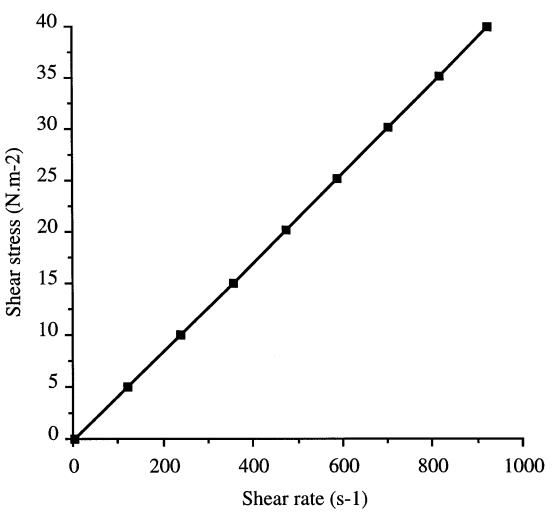

Fig. 3. Rheogram of a 27% aqueous solution of poloxamer 407 (■) at 10°C.

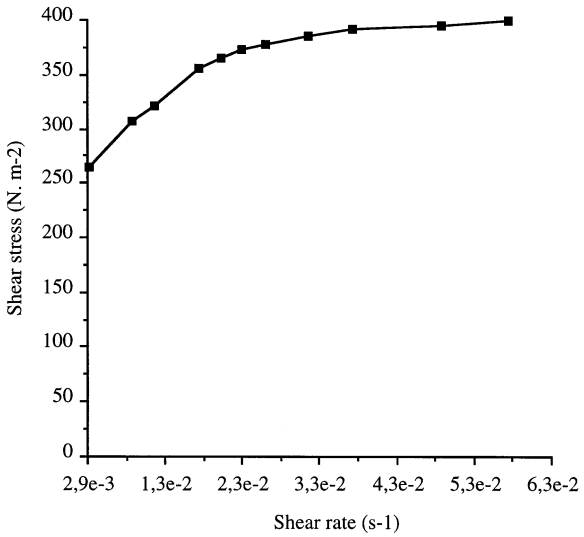

Fig. 4. Rheogram of a 27% aqueous solution of poloxamer 407 (■) at 37°C.

'gel' state and rheological behavior became non-Newtonian (Fig. 4). The rheograms are fitted by Herschel-Bulkley equation which describes a plastic behavior and are characterized by an important measured yield value (Tables 4 and 5). The experiments realized with eight 27% poloxamer solutions showed a good reproductibility of the results (Table 4). The influence of the presence of liposomes on the viscosity of poloxamer gels was then determined using 27% poloxamer 407 gels containing liposomes of PC:CHOL (70/30) or PC:CHOL:PEG-DSPE (64/30/06). The results obtained at 10 and 37°C with these systems containing 2 and 12 mM of lipids showed no difference in

Table 4
Herschel-Bulkley parameters resulting from 27% poloxamer solutions

Formulations	K (SI)	n	r
Poloxamer (1)	657	0.15	0.907
Poloxamer (2)	676	0.15	0.924
Poloxamer (3)	616	0.15	0.975
Poloxamer (4)	674	0.15	0.934
Poloxamer (5)	653	0.15	0.870
Poloxamer (6)	680	0.16	0.929
Poloxamer (7)	703	0.16	0.884
Poloxamer (8)	709	0.16	0.856

Table 5
Herschel Bulkley parameters resulting from a 27% poloxamer solution with liposomes ($n = 3$) or without ($n = 8$)

Formulations	τ_0 (N/m^2)	K (SI)	n
Poloxamer	240 ± 21	671 ± 30	0.15 ± 0.006
Poloxamer + PC:CHOL 2 mM	270 ± 16	657 ± 37	0.16 ± 0.02
Poloxamer + PC:CHOL 12 mM	250 ± 35	637 ± 75	0.14 ± 0.03
Poloxamer + PC:CHOL:PEG-DSPE 2 mM	240 ± 3	663 ± 32	0.15 ± 0.01
Poloxamer + PC:CHOL:PEG-DSPE 12 mM	240 ± 13	655 ± 9	0.15 ± 0.003

viscosity comparatively to the poloxamer 407 gels free of liposomes (Tables 3 and 5). As the concentration of liposomes increased, no significant differences in the rheological properties of the 27% poloxamer solutions were observed (Tables 3 and 5). The addition of liposomes did not induce the loss of thermosensible properties.

4. Discussion

Different studies dealing with the interaction of poloxamer 407 with liposomes (Jamshaid et al., 1988; Woodle et al., 1992) have shown that there was only a weak modification of the diameter of liposomes when coated with poloxamer 407. These studies were, however, carried out with too low concentrations of poloxamer 407 (0.001–0.85% w/v) for obtaining a viscous thermosensitive gel for ocular delivery. This is why, in this publication, we have investigated the stability of the liposomes into poloxamer 407 gels (up to 27%) with thermosensitive properties.

ζ-Potential and size measurements revealed that poloxamer adsorbed indifferently to negatively or positively charged or neutral liposomes. However, the thickness of the poloxamer coating layer was much more important with positive PC:CHOL:SA liposomes than with the other ones. Nevertheless, the presence of PEG-DSPE did not induce a complete repulsion of the adsorbed layer. One can easily imagine that, due to their similar chemical structure, polyethylene glycol and polyoxyethylene could interact together. However, the interaction between PEG and poloxamer molecules may be supposed to protect the lipid bilayer from the destabilization by poloxamer 407 (Fig. 5b). To investigate this point permeability studies were conducted showing that release of the fluorescent marker from liposomes in the presence of poloxamer coating material occurred only with conventional liposomes. This could be due to the intrusion of the diblock polymer into the phospholipid bilayer leading to the eventual formation of pores, fractures or region membrane fluidity through which calcein could leak out. Jamshaid et al. (1988) demonstrated that coating of small unilamellar liposomes (SUV) of egg phosphatidylcholine with polyoxyethylene-polyoxypropylene block copolymers (poloxamers) resulted in a rapid leakage of the entrapped aqueous materials. The preincubation of egg PC vesicles in the presence of 0.01% (w/v) poloxamer was sufficient to release more than 70% of their entrapped aqueous marker, thus indicating that liposome integrity was not retained. This study suggested clearly that poloxamers could penetrate the lipid bilayer of fluid egg PC liposomes and project their polyoxyethylene groups from the vesicle surface. In a study of Woodle et al. (1992), efflux of entrapped aqueous label increased in presence of poloxamer indicating that adsorption of this copolymer did occur at the surface of the liposomes. This effect was reduced when liposomes were prepared with high temperature phase transition lipid. The present publication provides evidences that PEG-DSPE could reduce dramatically the permeability of liposomes to calcein. As suggested by Torchilin et al. (1994) the flexible PEG on the liposome surface forms a conformational cloud with a very high density in its central part. Therefore, a relatively small number of water-soluble, very flexible polymer molecules can create a sufficient number

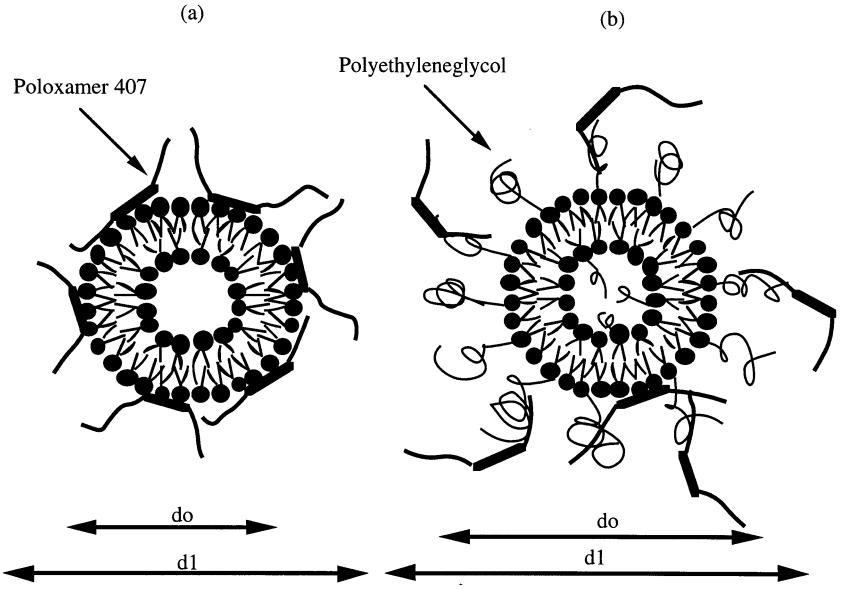

Fig. 5. Suggested mechanism for the interaction of poloxamer 407 with conventional (a) or sterically stabilized (b) liposomes. (d_0 = diameter of liposomes), (d_1 = diameter of liposomes in the presence of poloxamer 407).

of high density conformation clouds over the liposome surface, forming a protective 'umbrella'. The inclusion of PEG-DSPE in liposome formulations thus provide a steric barrier consequently reducing the possibility for poloxamer chains to meet the outer phospholipid bilayer as suggested in the proposed model (Fig. 5a, b).

Finally, the rheological properties of poloxamer 407 were not affected by the introduction of liposomes. Particularly, the thermosensitivity of the gels was kept constant which makes this system reliable for topical administration.

In conclusion, a new system based on a dispersion of sterically stabilized liposomes into a thermosensitive gel was developed. Due to its physicochemical characteristics (thermosensitivity and liposomal controlled delivery) this system may offer new possibilities for ocular delivery.

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