Department of Colloid Chemistry[1], Eötvös University, and Institute of Biophysics[2], Semmelweis Medical University, Budapest, Hungary

The influence of polymers on the physical stability and the thermal properties of dimyristoyl-phosphatidylcholine liposomes

F. L. Grohmann[1], F. Csempesz[1] and M. Szögyi[2]

The effect of some neutral polymers on the physical stability of dimyristoyl-phosphatidylcholine(DMPC)-liposomes and on the phase transition parameters of the phospholipid membrane was studied by size distribution measurements and differential scanning calorimetry. It was shown that uncharged macromolecules can be effective steric stabilizers for the vesicles. A close correlation was found between the thickness of the polymer layer formed around the vesicles and the physical stability of the liposomes. DSC measurements confirmed that the macromolecules interact with the membrane bilayer. The best steric stabilizer, poly(vinyl alcohol-co-vinyl butyral)copolymer eliminates pretransition of the membrane.

1. Introduction

Liposomes are promising drug carrier systems. There are a number of potential drug candidates for liposome encapsulation (antimicrobial drugs, cancer chemotherapeutic agents, etc.) [1–4].

The medical utility of so called conventional liposomes is limited by their rapid uptake by mononuclear phagocytic system (MPS). Interest in liposomes as drug carriers was rejuvenated by the introduction of sterically stabilized liposomes. Because of their reduced recognition and uptake by the immune system, these newly sophisticated liposomes have been referred to as "stealth" liposomes [5, 6]. The enhanced biological stability of sterically stabilized liposomes is a result of the inhibition of interactions with plasma proteins such as opsonins and lipoproteins. The polymer-coated liposomes have, therefore, a long lifetime in the bloodstream [7–12]. The polymer layer on the surface of liposome reduces attractive forces and increases the hardcore repulsion between the possible reactants.

Several reports demonstrated that liposomes containing phospholipid derivatized with a hydrophilic polymer of relatively low molecular weight (e.g. polyethylene glycol, PEG), exhibit prolonged circulation. Much less attention was paid to the use of high polymers in liposome stabilization. In this work the influence of some uncharged polymers with different chemical structures, on the physical stability of dimyristoyl-phosphatidylcholine (DMPC)-vesicles is studied with the aim of finding steric stabilizers for liposomes and of revealing the conditions for effective stabilization. Therefore, the change in time of the size and the zeta potential of vesicles with or without adsorbed polymer was measured by photon correlation spectroscopy (PCS) and laser Doppler anemometry (LDA), respectively [13]. For a more detailed understanding of the stabilizing effect, the possible interactions of lipid molecules with polymers were studied by differential scanning calorimetry (DSC).

2. Investigations, results and discussion

2.1. Characteristics of dimyristoyl-phosphatidylcholine-liposomes

Vesicle size and size distribution as well as the surface properties of liposomes are relevant features regarding both the organ distribution and the encapsulation or adsorption efficiency of liposomal drug carriers. In former studies [15–18] it was shown that using our standardized procedure for the preparation of DMPC-liposomes, small unilamellar vesicles (SUV) with well-defined size distribution can be prepared both in the absence and presence of polymer.

For an adequate characterization of the liposomes formed in different media, the volume-average mean vesicles size (d_0) calculated by multimodal analysis from the measured intensity distribution of scattered light from liposomes by PCS, the polydispersity index (PI) and also, the zeta potential of vesicles are shown in Table 1. It can be seen that soon after the preparation, the liposomes exhibit monomodal size distribution both in distilled water and physiological saline. The relatively low zeta potentials, however, show that the vesicles are electrostatically slightly stabilized, and their kinetic stability in the electrolyte solution is lower as expected.

Polymer addition to the dissolved lipid resulted in some increase in the vesicle size without perceptibly changing the polydispersity of the dispersion. This indicates that the uncharged macromolecules are attached to the DMPC-liposomes but they do not cause any vesicle aggregation. From the increase in the hydrodynamic diameter of the vesicles due to the attached macromolecules, the thickness

Table 1: Mean vesicle size (d_0) and zeta potential (ζ) of DMPC-liposomes

Medium	Vesikle size (d_0, nm)	Polydispersity (PI)	Zeta potential (ζ, mV)
Distilled water	48	0.41	-11.2 ± 1.1
0.15 M NaCl solution	57	0.40	$-\ 4.5 \pm 1.2$

Table 2: Average thickness of polymer adsorption layers on DMPC-liposomes

Liposome	Medium	Layer thickness (δ, nm)	Polydispersity (PI)
DMPC + PVA	distilled water	6 ± 1	0.41
	0.15 M NaCl	5 ± 1	0.41
DMPC + PVA-Al	distilled water	~ 1	0.41
	0.15 M NaCl	~ 1	0.38
DMPC + PVA-Prod	distilled water	4 ± 1	0.43
	0.15 M NaCl	~ 1	0.42
DMPC + PVA-Bul	distilled water	14 ± 2	0.40
	0.15 M NaCl	15 ± 2	0.34
DMPC + PVP K-30	distilled water	~ 2	0.42
	0.15 M NaCl	~ 1	0.40
DMPC + PVP K-90	distilled water	4 ± 1	0.36
	0.15 M NaCl	~ 2	0.34

(δ) of polymer layers formed around the liposomes could be estimated. The average hydrodynamic layer thicknesses are listed in Table 2.

These results show considerable differences in the layer thickness for the polymers. A significantly thicker macromolecular sheath, which is very favourable for steric stabilization is formed from the PVA-Bul (see 3.1) copolymer, a thinner one is formed around the vesicles from other polymers in both media. It is also demonstrated by these data that the molecules of PVA-based copolymers with systematically increasing side chains (while their molecular weight is nearly the same) are adsorbed in different conformations on the vesicle surfaces or their chain segments are incorporated to different extent in the lipid bilayers.

2.2. Physical stability of DMPC-liposomes

To preserve the stability of vesicles is one of the essential requirements for liposomal drug carriers [1–4, 12, 19, 20]. Aggregation and fusion of liposomes (which can be well regulated by the extent of electrical and/or steric repulsive forces operating between the vesicles) cause alike a shift in the mean size and size distribution towards higher values. Therefore, vesicle size is a useful marker for indicating changes in the physical stability of liposomes. From the change in time of size and especially of size distribution, the aggregation state of vesicles can be characterized.

To illustrate the differences in the long-term stability of vesicles stored in a refrigerator, the relative increase in the volume average mean size (d/d_0) of polymer-free and polymer-bearing DMPC-liposomes, various time periods after preparation, are shown in Figs. 1 and 2.

These results illustrate well that after longer storage, the vesicle stability decreases and the physical stability of bare DMPC-liposomes is much lower both in distilled water and physiological sodium chloride solution than that of polymer-containing vesicles. After some days storage, the polydispersivity of the polymer-free liposomes significantly increases, but the polymer layers can effectively retard vesicle aggregation or fusion. It can also be seen that among the polymers, the PVA-Bul copolymer is the best stabilizer for the DMPC-vesicles. The other polymers such as PVA, PVP K-90 and the PVA-Prol copolymer (see 3.1.) exerted still noticable stabilizing effect.

The influence of the temperature of storage on the liposome stability is shown in Table 3. The above statements on the stabilizing effect of polymers are supported by these data, as well. In accordance with theoretical considerations, the physical stability of liposomes stored at higher temperature is lowered, and again, the PVP K-90, the hydrolysed PVA and the PVA-Bul copolymer proved to be the most effective stabilizer for the liposomes.

Table 3: Changes in the mean size of liposomes stored for different times at 40 °C

Liposome	Relative increase of vesicle sizes (d/d_0)	
	30 d	60 d
DMPC	83	104
DMPC + PVA	1.7	6
DMPC + PVA-Al	1.8	53
DMPC + PVA-Prod	2	32
DMPC + PVA-Bul	1.4	26
DMPC + PVP K-30	9	105
DMPC + PVP K-90	1.4	2

The order of effectiveness estimated for the polymers as stabilizers closely correlates with the hydrodynamic thickness of the polymer layers formed around vesicles. It seems, therefore, reasonable to assume that the formation of a macromolecular sheath with proper thickness on the vesicle surface is a key factor for enhancing the physical stability of DMPC-liposomes.

2.3. Interaction of the macromolecules with the membrane phospholipid (dimyristoyl-phosphatidylcholine)

Differential scanning calorimetry (DSC) has become a standard method for studying the phase transition of lipid-

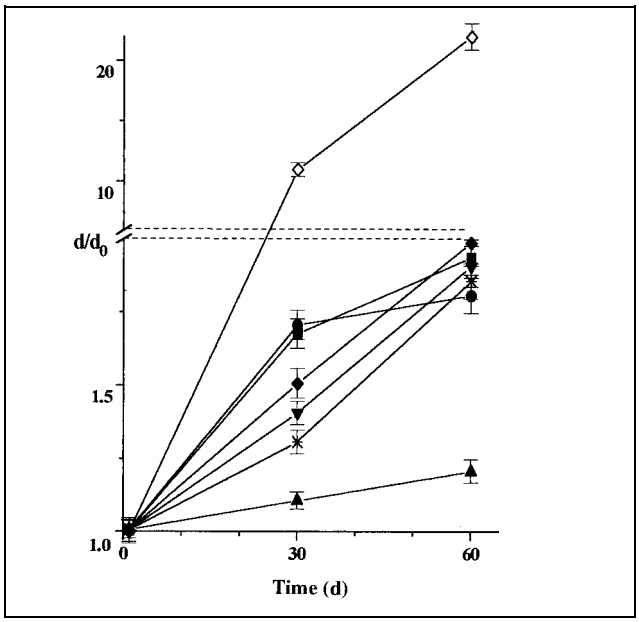

Fig. 1: Change in time of the volume average mean sizes of DMPC-liposomes stored in distilled water:
—◇— DMPC, —✳— DMPC + PVA, —◆— DMPC + PVA-Al,
—▼— DMPC + PVA-Prol, —▲— DMPC + PVA-Bul,
—●— DMPC + PVP K-30, —■— DMPC + PVP K-90

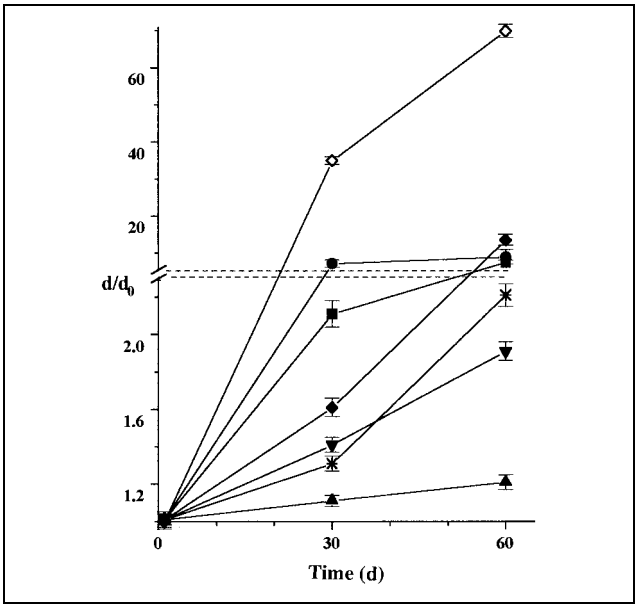

Fig. 2: Change in time of the volume average mean sizes of DMPC-liposomes stored in physiological NaCl solution:
—◇— DMPC, —✳— DMPC + PVA, —◆— DMPC + PVA-Al,
—▼— DMPC + PVA-Prol, —▲— DMPC + PVA-Bul,
—●— DMPC + PVP K-30, —■— DMPC + PVP K-90

layers from an ordered, crystallinelike state at low temperature (gel phase) to a liquid crystalline state at higher temperature. The pretransition temperature (T_p) which reflects a change in the phospholipid headgroup region and the main transition temperature (T_m) at which chain melting occurs, the half width of main transition ($\Delta T_{1/2}$) which is related to the cooperativity of phospholipid molecules and the enthalpy of main transition (ΔH_m) are all important parameters associated with the thermal behavior of lipids. The change of these transition parameters indicates the structural changes caused by the insertion of other molecules (proteins, drugs etc.) into the lipid bilayer [14–16].

The effect of some neutral polymers on the phase transition parameters of DMPC vesicles is compiled in Fig. 3 and Table 4.

A unique effect due to the macromolecules is that the pretransition of lipid disappears in the presence of PVA-Bul copolymer. This change proves that the copolymer molecules interact with the phospholipid headgroups. It correlates well with the result that the PVA-Bul copolymer proved to be the most effective steric stabilizer for the DMPC-vesicles.

The PVP K-30 polymer decreases all the pretransition temperature, the temperature and the enthalpy of main transition of DMPC. These effects show that the arrangement of the hydrocarbon chains of the lipid molecules is influenced presumably due to the surface binding and penetration of the polymer molecules into the membrane. Thus, the interaction of PVP molecules of low molecular weight with lipid molecules results in a disordered membrane structure which is reflected in the slightest stabilizing effect of this polymer, as well. The other polymers do not have significant influence on the phase transition parameters of the lipid membrane.

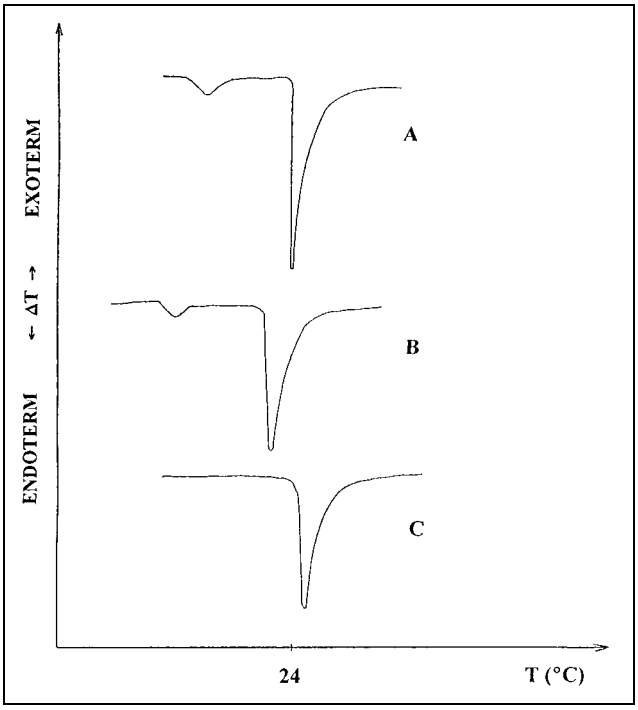

Fig. 3: DSC curves for DMPC-liposomes in the absence (A) and the presence (B, C) of polymers, B. DMPC/PVP K-30 (10 : 1 w/w), C: DMPC/PVA-Bul (10 : 1 w/w)

3. Experimental

3.1. Materials

Dimyristoyl-phosphatidylcholine (DMPC) were purchased from Sigma Chemical Co. St. Louis, MO. Water-soluble uncharged polymers, such as hydrolysed poly(vinyl)alcohol; PVA (products of Powal 420 Kuraray, Japan), poly(vinyl alcohol-co-vinyl acetal); PVA-Al, poly(vinyl alcohol-co-vinyl propional); PVA-Prol, poly(vinyl alcohol-co-vinyl butyral); PVA-Bul copolymers with 5% acetal, propional or butyral content and poly(vinyl)pyrrolidone; PVP K-30 and PVP K-90 (GAF GmbH, Germany), were used. All the polymers were fractionated samples, prepared from commercial products [21]. The degree of polymerization of the PVA, PVA-Al, PVA-Prol, PVA-Bul copolymers is alike 2450, but that of the K-30 and K-90 poly(vinyl)pyrrolidone is 280 and 8120, respectively.

3.2. Methods

3.2.1. Photon correlation spectroscopy

Mean vesicle size size distribution, and polydispersivity of the liposomes and also, the change in time of these variables were measured at 25 °C by an advanced technique of PSC using a Malvern Zetasizer 4 apparatus (Malvern Instruments, UK) with autosizing mode and auto sample time. The zeta potential of the liposomes was determined from electrophoretic mobility measurements with the Zetasizer 4 at 25 °C, using the Henry formula.

3.2.2. Differential scanning calorimetry

The DSC measurements were carried out on a Du Pont Thermoanalyser 990 (Barley Mill, Wilmington, Delaware, USA) at a heating rate of 5 °C/min and in the sensitivity range of 0.1–0.2 milliwatt/cm. The equipment was calibrated using indium.

3.2.3. Preparation of liposomes

For the stability measurements SUV liposomes were prepared by twofold ultrasonication from pure DMPC using the same procedure as described by Grohmann et al. [18]. The liposomes were stored at 5–7 °C in a refrigerator and warmed up to 25 °C before use, or at 40 °C in an air thermostat.

For the DSC measurements, the pure DMPC lipid was dissolved in a chloroform/methanol mixture (9 : 1, v/v), and then the solvent was evaporated under a stream of nitrogen at room temperature. Distilled water or polymer solution was then added to the sample. The samples were vigorously mixed for 30 min above the phase transition temperature of DMPC in a Vortex mixer. The phospholipid content in the final dispersion was 25% DMPC. The ratio of polymer/DMPC in each experiment was 1 : 10 (w/w).

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Table 4: Phase transition parameters of the polymer-free and polymer-containing DMPC membranes

DMPC/polymer 10 : 1 (w/w)	T_m (°C)	T_p (°C)	$\Delta T_{1/2}$ (°C)	ΔH_m (kJ/mol)	n*
DMPC	24.0 ± 0.1	15.3 ± 0.1	1.1 ± 0.1	24.5 ± 1.6	10
DMPC/PVA	23.8 ± 0.1	15.4 ± 0.2	1.2 ± 0.1	24.3 ± 1.7	7
DMPC/PVA-Al	23.7 ± 0.2	15.1 ± 0.1	1.5 ± 0.2	24.8 ± 1.4	8
DMPC/PVA-Prol	24.0 ± 0.1	15.1 ± 0.1	1.0 ± 0.1	23.4 ± 1.5	6
DMPC/PVA-Bul	24.1 ± 0.1	–	1.3 ± 0.1	24.8 ± 1.4	7
DMPC/PVP K-30	22.9 ± 0.2	14.1 ± 0.2	1.5 ± 0.1	23.1 ± 1.4	10
DMPC/PVP K-90	23.1 ± 0.2	14.5 ± 0.2	1.5 ± 0.1	24.7 ± 1.6	10

* n = number of parallel determinations

References

1 Marinkovic, S. S.; Mojovic, L.; Davinic, V.; Bugarski, B.: Drug Dev. Ind. Pharm. **23**, 1 (1997)
2 Qi, X. R.; Maitanai, Y.; Nagai, T.; Wei, S. L.: Int. J. Pharm. **146**, 31 (1997)
3 Zhn, G.; Oto, E.; Vaage, J.; Quinn, J.; Newman, M.; Engbers, C.; Uster, P.: Cancer Chemoth. Pharm. **39**, 138 (1996).
4 Northfelt, D. W.; Martin, F. J.; Worhing, P.; Volberding, P. A.; Russell, J.; Newmann, M.; Amantea, M. A.; Kaplan, L. D.: J. Clin. Pharm **36**, 55 (1996)
5 Lasic, D. D.; Martin, F. J. (Eds.): Stealt Liposomes p. 13. C. R. C., Boca Raton, F. L. 1995
6 Lasic, D. D.; Papahadjopoulos, D.: Science **267**, 1275 (1995)
7 Woodle, M. C.; Lasic, D. D.: Biochim. Biophys. Acta **1113**, 171 (1992)
8 Woodle, M.: Chem. Phys. Lipids **64**, 249 (1993)
9 Lasic, D. D.; Martin, F. J.; Gabiyon, A.; Huang, S. K.; Papahadjopoulos, D.: Biochim. Biophis. Acta **1070**, 187 (1991)
10 Kuhl, T. L.; Lechband, D. E.; Lasic, D. D.; Israelachvili, J. N.: Biophys. J. **66**, 1479 (1994)
11 Lasic, D. D.: Nature **387**, 26 (1997)
12 Allen, T. M.; Moase, E. H.: Adv. Drug Delivery Rev. **21**, 117 (1996)
13 Müller, R. H.: Colloidal Carriers for Controlled Drug Delivery and Targeting. Wissenschaftliche Verlagsgesellschaft, Stuttgart 1991
14 Biltonen, R. L.; Lichtenberg, D.: Chem. Phys. Lipids **64**, 129 (1993)
15 Szögyi, M; Cserháti, T.: J. Pharm. Biomed. Anal. **11**, 563 (1993)
16 Szögyi, M.; Cserháti, T.; Tölgyesi, F.: Lipids **28**, 847 (1993)
17 Grohmann, F. L.; Csempesz, F.; Szögyi, M.: Acta Pharm. Hun. **66** 197 (1996)
18 Grohmann, F. L.; Csempesz, F.; Szögyi, M.: Colloid Polym. Sci. **276**, 66 (1998)
19 Ellens, H.; Rustum, Y. M.; Mayhew, E.; Ledesma, E.: J. Pharm. Exp. Therap. **222**, 324 (1982)
20 Olsen, F.; Mayhew, E.; Maslow, D.; Rustum, Y. M.; Szoka, F.: Eur. J. Cancer Clin. Oncol. **18**, 167 (1982)
21 Csempesz, F.; Csáki, K.; Kovács, P.; Nagy, M.: Colloids Surfaces **101**, 113 (1995)

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Assoc. Prof. Dr. Ferenc Csempesz
Department of Colloid Chemistry
H-1518 Budapest 112
P.O. Box 32
Hungary

Department of Pharmaceutics, Al-Ameen College of Pharmacy, Bangalore, Karnataka, India

Ocular delivery systems of pefloxacin mesylate

S. Bharath, S. R. R. Hiremath

Ocular films of pefloxacin mesylate were prepared with the objectives of reducing the frequency of administration, to improve patient compliance, obtaining controlled release and greater therapeutic efficacy in the treatment of eye infections such as conjunctivitis, keratitis, kerato conjunctivitis, corneal ulcers etc. Polymers such as HPC, HPMC, PVP and PVA were used in different ratios to prepare the ocular films. They were evaluated for drug content which varied from 96–104%. Those which consisted of flexible and transparent films were subjected to *in vitro* release studies. The formulations which prolonged the release for eight hours were selected. The average weight and thickness of these were found to be 38.92–49.71 mg and 31.68–46.08 μm, respectively. The intactness of the formulations was confirmed by IR and TLC studies. *In vivo* studies carried out in the eyes of rabbits showed controlled release upto 8–9 h. There was a good correlation between the *in vitro* and *in vivo* data (r = 0.97–0.995). A minimum of 1 Mrad was found to be necessary for the sterilization of ocular films by gamma radiation. They were found to be stable at temperatures below 45 °C.

1. Introduction

Pefloxacin mesylate is a broad spectrum antibacterial agent useful in the treatment of eye infections [1] such as conjunctivitis, keratitis, kerato conjunctivitis, corneal ulcers etc. It is presently available as 0.3% eye drops. 1–2 drops every 15–30 min to 2–6 times daily have to be instilled for acute and moderately severe infections. Due to the increased frequency of administration, there is patient non-compliance and there is loss of drug from eye tear flow and nasal drainage in case of eye drops. Controlled release drug delivery systems such as ocular films of the drug were prepared with the objective of delivering the drug in a controlled manner for a specific period of time to obtain greater therapeutic efficacy by an increased contact time and to improve patient compliance by decreasing the frequency of administration.

Thus, in the present work, the ocular delivery systems of pefloxacin mesylate were prepared using HPC [2], HPMC [3], PVP [4], MC [5] and PVA [6] as the polymers and PEG-400, glycerine as plasticizers in different ratios and combinations. The formulations were evaluated for drug content, film characteristics, *in vitro* release, average weight and thickness, *in vivo* release characteristics and ocular irritancy. *In vitro/in vivo* correlation was investigated and sterility testing of the polymer films after gamma radiation sterilization was carried out followed by stability studies.

2. Investigations, results and discussion

The formula used for the preparations consisting of polymer and plasticizers and the formulation codes are shown in Table 1. Table 2 shows the formulations using mixture of polymers. Glycerine was used instead of PEG-400 in formulations F, G and H.

Among the 28 formulations prepared the following 18 were selected as they yielded good, transparent and flexible films. A-1 to A-4, B-1 to B-3, E-1 to E-5, F-1 to F-5 and H-3. The others yielded rigid and brittle films and hence were rejected. The drug content was determined