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International Journal of Pharmaceutics 320 (2006) 124–130
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

A calorimetric study of dimyristoylphosphatidylcholine phase transitions and steroid–liposome interactions for liposomes prepared by thin film and proliposome methods

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Received 4 January 2006; received in revised form 27 March 2006; accepted 26 April 2006
Available online 4 May 2006

Abstract

Using high sensitivity differential scanning calorimetry (HSDSC), the phase transitions of dimyristoylphosphatidylcholine (DMPC) liposomal bilayers and their interaction with the model steroid beclometasone dipropionate (BDP) were found to be dependent on the method of liposome manufacture. Ethanol-based proliposomes produced liposomes having no phospholipid pretransition, a main transition of high enthalpy and a low onset temperature, and a very low incorporation of the steroid (maximum 1 mol%). This was attributed to an alcohol-induced interdigitation of the bilayers, which was not apparently reversed by flushing the liposome dispersion with nitrogen in an attempt to remove ethanol. For liposomes manufactured by thin film or particulate-based proliposome methods, 1–2.5 mol% steroid was optimal for incorporation within bilayers, although the nature of the steroid interaction with the bilayers differed between the two methods. For liposomes manufactured by the thin film method, a higher steroid concentration resulted in a broadened main transition and a reduced melting cooperativity. This suggests that BDP formed separate domains within the bilayers which caused non-ideal mixing and phase separation at 5 mol% steroid. This observation was absent for liposomes generated from particulate-based proliposomes, indicating separate steroid domains were not formed and subsequent non-ideal mixing and phase separation did not occur. In addition, liposomes generated from particulate-based proliposomes showed reduced pretransition and main transition enthalpies. These differences were attributed to the employment of sucrose to manufacture the particulate-based proliposomes. This study has shown that the thermal behaviour of liposomes and their interaction with beclometasone dipropionate were dependent on the method of liposome manufacture. Moreover, particulate-based proliposomes may provide a reasonable alternative to the conventional thin film method in producing liposomes incorporating this steroid.

Keywords: Beclometasone dipropionate; Calorimetry; Dimyristoylphosphatidylcholine; Interdigitation; Liposome; Proliposome

1. Introduction

Calorimetry has been extensively employed to study the interaction of materials with liposomal bilayers (Jain et al., 1975; Fildes and Oliver, 1978; Mabrey et al., 1978; Lo and Rahman, 1995; Taylor and Morris, 1995; Budai et al., 2003). This involves measuring the changes such materials may produce in the phase transitions of bilayers when they are included within liposome formulations. It has generally been found that the transformation of phospholipid molecules from the subgel phase to the gel phase (pretransition endotherm) is more sensitive than the transformation from the gel phase to the liquid crystalline phase (main transition endotherm) when additives are included (Fildes and Oliver, 1978; Arrowsmith et al., 1983; Parmar, 1997; Castile et al., 1999; Korkmaz and Severcan, 2005). Maximal incorporation of a material in the liposomal bilayers may be determined as the concentration above which phase separation occurs (Fildes and Oliver, 1978) or at which no further changes in the phase transitions of liposomes are produced (Parmar, 1997). Steroids may be incorporated in the liposomal bilayers in a manner dependent on steroid molecular structure and liposome phospholipid composition. For instance,

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doi:10.1016/j.ijpharm.2006.04.015

using differential scanning calorimetry (DSC), the maximum incorporation in dipalmitoylphosphatidylcholine (DPPC) multilamellar liposomes (MLVs) was found to be 13.2 mol% (Fildes and Oliver, 1978) and 11.25 mol% (Arrowsmith et al., 1983) for hydrocortisone-21-palmitate and cortisone hexadecanoate respectively. By contrast, DSC has shown that the incorporation of other steroids such as beclometasone dipropionate (BDP) in MLVs was very low, and even minimal with some phospholipid bilayers (Parmar, 1997). Using high performance liquid chromatography (HPLC), the maximum incorporation of BDP in DPPC MLVs was found to be 2.52 mol% (Batavia et al., 2001) or 2.24 mol% (Darwis and Kellaway, 2001). Compared with HPLC, calorimetry may offer considerable advantages in investigating the incorporation of steroids within bilayer structures, since the separation of the unentrapped drug from the liposome-entrapped fraction is not required. Liposomes are commonly manufactured by the thin film hydration method (Bangham et al., 1965). However, this method is considered unsuitable for liposome production on a large scale. Proliposome technologies may be suitable for producing liposomes on a large scale (Chen and Alli, 1987; Turánek et al., 1997), and hence represent reasonable and convenient alternatives to the thin film method. Proliposome formulations are of two types. Firstly, particulate-based proliposomes comprise aqueous soluble carrier particles (e.g. carbohydrates) coated with phospholipid (Payne et al., 1986). Secondly, ethanol-based proliposomes are concentrated ethanolic solutions of the phospholipid (Perrett et al., 1991). The addition of aqueous phase to proliposomes above the main phase transition temperature (T_m) of the phospholipid and shaking results in the formation of liposomes. In this study, high sensitivity differential scanning calorimetry (HSDSC) was employed to investigate the phase transitions of liposomes generated from both types of proliposome formulations. The thermal behaviour of liposomes generated from proliposomes was compared with liposomes produced by the conventional thin film method. The interaction of the model steroid beclometasone dipropionate (BDP) with the liposomal bilayers was also investigated.

2. Materials and methods

2.1. Materials

Sucrose, chloroform, and absolute ethanol were AnalaR grade and purchased from BDH, UK. Ammonium thiocyanate and ferric chloride used to perform the Stewart assay (Stewart, 1980) were also AnalaR grade (BDH, UK). Dimyristoylphosphatidylcholine (DMPC, approx. 99%) was purchased from Sigma–Aldrich, UK. Beclometasone dipropionate (BDP) was supplied by GlaxoSmithKline, UK.

2.2. Manufacture of liposomes using the thin film method

DMPC (250 mg) was accurately weighed in a round-bottomed flask, and chloroform was added to give a 60 mg/ml phospholipid solution. The flask was attached to a rotary evaporator (Büchi Rotavapor R-114, Büchi, Switzerland) and a vacuum was applied using a vacuum pump (Büchi Vac V-501 Büchi, Switzerland). The rotation speed was set at maximum and the water bath was set at 35 °C. After 1 h, the vacuum pump was turned off, negative pressure released, and the flask detached. The thin film formed was flushed for few min with nitrogen to fully remove solvent residues. Deionised water (40 °C) was added to give a phospholipid concentration of 62.5 mg/ml, and hydration took place by vigorous hand shaking for 10 min with intermittent immersion of the flask in the water bath (35 °C). This was followed by 15 min flask immersion in the water bath and finally 10 min hand shaking to assure liposome formation and deaggregation. The preparation was left in the water bath (35 °C) for 2 h to anneal before being stored overnight at 6 ± 2 °C. This procedure was repeated with inclusion of 1, 2.5, or 5 mol% BDP in the phospholipid phase. Thin films without BDP were also manufactured using absolute ethanol as a phospholipid solvent rather than chloroform. The thin film formed was hydrated as described previously.

2.3. Manufacture of liposomes using the particulate-based proliposome method

Sucrose was ground in a ball mill (Pascall Engineering Co. Ltd., UK) rotated on a roll mixer (1600-VS-A roll mixer, Pascall Engineering Co. Ltd., UK) at medium speed for 10 min. The resultant powder was sieved and 300–500 μm sucrose particles were collected to manufacture proliposomes having a 1:5 w/w phospholipid to carrier ratio. Sucrose carrier particles (1.25 g) were placed in a 100 ml pear-shaped flask and attached to a modified rotary evaporator (Büchi Rotavapor R-114, Büchi, Switzerland) with a feed-line tube (Büchi, Switzerland), and immersed in the water bath (35 °C). The vacuum was applied and rotation speed was set at maximum. A chloroformic solution of DMPC (60 mg/ml) was injected in portions (0.5–1 ml each) via the feed-line. After each addition, evaporation of chloroform was achieved before injecting the next portion. After complete addition of the chloroformic solution, solvent evaporation took place for at least 30 min before releasing the vacuum, detaching the flask, and collecting the proliposomes. Proliposomes were stored in a glass vial at −18 °C. This procedure was repeated with inclusion of 1, 2.5, or 5 mol% BDP in the lipid phase. Hydration of the proliposomes and annealing and storage of the generated liposomes were all carried out as described for the thin film method.

2.4. Quantification of DMPC in liposome dispersions produced from particulate-based proliposomes

Coating sucrose particles with DMPC via the feed-line may result in some loss of phospholipid in the feed-line or the flask. For accurate quantification of phospholipid for HSDSC, the DMPC concentration in the aqueous phase was determined. A 100 μl sample was taken from liposomes generated from particulate-based proliposomes and made up to 10 ml with deionised water. A sample of the diluted liposome dispersion was added to absolute ethanol (1 ml) in a 15 ml glass tube to convert liposomes into an ethanolic solution of phospholipid. This was placed overnight in an oven (90 °C) to evaporate the

Table 1
Phase transitions and size distribution of DMPC liposomes prepared using thin film and proliposome methods, with a range of steroid concentrations ($n = 3 \pm$ S.D.)

BDP concentration (mol%)	Pretransition		Main transition		Size distribution	
	T_{pre} (°C)	ΔH_{pre} (kJ/mol)	T_m (°C)	ΔH_m (kJ/mol)	VMD (μm)	Span
Liposomes prepared using thin film method						
0	15.17 ± 0.02	3.30 ± 0.26	24.84 ± 0.05	21.04 ± 0.70	7.48 ± 0.18	1.90 ± 0.06
1	9.85 ± 0.45	0.83 ± 0.10	24.06 ± 0.52	21.93 ± 2.02	7.40 ± 0.09	2.00 ± 0.11
2.5	Not detected	Not detected	24.77 ± 2.83	23.09 ± 1.64	7.21 ± 0.13	2.07 ± 0.15
5	Not detected	Not detected	22.70 ± 0.40	25.00 ± 0.27	7.75 ± 0.89	1.84 ± 0.03
Liposomes prepared using particulate-based proliposome method						
0	16.73 ± 0.15	1.91 ± 0.01	25.35 ± 0.07	19.66 ± 0.93	5.21 ± 0.02	0.99 ± 0.06
1	14.38 ± 0.45	0.66 ± 0.11	24.63 ± 0.12	17.50 ± 0.37	4.93 ± 0.13	1.07 ± 0.10
2.5	14.10 ± 0.50	0.76 ± 0.23	24.33 ± 0.25	16.49 ± 0.39	5.23 ± 0.10	1.09 ± 0.01
5	14.70 ± 0.60	1.18 ± 0.28	24.98 ± 0.12	16.11 ± 0.48	5.13 ± 0.10	1.17 ± 0.04
Liposomes prepared using ethanol-based proliposome method						
0	Not detected	Not detected	23.08 ± 0.06	25.03 ± 1.05	3.88 ± 0.13	1.26 ± 0.02
1	Not detected	Not detected	22.99 ± 0.02	23.43 ± 0.36	5.33 ± 0.11	1.26 ± 0.11
2.5	Not detected	Not detected	22.37 ± 0.65	24.09 ± 1.16	5.76 ± 0.40	1.54 ± 0.02
5	Not detected	Not detected	23.03 ± 0.31	24.12 ± 0.64	5.09 ± 0.40	1.56 ± 0.05

solvent and obtain a film of phospholipid depositing on the wall of the glass tube. Phospholipid was then quantified colorimetrically using the Stewart assay (Stewart, 1980).

2.5. Manufacture of liposomes using the ethanol-based proliposome method

DMPC (165 mg) was dissolved in absolute ethanol in a 7 ml glass vial to obtain a solution having 5:4 w/w phospholipid to ethanol ratio. Deionised water (40 °C) was added and vigorous hand-shaking took place for 10 min to generate liposomes comprising a phospholipid concentration of 62.5 mg/ml. The preparation was left in the water bath (35 °C) for 2 h before storage overnight at 6 ± 2 °C. This procedure was repeated with inclusion of 1, 2.5, or 5 mol% BDP in the phospholipid phase. In order to investigate the effect of ethanol on vesicle size distribution and thermotropic behaviour of the bilayers, a liposome preparation without BDP was flushed with nitrogen for 2 min in an attempt to remove ethanol.

2.6. Laser diffraction size analysis of liposomes

The size distribution of liposomes was determined using laser diffraction (Malvern Mastersizer S, Malvern Instruments Ltd., UK). The volume median diameter (VMD), and Span were recorded. Span = (90% undersize − 10% undersize)/VMD.

2.7. High sensitivity differential scanning calorimetry (HSDSC)

HSDSC was conducted using a Micro DSCIII (Setaram, France) and Hastelloy-made vessels (1 cm^3) (Setaram, France). Liposome dispersions (approx. 0.8 ml) containing 50 mg DMPC were loaded in the sample vessel. The reference vessel was filled with the same components of the sample excluding the lipid phase (i.e. DMPC and BDP). Nitrogen was supplied in order to prevent vapour condensation on the vessels during cooling, and scanning was performed between 6 and 45 °C at a rate of 1 °C/min. The thermal behaviour of liposomes was evaluated by determining the linear onset temperature of the pretransition (T_{pre}) and main transition (T_m), and by calculating the enthalpy of the pretransition (ΔH_{pre}) and main transition (ΔH_m) as kJ/mol of DMPC molecules in the sample.

3. Results and discussion

3.1. Influence of preparation method on the phase transitions of liposomes

The phase transitions and size distribution of DMPC liposomes were dependent on the formulation approach employed (Table 1). For preparations without BDP, liposomes produced by the thin film method showed a larger pretransition enthalpy (ΔH_{pre}) (Table 1, Fig. 1) and a sharper main transition (Fig. 1) compared to liposomes formed by the proliposome methods.

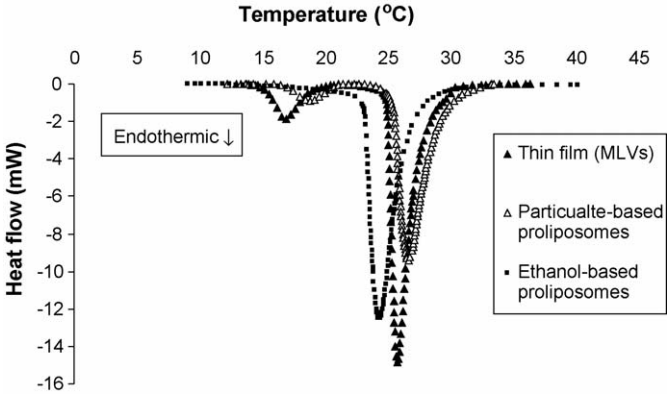

Fig. 1. Phase transitions of DMPC liposomes generated by the thin film method and proliposome methods.

Table 2
Phase transitions and size distribution of DMPC liposomes prepared by ethanol-based proliposome and thin film (ethanolic) methods ($n = 3 \pm$ S.D.)

Liposome preparation	Pretransition		Main transition		Size distribution	
	T_{pre} (°C)	ΔH_{pre} (kJ/mol)	T_m (°C)	ΔH_m (kJ/mol)	VMD (μm)	Span
Proliposomes (unflushed)	Not detected	Not detected	23.08 ± 0.06	25.03 ± 1.05	3.88 ± 0.13	1.26 ± 0.02
Proliposomes (flushed)	Not detected	Not detected	24.75 ± 0.03	26.63 ± 0.47	6.87 ± 0.66	1.42 ± 0.00
Thin film (ethanolic)	15.19 ± 0.12	3.27 ± 0.26	24.94 ± 0.02	20.78 ± 0.87	9.10 ± 0.13	1.68 ± 0.03

These differences may be attributed to the inclusion of sucrose or ethanol in the proliposome formulations, or to differences in bilayer packing resulting from different methods of liposome manufacture being employed. In contrast to liposomes produced by the thin film or particulate-based proliposome methods, vesicles generated from ethanol-based proliposomes produced no pretransition endotherm (Table 1, Fig. 1). Phospholipid pretransition may be depressed by the presence of alcohols and completely removed when alcohol concentration exceeds a threshold value (Pringle and Miller, 1979; Veiro et al., 1987). This has been attributed to an alcohol-induced interdigitation which is an unusual gel phase in which the phospholipid alkyl chains form opposing fully interdigitated monolayers (Simon and McIntosh, 1984; Veiro et al., 1987). Compared to the thin film and particulate-based proliposome methods, liposomes generated from ethanol-based proliposomes had a significantly ($P < 0.05$) higher ΔH_m and lower T_m, as the main transition was shifted to the lower temperature side (Table 1, Fig. 1). It has been previously shown that alcohols may reduce the temperature of the main transition (Rowe, 1983; Rowe, 1985; Almeida et al., 1986; Veiro et al., 1987), and increase bilayer fluidity (Almeida et al., 1986) and permeability (Zeng et al., 1993; Engelke et al., 1997). However, liposomes generated from particulate-based proliposomes showed significantly ($P < 0.05$) higher T_m, and a trend of ΔH_m decrease compared to vesicles generated by the other two methods. These liposomes contain dissolved sucrose, and it is known that disaccharides such as trehalose or sucrose can interact with the polar headgroup of the phospholipid molecules, depressing the T_m, and maintaining the phospholipid in a fluid state following dehydration (Crowe and Crowe, 1988; Van Winden et al., 1998). In the hydrous state it has been previously reported that sucrose may cause a slight elevation in the T_m and a reduction in the ΔH_m of DPPC liposomes (Chowdhry et al., 1984; Crowe and Crowe, 1991), with a second peak at a lower temperature appearing at very high concentrations (Crowe and Crowe, 1991). This suggests that sucrose present in the particulate-based proliposomes was responsible for the observed changes in the thermal profile of the liposomes formed from this system. In addition, sucrose may be responsible for the reduction of liposome size and size distribution (Table 1). It has been reported previously that sucrose may reduce the polydispersity of DMPC liposomes (Kiselev et al., 2003). Liposomes generated from ethanol-based proliposomes have been shown to be predominantly oligolamellar (Perrett et al., 1991). Consequently, the reduced size of liposomes generated by this method (Table 1) might be attributed to the presence of fewer bilayers compared to vesicles formed by thin film or particulate-based proliposome methods.

3.2. Effect of ethanol on the phase transitions of liposomes

Flushing the liposome dispersion with nitrogen in an attempt to remove residual ethanol did not markedly change the phase behaviour of liposomes generated from ethanol-based proliposomes (Table 2, Fig. 2). It seems that a small amount of residual ethanol remained after flushing and that this was sufficient to induce interdigitation and eliminate the pretransition. The high repulsion in the interfacial region between phospholipid molecules and aqueous phase may force the lipid hydrophobic chain to tilt and this may be responsible for the occurrence of the pretransition (Nagel et al., 1992). It is hence possible that the amphiphilic property of ethanol has prevented the pretransition by reducing the interfacial tension between the lipid and aqueous phases. Löbbecke and Cevc (1995) have reported that short chain alcohols such as ethanol may induce lateral expansion in the phospholipid headgroup interfacial region, which may lower the pretransition temperature and finally cause complete removal of the pretransition endotherm. In this study, flushing with nitrogen increased the T_m to a level similar to that for liposomes prepared by the thin film method (Table 1), suggesting some ethanol was removed. HSDSC of liposomes formed by the thin film method using ethanol as the phospholipid solvent (Table 2, Fig. 2) showed that the pretransition was not removed, suggesting that residual ethanol, if any, was not sufficient to induce bilayer interdigitation and subsequent pretransition removal. It has been found that a certain alcohol concentration in liposome formulations is needed to start depressing the pretransition, whilst below that critical level the pretransition was independent of alcohol concentration (Veiro et al., 1987). The ΔH_m was significantly different ($P < 0.05$) between preparations, with markedly smaller values for liposomes formed by the ethanolic thin film method

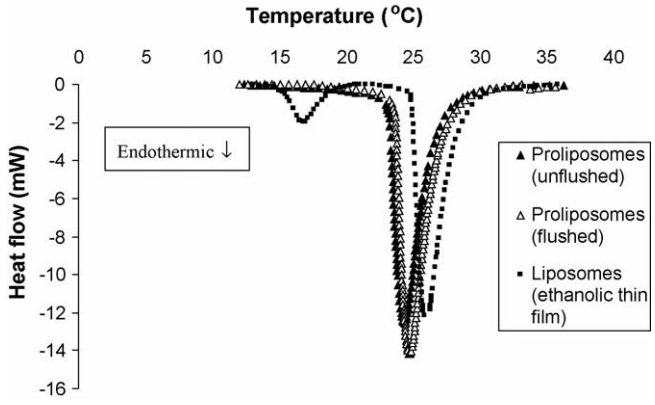

Fig. 2. Effect of ethanol on the phase transitions of DMPC liposomes.

(Table 2). This indicates that ethanol concentration within the preparations was responsible for the high ΔH_m values. However, when the overall enthalpy (i.e. ΔH_{pre} and ΔH_m) was considered, the difference between the three preparations was small. This suggests that the concentration of ethanol present in either ethanol-based proliposome formulation has delayed the uptake of energy by phospholipid molecules present in the interdigitated phase, without changing the overall energy required to reach the liquid crystalline phase from the gel state. Liposome median size (VMD) and size distribution (span) were dependent on the preparation method (Table 2). The large size produced by the thin film (ethanolic) method might be attributed to the presence of large liposomes or liposome aggregates. Flushing with nitrogen resulted in larger liposomes compared to those in the unflushed formulation (Table 2), suggesting that ethanol was responsible for liposome size reduction.

3.3. Steroid interaction with liposomes prepared by the thin film method

Inclusion of increasing amounts of BDP into MLVs resulted in a more marked effect on the pretransition compared to the main transition (Table 1, Fig. 3). Compared to the formulation without steroid, that containing 1% BDP had a significant ($P<0.05$) reduction in T_{pre} and ΔH_{pre} (Table 1, Fig. 3). Higher steroid concentrations resulted in complete removal of the pretransition endotherm (Table 1, Fig. 3). This indicates that the steroid concentration required to remove the pretransition was between 1% and 2.5% in liposomes manufactured by the thin film method. Similar effects have been observed for a number of different steroids for DPPC MLVs (Fildes and Oliver, 1978; Arrowsmith et al., 1983; Parmar, 1997; Korkmaz and Severcan, 2005). For instance, 3.8% of hydrocortisone-21-palmitate (Fildes and Oliver, 1978), 2.5% of cortisone hexadecanoate (Arrowsmith et al., 1983), 1–2.5% of BDP (Parmar, 1997), and 3% progesterone (Korkmaz and Severcan, 2005) were demonstrated to be sufficient concentrations to remove the pretransition endotherm. Thus, very low concentrations of steroid are sufficient to remove the pretransition of liposome bilayers. Although the steroid exerted a smaller effect on the main transition parameters (i.e. T_m and ΔH_m), the main transition peak broadened as BDP concentration was increased within formulations (Fig. 3). At 5% BDP, a significant ($P<0.05$) increase in ΔH_m and a decreasing tendency of T_m were observed (Table 1) which was accompanied by a broadening of the main transition and the occurrence of a slight shoulder or flattening on the lower temperature side (Fig. 3). These findings suggest that after the pretransition was removed (i.e. at 1–2.5% BDP), the steroid started to form separate domains in the bilayers. This resulted in a reduced "melting" cooperativity of the lipid chain at 2.5% BDP, and phase separation at 5% BDP as non-ideal mixing between phospholipid and steroid in the bilayers started to occur. The incorporation of BDP as separate domains within liposomal bilayers has been previously described by Parmar (1997) after removal of the pretransition for DPPC MLVs. Thus, it is suggested that 1–2.5% BDP may be optimal for inclusion in this liposome formulation. Laser diffraction size analysis showed that BDP concentration had only a small effect on the measured VMD and Span of liposomes produced by the thin film method (Table 1).

3.4. Steroid interaction with liposomes generated from particulate-based proliposomes

Compared to the formulation without steroid, that containing 1% BDP showed a significant ($P<0.05$) decrease in the ΔH_{pre} and T_{pre} (Table 1, Fig. 4). At 2.5% BDP, no changes in the pretransition were observed compared to the formulation containing 1% steroid. However, the 5% BDP formulation showed a small but significant ($P<0.05$) increase in the ΔH_{pre} (Table 1, Fig. 4). For the main transition, the ΔH_m significantly differed ($P<0.05$) between formulations, with a trend of continuous decrease with progressive increase of BDP concentration (Table 1). Each increase in steroid concentration resulted in a significant ($P<0.05$) lowering of the ΔH_m except for 5%, for which the ΔH_m was not significantly reduced compared with formulation containing 2.5% BDP (Table 4). This suggests that the interaction of BDP with the bilayers takes place in two different modes; one was responsible for the interference with the pretransition and the other for the interference with the main

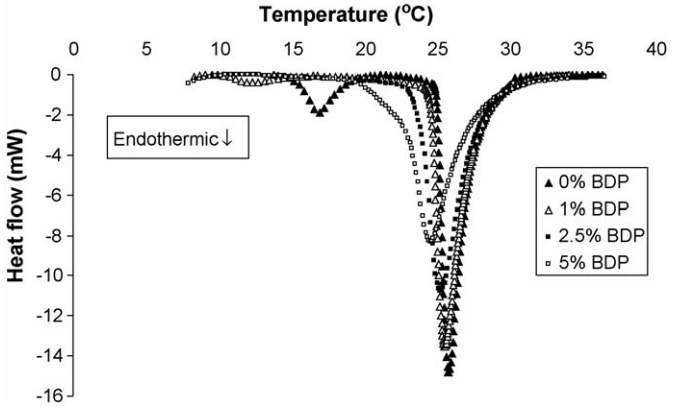

Fig. 3. Phase transitions of DMPC liposomes prepared by the thin film method using a range of steroid concentrations.

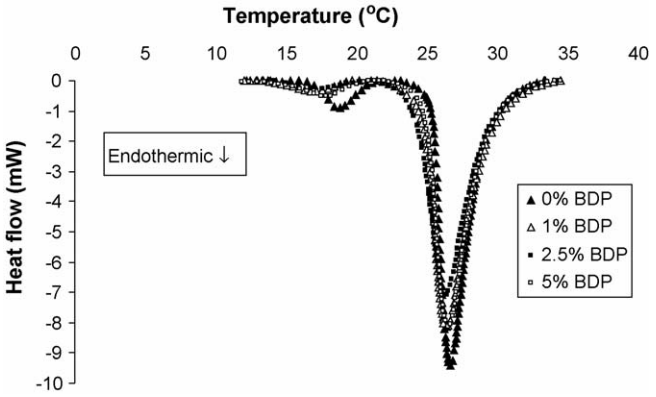

Fig. 4. Phase transitions of liposomes prepared by the particulate-based proliposome method using a range of steroid concentrations.

transition. The maximum interaction affecting the pretransition was at 1 mol% BDP, whilst that affecting the main transition was at 2.5%. Above 2.5% BDP, no significant changes ($P > 0.05$) were observed in the main transition whilst the pretransition enthalpy increased, suggesting that the steroid partitioned out of the bilayers at this concentration. This agrees with the study conducted by Batavia et al. (2001), demonstrating the tendency of BDP to form crystals in the aqueous phase after achieving the maximum incorporation in the liposomal bilayers. Unlike vesicles manufactured by the thin film method, liposomes generated from particulate-based proliposomes showed no broadening in the main transition at 5 mol% BDP. This suggests that the employment of sucrose as the particulate carrier prevented the formation of separate domains of BDP within the bilayers, non-ideal mixing, and subsequent phase separation. It is unclear how this is mediated, but may be due to the viscosity effect of the dissolved sugar. Thus, according to the present findings, optimal incorporation in liposomes generated from particulate-based proliposomes is between 1% and 2.5%. This is similar to the incorporation of this steroid in the liposomes made by the thin film method, although the mode of incorporation differed. Laser diffraction showed a minimal effect of BDP concentration on the measured VMD and span of liposomes generated from particulate-based proliposomes (Table 1).

3.5. Steroid interaction with liposomes generated from ethanol-based proliposomes

All ethanol-based proliposome formulations generated liposomes with no detectable pretransition (Table 1, Fig. 5). This indicates that BDP inclusion produced no apparent effect on the ethanol-induced interdigitation at the steroid concentrations investigated. Moreover, the increase of BDP concentration produced no significant change ($P > 0.05$) in the T_m or ΔH_m (Table 1), indicating no interaction was detected between this steroid and the liposomal bilayers. However, BDP inclusion at 1 mol% showed a trend for a decrease in the ΔH_m, which was accompanied by a significant ($P < 0.05$) increase in the measured VMD of liposomes. This suggests that some interaction of BDP with the bilayers had occurred. At BDP concentrations above 1%, the VMD did not change whilst the span increased significantly ($P < 0.05$) (Table 1), indicating some aggregation of the liposomes. Overall, results suggest that liposomes generated using this method may not incorporate more than 1% BDP. This might be attributed to the thinness of the interdigitated bilayers as a result of ethanol inclusion. Alternatively, ethanol may have enhanced the solubility of BDP in the aqueous phase by acting as a cosolvent and decreased the partitioning of the steroid into the phospholipid bilayers.

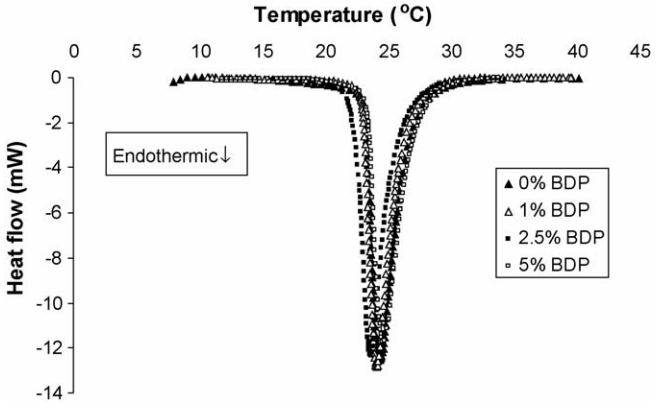

Fig. 5. Phase transitions of liposomes prepared by the ethanol-based proliposome method using a range of steroid concentrations.

4. Conclusions

This HSDSC study has shown that the phase transitions of liposomal phospholipids and bilayer interaction with the model steroid beclometasone dipropionate were dependent on the formulation approach adopted. This was attributed to the presence of sucrose for liposomes generated from particulate-based proliposomes and ethanol for vesicles generated from ethanol-based proliposomes. The presence of such excipients (sucrose or ethanol) within liposome formulations had a greater effect on the pretransition than the main transition of the phospholipid employed. HSDSC of liposomes manufactured by the thin film or particulate-based proliposome methods showed that optimal incorporation in the bilayers was achieved at a steroid concentration between 1 and 2.5 mol%. By contrast, for liposomes generated from ethanol-based proliposomes, the maximum incorporation of this steroid in the bilayers was found not to exceed 1 mol%, which was attributed to an ethanol-induced interdigitated phase of the bilayers. Whilst further work is required to enhance the incorporation of this steroid in liposomes generated from ethanol-based proliposomes, the other two methods were in agreement with literature findings for the incorporation of this steroid in phospholipid structures. This suggests that particulate-based proliposomes may possess the potential for manufacturing liposomes on a large scale without compromising the incorporation of the steroid employed in this study. Moreover, the presence of sucrose in liposomes generated from particulate-based proliposomes may prevent the formation of separate steroid domains within the bilayers and subsequent phase separation.

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