ELSEVIER

International Journal of Pharmaceutics 221 (2001) 197–209

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

The influence of incubation temperature and surfactant concentration on the interaction between dimyristoylphosphatidylcholine liposomes and poloxamer surfactants

Jonathan D. Castile, Kevin M.G. Taylor *, Graham Buckton

Centre for Materials Science, Department of Pharmaceutics, School of Pharmacy, University of London, 29-39 Brunswick Square, London, WC1N 1AX, UK

Received 23 August 2000; received in revised form 3 April 2001; accepted 3 April 2001

Professor Michael Newton, has recently retired as Head of the Department of Pharmaceutics, School of Pharmacy, University of London. The work described in this paper was carried out in that Department and all three authors are grateful to Professor Newton for the help and guidance he gave them at various stages of their scientific careers.

Abstract

Differential scanning calorimetry and photon correlation spectroscopy have been used to study the interaction between poloxamers P338 and P407 and dimyristoylphosphatidylcholine (DMPC) liposomes. The extent of the interaction was found to be dependent on the incubation temperature in addition to the poloxamer concentration. At low poloxamer concentrations (0.1–1.0% w/v) an interaction with the phospholipid bilayer was detected by a reduction of the pre-transition enthalpy of DMPC. At higher concentrations (2.0–5.0% w/v), the main phase transition temperature of the liposomes decreased and the endotherm broadened with a shoulder on the high temperature side, indicative of phase separation. Maximum increases in the diameter of small freeze–thaw extruded liposomes were shown to occur at temperatures close to the poloxamer critical micelle temperatures. At higher temperatures and surfactant concentrations there was evidence of solubilization of phospholipid into mixed micelles.

Keywords: Differential scanning calorimetry; Liposome; Micelle; Phase transition temperature; Phospholipid; Poloxamer; Surfactant

1. Introduction

Poloxamers are non-ionic surface-active block copolymers having two hydrophilic polyoxyethylene (POE) moieties and a hydrophobic polyoxypropylene (POP) moiety. They have been

* Corresponding author. Tel.: +44-020-77535853; fax: +44-020-77535920.

E-mail address: kevin.taylor@ulsop.ac.uk (K.M.G. Taylor).

0378-5173/01/$ - see front matter
PII: S0378-5173(01)00685-8

included in liposomal drug delivery systems, to sterically stabilise liposomes, prolonging the duration of their circulation in the bloodstream (Woodle et al., 1992). The interaction between poloxamers and liposomes is poorly understood, but may result in sterically stabilised systems which are less stable than those employing PEG-lipids (Woodle et al., 1992), which have been used successfully in commercially available formulations. In the presence of poloxamers, the bilayer permeability of egg phosphatidylcholine (eggPC) liposomes was shown to increase with loss of encapsulated marker (Jamshaid et al., 1988) though this was not observed for hydrogenated soybean PC/cholesterol liposomes (Woodle et al., 1992) or dipalmitoylphosphatidylcholine (DPPC)/cholesterol liposomes (Khattab et al., 1995). Jamshaid et al. (1988) suggested that poloxamers associate with relatively fluid eggPC bilayers in a way that results in the POE groups projecting from the liposome surface resulting in increased particle size. Such increases were not observed when poloxamers were added to gel state (distearoylphosphatidylcholine; DSPC) liposomes (Moghimi et al., 1991). This suggests that the interaction between poloxamers and liposomes may not be the result of surfactant adsorption onto the liposome surfaces, but rather involves penetration of poloxamer into liquid crystalline state bilayers.

Baekmark et al. (1997) used differential scanning calorimetry (DSC) to study the interaction of DPPC liposomes with POE-containing lipopolymers and POE–polystyrene–POE triblock copolymers. The main phospholipid gel to liquid crystalline phase transition endotherm, measured as the half height width (HHW), broadened as POE-containing lipopolymer concentration was increased. The results were indicative of conventional impurity-induced depression of the temperature of the main phase transition (T_c), and since aqueous solutions of POE molecules do not bind to liposome surfaces (Evans and Needham, 1988), it was concluded that the copolymer incorporated into the lipid bilayer (Baekmark et al., 1997). This has been confirmed with high sensitivity differential scanning calorimetry (HSDSC) investigations of the interaction between poloxamers P338 and P407 and dimyristoylphosphatidylcholine (DMPC) and DPPC liposomes (Castile et al., 1999). The phospholipid pre-transition was shown to be more sensitive to the association between poloxamers and liposomes than the main transition, with the enthalpy of the pre-transition reduced in the presence of poloxamer for liquid–crystalline state, but not gel state liposomes.

2. Materials and methods

2.1. Materials

Poloxamer P338 was obtained from ICI, France and poloxamer P407 from Blagden, UK. Details of the composition, molecular mass and critical micelle concentration (cmc) of these poloxamers are shown in Table 1. DMPC (99%+ pure) was obtained from Sigma Chemicals Ltd, UK and chloroform (HiPerSolv) from BDH Chemicals, UK. Bi-distilled deionised water was produced (Model WP700, Whatman, UK) and further purified by passing through an Elgastat Ultra High Quality Purification System (Elga Ltd., UK).

Table 1
Properties for poloxamers P338 and P407 (sources: Alexandridis et al., 1994; Buckton and Machiste, 1996)

Poloxamer	P338	P407
Composition	$(POE)_{128}(POP)_{54}(POE)_{128}$	$(POE)_{98}(POP)_{67}(POE)_{98}$
Molecular mass (nominal)	14 000	11 500
CMC (%w/v) in water at		
20°C	–	4.0
25°C	4.5	0.7
30°C	0.8	0.1
35°C	0.15	0.0025
40°C	0.04	0.008
45°C	0.008	–

2.2. Critical micelle temperature of poloxamers

The endothermic peak associated with micellization of P338 and P407 was measured using HSDSC. CMTs were determined at poloxamer concentrations of 0.5, 1, 2, 5 and 10% w/v in bi-distilled water. A 500 μl aliquot of poloxamer solution was pipetted into an aluminium crucible with screw-top lid (Setaram, France), weighed and sealed. A crucible containing an equal mass of bi-distilled water was prepared as a reference. Samples were analysed between 5 and 70°C at a heating rate of 1°C/min as previously described (Mitchard et al., 1992; Armstrong et al., 1994; Paterson et al., 1997). Temperature and enthalpy calibration was performed using indium (Perkin Elmer, UK) as the calibrant.

2.3. Preparation of liposomes

Multilamellar vesicles (MLVs) were prepared by weighing appropriate amounts of DMPC into a round bottomed flask and dissolving in chloroform. Chloroform was removed by rotary evaporation under vacuum, in a water bath at 55°C for 15 min. The flask was then flushed with nitrogen to remove traces of residual organic solvent. An appropriate volume of filtered [100 nm pore filter (Poretics, USA)], bi-distilled deionised water was added to the dry film in the flask to give the appropriate phospholipid concentration. Glass beads were added to aid dispersion, the flask was flushed with nitrogen, gently rotated for 30 min in the water bath, and shaken to produce MLVs. The suspension was annealed for a further 2 h in the water bath before storage under nitrogen in a refrigerator at 4°C.

2.3.1. Standardised freeze-thaw procedure

MLVs, with water as the aqueous phase, were extruded ten times through a 2 μm polycarbonate filter (Cyclopore Ltd, UK), held in a 25 mm syringe filter holder, to standardize the size of the liposome population. Each dispersion (4 ml) was frozen in liquid nitrogen at −196°C for 3 min and then thawed for 3 min at 50°C in a water bath. Frozen and thawed MLVs (FATMLVs) were then extruded ten times at 35°C through double stacked 100 nm pore filters, held in a LiposoFast Miniextruder (Avestin, Canada) after initial passage twice through a single 100 nm pore filter, to produce unilamellar vesicles.

2.4. The effect of poloxamer on the thermal profile of DMPC MLVs

Conventional differential scanning calorimetry (DSC; DSC7, Perkin Elmer) and high sensitivity differential scanning calorimetry (HSDSC; Micro DSC III, Setaram, France) were used to study liposome/poloxamer interactions by determining the effect of poloxamers on the phase transition behaviour of DMPC MLVs. Poloxamers P338 and P407 were dissolved in filtered (100 nm pore filter; Poretics) bi-distilled water and added to DMPC liposomes to give a final lipid concentration of 50 mg/ml and poloxamer concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, 3.0 and 5.0% w/v. Liposome dispersions and poloxamer solutions were equilibrated at the incubation temperature for 30 min prior to preparation of the final dispersion. In addition, liposomes were prepared in the absence of poloxamer. All samples were incubated for 24 h at 4, 18, 25 or 37°C for 24 h before DSC analysis. Each suspension (20 or 100 μl for DSC and HSDSC analysis respectively) was pipetted into the sample container: hermetically sealed pans (TA instruments, UK) for conventional DSC, aluminium crucible with screw-top lid (Setaram) for HSDSC, weighed and sealed. A reference pan (DSC) or crucible (HSDSC) containing an equal volume of bi-distilled water was also prepared and placed in the DSC apparatus and heated at a scan rate of 5°C/min (DSC) or 1°C/min (HSDSC) from 3 to 45°C. Temperature and enthalpy calibration for DSC was performed using indium (Perkin Elmer) as the calibrant.

2.5. Effect of incubation temperature on the mean diameter of small DMPC liposomes

Extruded DMPC liposomes and P338 and P407 solutions were equilibrated at a specific in-

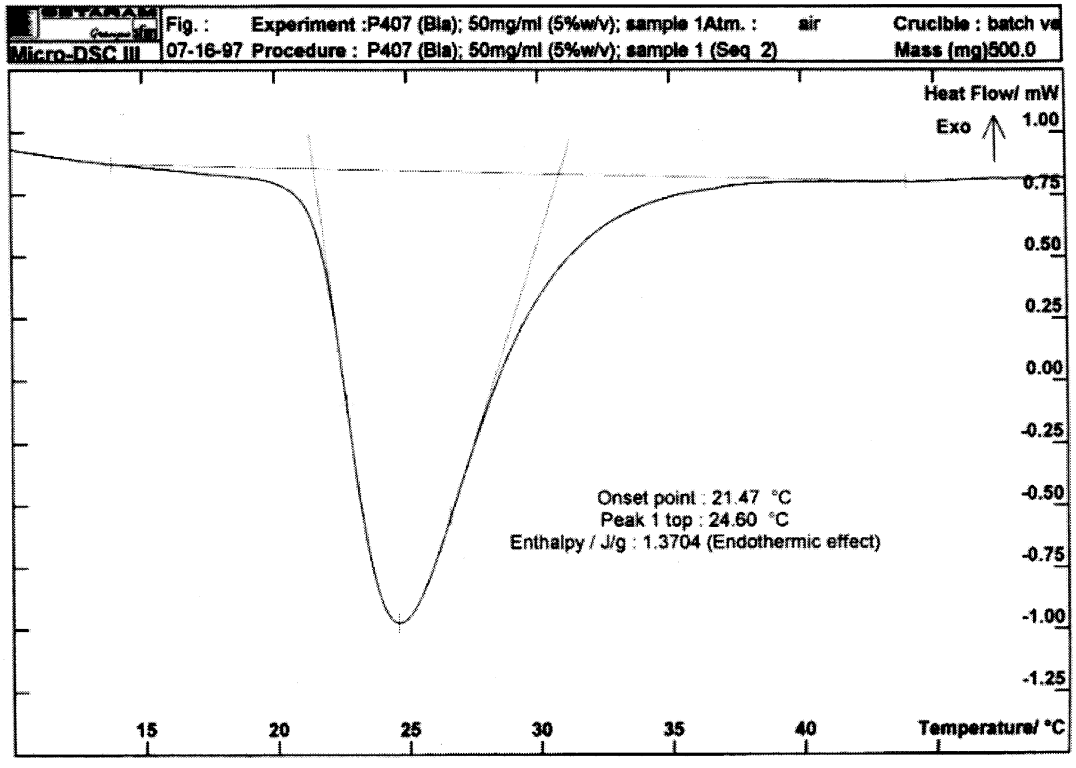

Fig. 1. Typical HSDSC scan for a 5% w/v aqueous solution of poloxamer P407.

cubation temperature for 30 min before being mixed to give final poloxamer concentrations of 0.05, 0.1, 0.2, 0.3, 0.5 and 1% w/v and a final lipid concentration of 2 mg/ml. Resultant dispersions were incubated in a shaking water bath at 18, 25 or 37°C for 24 h before size analysis by photon correlation spectroscopy (PCS) using a Malvern Autosizer 2C (Malvern Instruments, UK) with a thermostatically controlled sample chamber set at the respective incubation temperature prior to analysis. PCS measurements were made at a scattering angle of 90° and a hydrodynamic diameter calculated by the instrument software by reference to the Stokes–Einstein equation. Generally submicron particles are well characterised by PCS, though accuracy of measurement may be compromised with polydispersed systems, and care should be taken when interpreting data generated.

3. Results and discussion

3.1. Critical micelle temperatures (CMTs) of poloxamers

A typical HSDSC scan of P407 (5% w/v) is shown in Fig. 1. Mitchard et al. (1992) demonstrated a good agreement between CMTs determined using HSDSC and theoretically derived values, so onset temperature (T_0) and peak temperature (T_{min}) values were extracted from each endotherm as a measure of the CMT for each poloxamer at a particular concentration. For a thermal transition, such as the melt of a crystal, the onset temperature would be the appropriate descriptor for the melting point, whereas for a polydisperse polymer the peak temperature may be more suitable. In this instance, the micellization of

a polydisperse surfactant is probably best characterised by the peak temperature, rather than the onset, though for thoroughness both parameters have been used in calculations. The T_0 and T_{min} values of each poloxamer decreased as concentration increased (plot of T_0 against concentration is shown in Fig. 2), allowing the relationship between T_0 or T_{min} and concentration to be applied to a linear regression model in order to determine the extrapolated y-intercept temperature, which is the CMT of the poloxamer solution at infinite dilution (Hecht and Hoffman, 1995). At infinite dilution P338 had a CMT of 25.92 ± 0.69°C, calculated using T_0 and a CMT of 32.17 ± 0.40°C calculated using T_{min}, compared to P407 with a CMT of 25.80 ± 0.51°C calculated using T_0 and a CMT of 29.23 ± 0.39°C calculated using T_{min}.

The plots of T_0 and T_{min} against concentration had R values ≥ 0.98 indicating good linearity, agreeing with previous findings (Wanka et al., 1994; Hecht and Hoffman, 1995). At low concentrations of poloxamer the plots showed some deviation from linearity, as previously described (Hecht and Hoffman, 1995). P407 has a greater number of POP units per monomer than P338 (Table 1) and would thus be predicted to have a lower CMT at a given concentration since CMT should decrease as the molecular weight of the POP moiety increases. The heat of micellization is due to the dehydration of POP moieties and so is strongly correlated to the size of the POP block (Beezer et al., 1992; Wanka et al., 1994). Consequently, the CMT at infinite dilution, calculated using T_{min} data seem to be most reliable.

3.2. Effect of poloxamers on the thermal transitions of DMPC MLVs

The effects of poloxamers on the enthalpy of the phospholipid pre-transition in the concentration range 0.1–1.0% w/v were assessed. This approach has been used previously to assess the interaction of various molecules with liposomal phospholipid bilayers (Jain and Wu, 1977; Fildes and Oliver, 1978; Posch et al., 1983). The enthalpy of the pre-transition of formulations that included poloxamer (ΔH_p) was divided by the enthalpy of the pre-transition of formulations without poloxamer (ΔH) to normalise any differences between replicate phospholipid suspensions. The $\Delta H_p/\Delta H$ values of dispersions incubated with P338 and P407 (shown in Fig. 3A and B) showed similar decreases as poloxamer concentration was increased at 18, 25

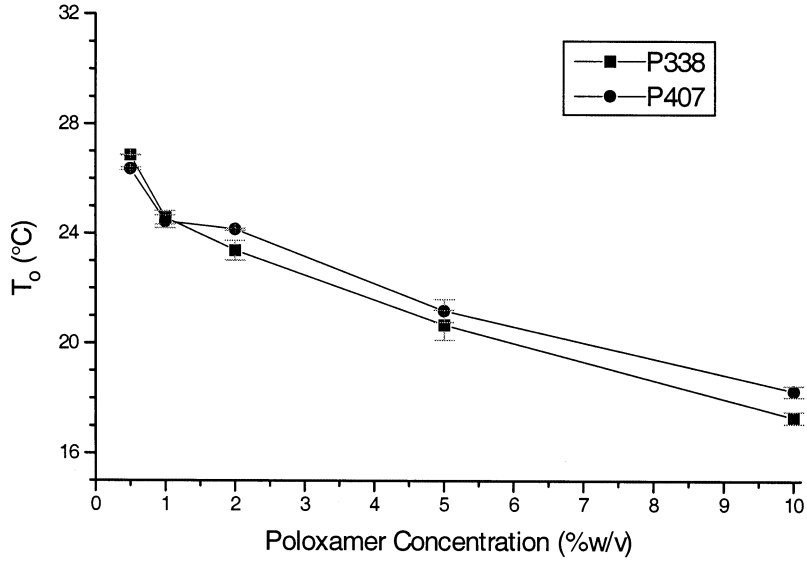

Fig. 2. CMT onset temperature (T_0) for poloxamer solutions ($n = 3 \pm$ S.D.).

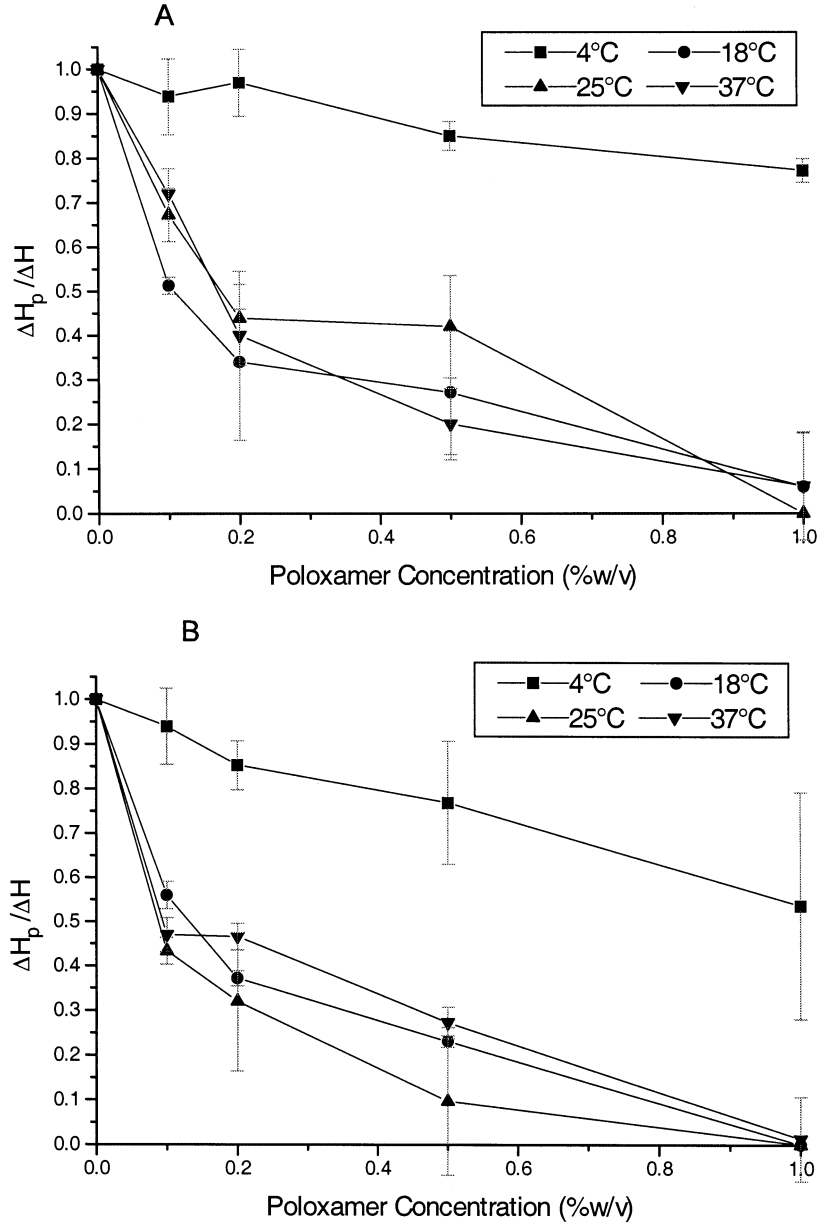

Fig. 3. $\Delta H_p/\Delta H$ for the pre-transition of DMPC (50 mg/ml) MLVs incubated with (A) P338 and (B) P407 at 4,18, 25 or 37°C for 24 h ($n = 4 \pm$ S.D.).

and 37°C. This is indicative of an interaction between poloxamers and bilayers at temperatures near to and above the gel to liquid–crystalline phase transition temperature (24.04–24.23°C; Table 2) of DMPC MLVs. Interestingly, there was no significant difference for data ($P < 0.05$) extracted at any of these temperatures following incubation with either poloxamer, suggesting that the magnitude of the interaction at 18°C [ie. above the pre-transition of 15.5°C (Castile et al., 1999) but below the main transition] was not different to that detected following incubation

above the main transition temperature. The $\Delta H_p/\Delta H$ values were relatively unaffected following incubation with either poloxamer at 4°C, with $\Delta H_p/\Delta H$ values significantly higher than at 18, 25 and 37°C ($P < 0.05$), indicating that the rigid structure of the gel state inhibited poloxamer penetration and interaction with the bilayer.

3.3. Effect of high concentrations of poloxamers on the thermal transitions of DMPC MLVs

Addition of poloxamers in the concentration range 2.0–5.0% w/v had a much greater effect on the thermal profile than was observed in the lower concentration range. Fig. 4 shows a typical HSDSC trace of DMPC MLVs incubated at 25°C for 24 h with 5% w/v P407. Poloxamers P338 and P407 affected both the co-operativity and temperature of the main transition. HHW values were calculated by measuring the width of the main transition peak (°C) at half its height and are used to assess the co-operativity of the transition (Jain and Wu, 1977).

The HHWs of the main phospholipid phase transition peak of DMPC MLVs incubated at 18, 25 and 37°C with up to 5% w/v P338 and P407 are shown in Fig. 5A and B. At concentrations above 2% w/v, the HHW of formulations containing either poloxamer increased significantly ($P < 0.05$). Preparations incubated with P338 (Fig. 5A) at 25°C had significantly higher HHWs than those incubated at 18 and 37°C ($P < 0.05$), indicating that incubation at 25°C facilitated penetration of this poloxamer molecule into the bilayer. This temperature is close to the main gel to liquid crystalline phase transition temperature for DMPC, at which the bilayer should be particularly permeable to extraneous solutes (Deamer and Bramhall, 1986). Large increases in HHW were also obtained for preparations incubated with P407 at poloxamer concentrations above 2% w/v (Fig. 5B). The HHW of formulations incubated with the more hydrophobic P407 at 25°C were not significantly different ($P < 0.05$) to those incubated at 18 or 37°C.

The main T_c of DMPC MLVs decreased when incubated with P338 and P407 at concentrations above 2% w/v (Table 2). Following incubation at 18°C, significant reductions in T_c were observed for formulations containing 5% w/v P338 and $\geq 3\%$ w/v P407. The greatest reductions were observed for dispersions incubated at 25 and 37°C (significant, $P < 0.05$, at all concentrations of P338 and P407 except for the formulation incubated with 2% w/v P407 at 25°C). The reduction of T_c with increasing poloxamer concentration coincides with the development of a broad shoulder on the high temperature side of the main transition peak (Fig. 4). This was a feature of the scans of systems incubated at all temperatures studied, but was especially evident for those incubated at 25°C. It is indicative of an impurity-induced depression of the T_c (Cater et al., 1974), which suggests that poloxamer interferes with the packing order of the bilayer, by incorporating into the phospholipid bilayer. However, the data do not rule out the formation of a mixed micellar phase or disk-like aggregates. Inclusion of PEG-lipid conjugates within the bilayers of DPPC liposomes (Bedu-

Table 2
Main transition temperature (T_c) of DMPC MLVs following incubation at 18, 25 or 37°C for 24 h with P338 or P407 ($n = 3 \pm$ S.D.)

Formulation	T_c (°C)		
	(incubation at 18°C)	(incubation at 25°C)	(incubation at 37°C)
No poloxamer	24.04 ± 0.17	24.23 ± 0.04	24.18 ± 0.06
+2% P338	23.93 ± 0.04	22.34 ± 0.10	24.03 ± 0.04
+3% P338	23.63 ± 0.24	23.22 ± 0.27	23.61 ± 0.31
+5% P338	22.32 ± 0.11	22.34 ± 0.29	22.49 ± 0.02
+2% P407	23.35 ± 0.45	22.71 ± 1.03	22.98 ± 0.06
+3% P407	22.49 ± 0.06	22.44 ± 0.01	22.46 ± 0.03
+5% P407	21.28 ± 0.47	21.15 ± 0.27	21.49 ± 0.07

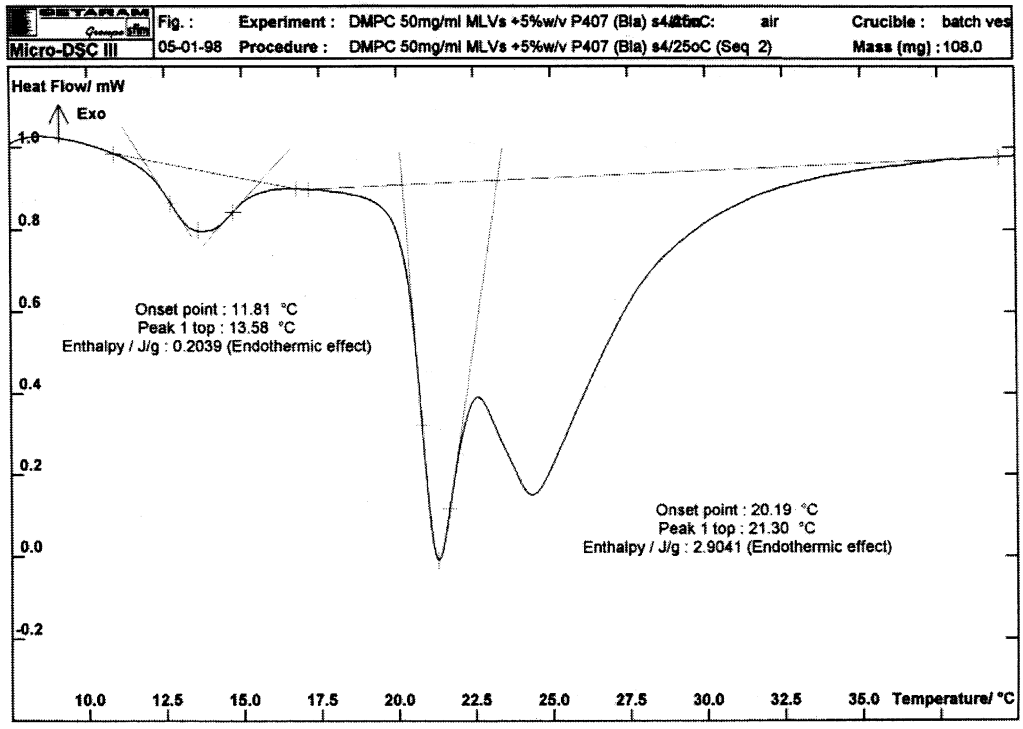

Fig. 4. Typical HSDSC scan of DMPC (50 mg/ml) MLVs + 5% w/v P407 incubated at 25°C for 24 h.

Addo et al., 1996) or DSPC liposomes (Kenworthy at al., 1995) has previously been shown to result in a shoulder on the high temperature side of the main phase transition endotherm. This was ascribed to the gradual solubilization of liposomes from the lamellar state to a mixed micellar state as the concentration of polymer conjugate was increased. The theory was supported by PCS analysis which indicated that there was an appreciable decrease in particle size with 17 mol% PEG(3000)-PE (Bedu-Addo et al., 1996). P407 has a larger hydrophobic POP group than P308, and thus reduces the temperature of the main transition to a greater extent.

Interestingly, a pre-transition endotherm was apparent at high poloxamer concentrations possibly indicating separation into poloxamer-rich and poloxamer poor phases. Following incubation at 25°C with 5% w/v P338 or P407, it was not possible to reproducibly measure the diameter of liposomes using laser particle size analysis, suggesting that vesicles may have been solubilized to mixed micelles. This has been previously described for other poloxamers (Johnsson et al., 1999) and for high concentrations of PEG–lipid conjugates included in liposome formulations (Hristova et al., 1995; Kenworthy et al., 1995; Bedu-Addo et al., 1996). It has also recently been demonstrated using cryo-transmission electron microscopy that poloxamers containing large POE blocks, such as are present in P338 and P407 when included in liposome bilayers can induce the formation of disk-like aggregates, which may co-exist with the liposomes (Johnsson et al., 1999).

Jain and Wu (1977) assigned the non-ionic surfactants they studied to a type A profile: broadening of the endothermic peak accompanied by a lowering of the T_c. The types of molecules that produce type A profiles are generally straight chain structures with a polar and nonpolar end, which disrupt both the packing and co-operativity of the bilayer, and are localized in the vicinity of the first eight carbons of the phospholipid hydrocarbon chains. This would require them to be

relatively large and moderately amphipathic. The POP moiety of P338 or P407 could extend into the hydrocarbon chain region of the bilayer and upset the molecular packing. At low concentrations this would be symbolized by a reduction of pre-transition enthalpy. Only at higher concentrations would there be enough poloxamer molecules present to significantly change the T_c and reduce bilayer co-operativity (increase HHW). In addition, the reduction in the T_c is said to indicate a preferential localisation in liquid crystalline regions of the bilayer (Van Osdol et al., 1992; Biltonen and Lichtenberg, 1993), although further studies are required to verify this hypothesis.

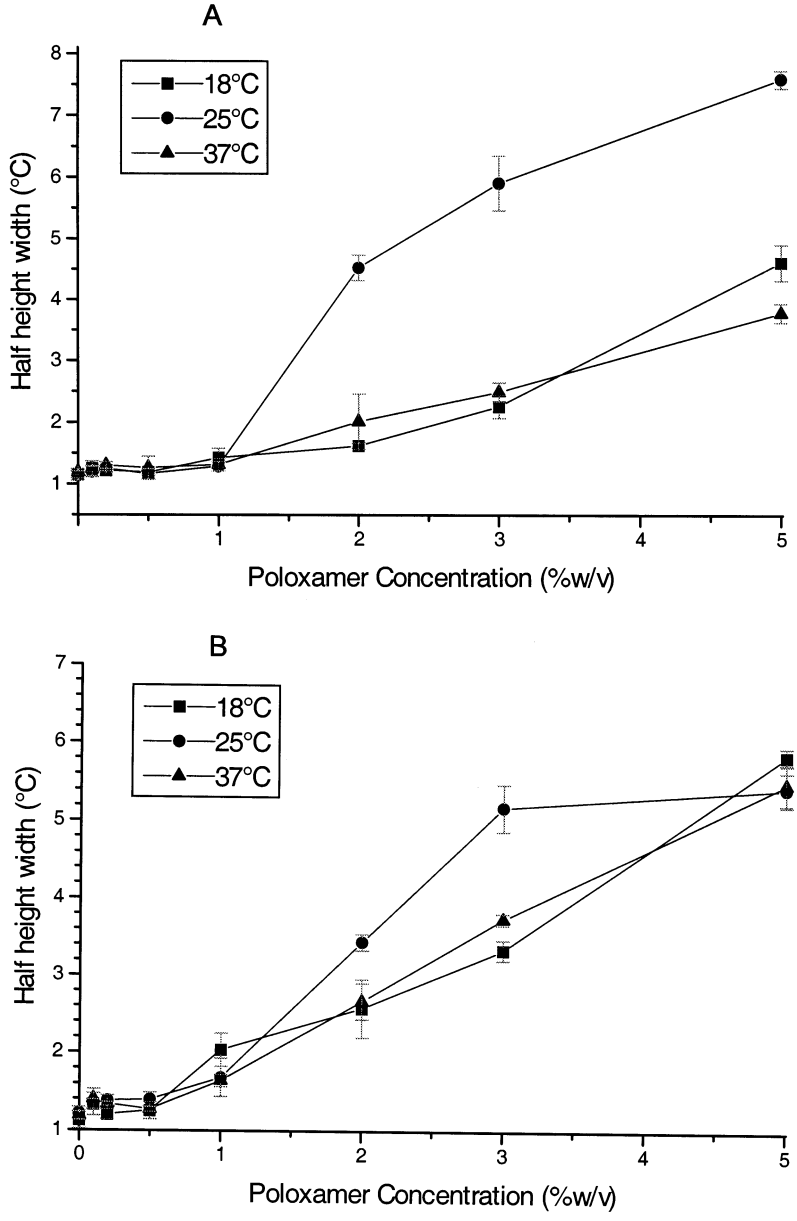

Fig. 5. Half height width (°C) of the main transition of DMPC (50 mg/ml) MLVs incubated with (A) P338 and (B) P407 at 18, 25 or 37°C for 24 h ($n = 3 \pm$ S.D.).

3.4. Effect of incubation temperature on the mean diameter of small DMPC liposomes

Preliminary experiments (data not shown) showed that small increases in mean liposome size were observed until approximately 15–24 h after addition of poloxamer, after which no further increases were observed. Consequently, 24 h was chosen as a standardised incubation time for further experiments. Populations of liposomes with polydispersities of 0.1 or less can be regarded as monodispersed (Elorza et al., 1993). All liposomes in this study had mean polydispersities less than 0.1 and they did not significantly change during incubation ($P < 0.05$), indicating that aggregation or fusion of vesicles due to the presence of poloxamers, or storage did not occur to any great extent.

The mean diameters of freeze–thaw extruded 2 mg/ml DMPC liposomes incubated with P338 and P407 at 18, 25 and 37°C are shown in Fig. 6A and B. At 18°C, an increase in mean diameter was observed as poloxamer concentration was increased, with significant changes ($P < 0.05$) in mean diameter at $\geq 0.1\%$ w/v P338 (Fig. 6A) and $\geq 0.3\%$ w/v P407 (Fig. 6B). A temperature of 18°C is below the CMTs of both poloxamers at all concentrations studied here (Fig. 2), possibly explaining why the size increases were less than at higher temperatures, since poloxamers are more thermodynamically stable in water at lower temperatures (Beezer et al., 1992; Mitchard et al., 1992; Wanka et al., 1994; Hecht and Hoffman, 1995). Under these conditions DMPC bilayers are likely to adopt the $P_{\beta'}$ ('ripple') conformation (Nagle and Scott, 1978) as 18°C is between the pre- and main transition temperatures of DMPC liposomes, suggesting that the level of disorder within the bilayers is sufficient to facilitate significant poloxamer penetration.

Larger size increases were observed at 25 and 37°C following incubation with both P338 and P407. At both temperatures, maximal diameters were detected at 0.5% w/v P338, above which there were decreases in the mean diameter. Changes in mean diameter were significant at concentrations of 0.05–0.5% w/v P338 ($P < 0.05$) at 25 and 37°C. At 25°C the mean diameter of liposomes was highest at 0.3% w/v P407, after which there was a decrease in the vesicle mean diameter. At 37°C, the highest mean diameter was detected at 0.1% w/v P407, after which there was a steady decrease in mean diameter. Changes in mean diameter were significant for 0.05–0.5% w/v P407 at both 25 and 37°C ($P < 0.05$).

The greater size increases at 25°C than 37°C seen following incubation with P338 may be due to the proximity of the incubation temperature to the T_c of DMPC liposomes, at which point bilayers are most permeable due to a maximum lateral compressibility caused by head group fluctuations and by boundary defects formed between gel-state phospholipid molecules and liquid–crystalline state molecules during melting (Doniach, 1978; Nagle and Scott, 1978; Deamer and Bramhall, 1986). This may facilitate the penetration of the hydrophobic moiety of the poloxamer into the hydrophobic region of the bilayer. However, size increases were greater at 37°C than 25°C following incubation with P407, indicating that incubation near to or above that poloxamer CMT was a more influential parameter. It is clear that both poloxamer CMT and liposome state influenced the magnitude of size increase for DMPC liposomes, with bilayers most disordered around the T_c and poloxamers seemingly more likely to interact with the bilayer at temperatures near to their CMT. Low concentrations of poloxamers incorporated into egg phosphatidylcholine liposomes, have been reported to result in the formation of bilayer disks and disk-like aggregates, though the size of these structures was not determined (Johnsson et al., 1999). It is thus possible that the increase in size seen in this study at low concentrations may be indicative of disk formation.

The decrease in size detected for preparations incubated with 1% w/v P338 at 25 and 37°C may indicate that some solubilization of liposomes by the poloxamer had occurred. Kostarelos et al. (1995) ascribed similar decreases in the diameter of soybean phosphatidylcholine liposomes at high poloxamer concentrations to the formation of mixed micelles.

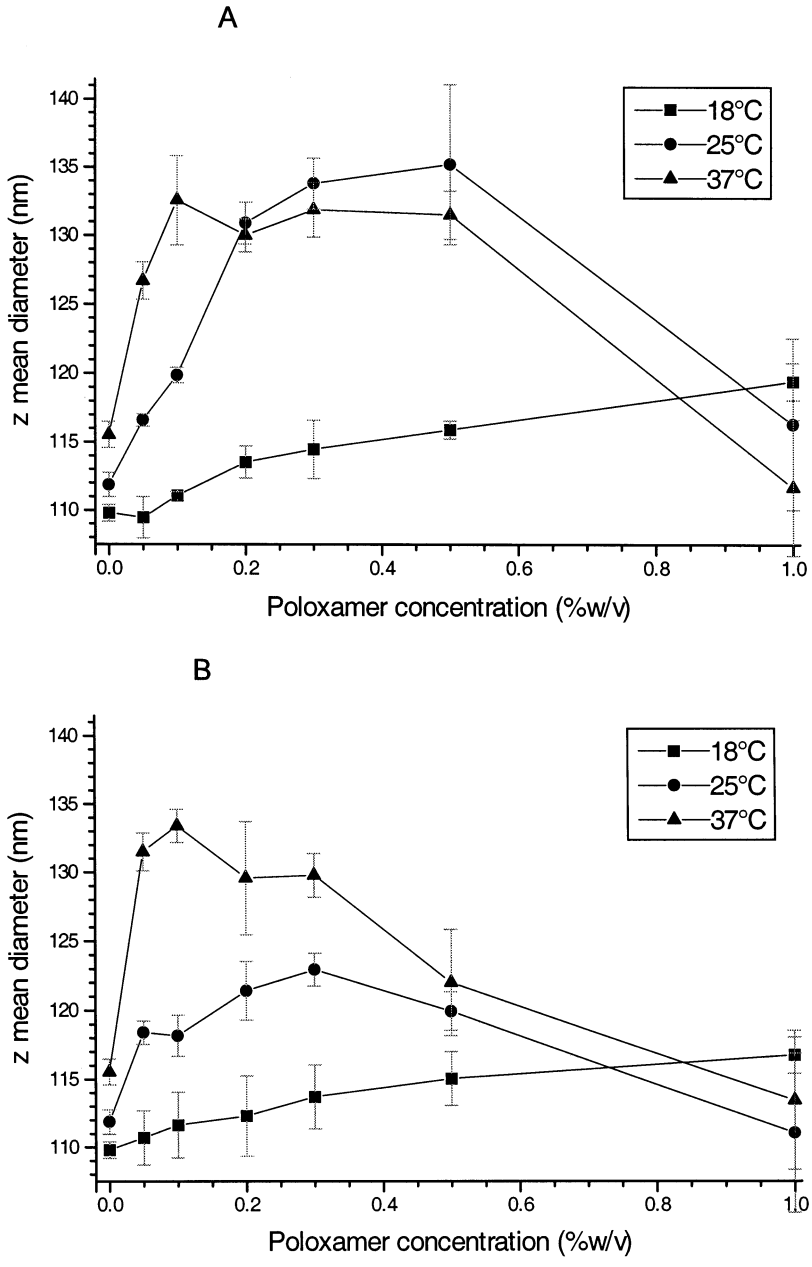

Fig. 6. Mean diameter of FT extruded 2 mg/ml DMPC liposomes incubated with (A) P338 and (B) P407 at 18, 25 or 37°C for 24 h ($n = 4 \pm$ S.D.).

4. Conclusion

Differential scanning calorimetry combined with particle size analysis has proved useful for exploring the nature of the interaction of poloxamers P338 and P407 with DMPC liposomes. At low poloxamer concentrations (up to 1% w/v) a concentration dependent reduction in the en-

thalpy of the DMPC pre-transition was observed at 18, 25 and 37°C. This demonstrated that poloxamers interact directly with the liposome bilayer at temperatures close to, or in excess of, the main phospholipid temperature. However, such interaction was minimal following incubation of liposomes with either poloxamer at 4°C. A direct interaction of the hydrophobic POP moiety of poloxamers with liposomal bilayers, rather than adsorption of the surfactant on the liposome surface is likely to be desirable to achieve steric stabilisation of these systems in vivo. Higher concentrations of poloxamers reduced the temperature of the main transition endotherm, the shape of which was indicative of phase separation. This was confirmed by size analysis which showed a decrease in mean vesicle size of liposomes at relatively high poloxamer concentrations, suggesting the presence of mixed micelles. Addition of lower poloxamer concentrations (up to 0.5%), resulted in an increase in the size of the vesicles, which was temperature dependent, with maximal increases at temperatures close to the critical micelle temperature of the surfactant. Further study is required to determine whether the concentration and temperature dependance of the interaction between poloxamer and liposome can be exploited to optimize the location of the surfactant within the bilayer and minimize leakage of entrapped materials, and to establish whether structural changes in the liposomes, for instance the formation of disks is occurring in these systems.

Acknowledgements

The authors wish to thank the BBSRC for their financial support.

References

Alexandridis, P., Holzwarth, J.F., Hatton, T.A., 1994. Micellization of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers in aqueous solutions: thermodynamics of copolymer association. Macromolecules 27 (241), 424–425.

Armstrong, J.K., Chowdhry, B.Z., Beezer, A.E., Mitchell, J.C., Leharne, S., 1994. Thermodynamic model fitting of differential scanning calorimetry traces obtained for aqueous solutions of oxyethylene–oxypropylene ABA block copolymers. J. Chem. Research S, 364–365.

Baekmark, T.R., Pedersen, S., Jørgensen, K., Mouritsen, O.G., 1997. The effects of ethylene oxide containing lipopolymers and tri-block copolymers on lipid bilayers of dipalmitoylphosphatidylcholine. Biophys. J. 73, 1479–1491.

Bedu-Addo, F.K., Tang, P., Xu, Y., Huang, L., 1996. Effects of polyethylene glycol chain length and phospholipid acyl chain composition on the interaction of polyethylene–phospholipid conjugates with phospholipid: implications in liposomal drug delivery. Pharm. Res. 13, 710–717.

Beezer, A.E., Mitchard, N., Mitchell, J.C., Armstrong, J.K., Chowdhry, B.Z., Leharne, S., Buckton, G., 1992. High sensitivity differential scanning microcalorimetry of phase transitions in dilute solutions of poly(oxyethylene) + poly(-oxypropylene) + poly(oxyethylene) block copolymers. J. Chem. Res. S, 236–237.

Biltonen, R.L., Lichtenberg, D., 1993. The use of differential scanning calorimetry as a tool to characterize liposome preparations. Chem. Phys. Lipids 64, 129–142.

Buckton, G., Machiste, E.O., 1996. Differences between dynamic equilibrium surface tension of poly(oxyethylene)–poly(oxypropylene)–poly(oxyethylene) block copolymer surfactants (Poloxamers P407, P237 and P338) in aqueous solution. J. Pharm. Sci. 86, 163–166.

Castile, J.D., Taylor, K.M.G., Buckton, G., 1999. A high sensitivity differential scanning calorimetry study of the interaction between poloxamers and dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine liposomes. Int. J. Pharm. 182, 101–110.

Cater, B.R., Chapman, D., Hawes, S.M., Saville, J., 1974. Lipid phase transitions and drug interactions. Biochim. Biophys. Acta 363, 54–69.

Deamer, D., Bramhall, J., 1986. Permeability of lipid bilayers to water and ionic solutes. Chem. Phys. Lipids 40, 167–188.

Doniach, S.J., 1978. Thermodynamic fluctuations in phospholipid bilayers. J. Chem. Phys. 68, 4912–4916.

Elorza, B., Elorza, M.A., Sainz, M.C., Chantrez, J.R., 1993. Comparison of particle size and encapsulation parameters of three liposomal preparations. J. Microencaps. 10, 237–248.

Evans, E., Needham, D., 1988. Attraction between lipid bilayer membranes in concentrated solutions of nonadsorbing polymers: comparison of mean-field theory with measurements of adhesion energy. Macromolecules 2, 822–1831.

Fildes, F.J.T., Oliver, J.E., 1978. Interaction of cortisol-21-palmitate with liposomes examined by differential scanning calorimetry. J. Pharm. Pharmacol. 30, 337–342.

Hecht, E., Hoffman, H., 1995. Kinetic calorimetric investigations on micelle formation of block copolymers of the poloxamer type. Colloids and Surfaces 96, 181–197.

Hristova, K., Kenworthy, A., McIntosh, T.J., 1995. Effect of bilayer composition on the phase-behaviour of liposomal suspensions containing poly(ethylene glycol) lipids. Macromolecules 28, 7693–7699.

Jain, M.K., Wu, N.M., 1977. Effect of small molecules on the dipalmitoyl lecithin liposomal bilayer. III. Phase transition in lipid bilayer. J. Membrane Biol. 34, 157–201.

Jamshaid, M., Farr, S.J., Kearney, P., Kellaway, I.W., 1988. Poloxamer sorption on liposomes: comparison with polystyrene latex and influence on solute efflux. Int. J. Pharm. 48, 125–131.

Johnsson, M., Silvander, M., Karlsson, G., Edwards, K., 1999. Effect of PEO-PPO-PEO triblock copolymers on structure and stability of phosphatidylcholine liposomes. Langmuir 15, 6314–6325.

Kenworthy, A.N., Simon, S.A., McIntosh, T.J., 1995. Structure and phase behaviour of lipid suspensions containing phospholipids with covalently attached poly(ethylene glycol). Biophys. J. 68, 1903–1920.

Khattab, M.A., Farr, S.J., Taylor, G., Kellaway, I.W., 1995. The in vitro characterisation of some non-ionic surfactant coated liposomes in the rabbit. J. Drug Targeting 3, 39–49.

Kostarelos, K., Luckham, P.F., Tadros, Th.F., 1995. Addition of block copolymers to liposomes prepared using soybean lecithin. Effects on formation, stability and the specific localisation of the incorporated surfactants investigated. J. Liposome Research 5, 117–130.

Mitchard, N.M., Beezer, A.E., Mitchell, J.C., Armstrong, J.K., Chowdhry, B.Z., Leharne, S., Buckton, G., 1992. Thermodynamic analysis of scanning calorimetric transitions observed for dilute aqueous solutions of ABA block copolymers. J. Phys. Chem. 96, 9507–9512.

Moghimi, S.M., Porter, C.J.H., Illum, L., Davis, S.S., 1991. The effect of poloxamer-407 on liposome stability and targeting to bone marrow: comparison with polystyrene microspheres. Int. J. Pharm. 68, 121–126.

Nagle, J.F., Scott, H.L., 1978. Lateral compressibility of lipid mono- and bilayers. Theory of membrane permeability. Biochim. Biophys. Acta 513, 236–243.

Paterson, I., Armstrong, J.K., Chowdhry, B.Z., Leharne, S., 1997. Thermodynamic model fitting of the calorimetric output obtained for aqueous solutions of oxyethylene–oxypropylene–oxyethylene triblock copolymers. Langmuir 13, 2219–2226.

Posch, M., Rakusch, C., Mollay, C., Laggner, P., 1983. Co-operative effects in the interaction between melittin and phosphatidylcholine model membranes. J. Biol. Chem. 258, 1761–1766.

Van Osdol, W.W., Ye, Q., Johnson, M.L., Biltonen, R.L., 1992. Effects of the anesthetic dibucaine on the kinetics of the gel–liquid crystalline transition of dipalmitoylphosphatidylcholine multilamellar vesicles. Biophys. J. 63, 1011–1017.

Wanka, G., Hoffman, H., Ulbricht, W., 1994. Phase diagrams and aggregation behaviour of poly(oxyethylene)–poly(oxypropylene)–poly(oxyethylene) triblock copolymers in aqueous solutions. Macromolecules 27, 4145–4159.

Woodle, M.C., Newman, M.S., Martin, F.J., 1992. Liposome leakage and blood circulation: Comparison of adsorbed block copolymers with covalent attachment of PEG. Int. J. Pharm. 88, 327–334.