

A Study of the Phase Transition Behaviours of Cholesterol and Saturated Egg Lecithin, and Their Interaction by Differential Scanning Calorimetry

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The thermal behaviours of hydrogenated egg lecithin (PC) and cholesterol (C) in anhydrous form and in aqueous dispersions were studied by differential scanning calorimetry. The role of C in phase transitions of C-PC mixtures prepared by physical mixing or coprecipitating from chloroform has been examined. C underwent a phase transition at 34°C and a second one at 76–78°C which depended on the thermal history of the samples. C lowered the gel to liquid crystalline phase transition temperature (T_i) of the aqueous dispersions prepared from C-PC coprecipitates, broadened the endothermic peak at the T_i , and at 1:1 molar ratio the C-PC system did not undergo the phase transition. The C-PC interaction was observed below the T_i . In aqueous dispersions prepared from C-PC physical mixtures, C did not influence the phase transition and acted like an inert diluent. Pellets prepared from C-PC mixtures formed myelin bodies as a result of their erosion in 0.185 M borate buffer (pH 7.4) at below T_i , but the thermal behaviours of these myelin bodies were different for pellets prepared differently. Addition of bovine serum albumin (BSA) (10%, w/w) to the physical mixtures or coprecipitates of C-PC, or to PC alone did not show any effect on the thermal behaviours of their aqueous dispersions at the T_i .

Keywords lyotropic liquid crystal; cholesterol; lecithin; myelin body; polymorphism; phosphatidylcholine bilayer; biodegradable implant; model membrane; implant erosion; lipid implant

Introduction

The widespread occurrence of cholesterol (C) in association with lecithin and other phospholipids in biological membranes has prompted studies of their interactions in relatively simple systems. During the last few decades C-phospholipid and lipid-protein interactions have been studied using differential scanning calorimetry,^{1–5} X-ray diffraction,^{6–9} nuclear magnetic resonance,^{4,10} and other physical techniques. Most of these studies suggest that C interacts with lecithin in the presence of water, lowers the gel to liquid crystalline phase transition temperature (T_i) of the lecithin-water system and has a condensing effect on lecithin molecules in the system. The C-lecithin interaction has been interpreted as due to particular arrangements of C and lecithin molecules variously described as C-lecithin complex,^{10–13} association,⁶ homogeneous mixed bilayer structure³ or otherwise.^{9,14,15} While the qualitative aspects of the influence of C on lecithin bilayers have generally been agreed upon, the quantitative aspects remain controversial. There is disagreement about the maximum amount of C which can be incorporated into the C-lecithin bilayers with suggestions of maximum C-lecithin molar ratios of 1:4,¹⁶ 1:2,^{8,17} 1:1^{1,6,12,18} and 2:1.¹⁹ Recently, Ipsen *et al.*²⁰ and Sankaram and Thompson¹⁵ suggested that the influence of C on phospholipid-water systems does not require complex formation but can be explained by time-averaged arrangements of C and phospholipid molecules. Sankaram and Thompson¹⁵ described a hypothetical C-phospholipid bilayer structure for systems with C-lecithin molar ratios of 1:3, 1:2 and 1:1, but made no comments on what happens if C content is increased further to a 2:1 ratio or more.

In most of these studies, C and lecithin were mixed by dissolving them in organic solvents, and aqueous dispersions were heated above the phase transition temperature of the phospholipid in order to have the fatty acid chains of the phospholipid in melted state. This was considered a

precondition for hydration of the phospholipid to form myelin bodies (bilayer structures) and for C-lecithin interaction.^{1,6,21} There have been no reports of phase transition behaviour of physically mixed C-lecithin either in anhydrous form or in the presence of water, and the possibility of formation of myelin bodies below the phase transition temperature of the phospholipids.

The use of C^{22,23} and lecithin^{24,25} separately and in combination^{26–29} as drug carriers makes it important to study the physico-chemical properties of C and lecithin, and their interactions. Lecithin extracted from natural sources (*e.g.*, egg lecithin) is not suitable for solid dosage forms because it is waxy and unstable in the presence of air.²⁵ The lability of lecithin largely depends on the degree of unsaturation,³⁰ hence hydrogenated lecithin offers an advantage in preparation of pharmaceutical dosage forms.^{24,25,27,31,32} Recently, we described the use of hydrogenated egg lecithin (PC) in conjunction with C for sustained delivery of a model antigen-bovine serum albumin (BSA).^{28,29} However, the phase-transition behaviour of PC and influence of C on PC-water system have not been reported.

In this paper we report a differential scanning calorimetric study of C-PC interactions in powder form and in aqueous dispersions, the effect of preparation by physical mixing and coprecipitating from chloroform, the influence of BSA as a component, and the temperature effect in preparing the C-PC-water systems. C when coprecipitated with PC was found to lower the gel to liquid crystalline phase transition temperature of the PC in the presence of excess water, and at 1:1 molar ratio the C-PC system did not undergo the phase transition. PC formed bilayer structures in the form of myelin bodies below its T_i , and C-PC interaction occurred below the T_i .

Materials and Methods

Materials Hydrogenated egg lecithin (PC) (technical grade, iodine value=0) was supplied by Asahi Chemical Industry Co., Ltd., Tokyo,

Japan. Cholesterol (C) (No. C-8503) was bought from Sigma Chemical Co., St. Louis, U.S.A., and recrystallised from anhydrous ethanol (once) and methanol (twice) to get the needle-shaped crystals of pure anhydrous C with mp 149°C. Purity was checked using thin layer chromatography.³³⁾ BSA (No. A-7030) was bought from Sigma Chemical Co. Fractionated chloroform was used to prepare the coprecipitates of C and PC.

Preparation of Binary Mixtures of C and PC Physical mixtures of C and PC with various C-PC ratios (0:1, 1:20, 1:15, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1, 8:1, 1:0, w/w) were made by crushing and mixing appropriate amounts of C and PC in a mortar. Coprecipitates of C and PC with the same series of C-PC ratios were prepared by dissolving the appropriate amounts of C and PC in chloroform (125–150 mg/ml) and then evaporating the solvent as described elsewhere.²⁸⁾

Incorporation of BSA (10%, w/w, when used) in both the physical mixtures and crushed coprecipitates of C-PC was carried out by geometric progression.

Preparation of Dispersions To prepare the aqueous dispersions of physical mixtures or coprecipitates, 80 mg of the powders was dispersed in 400 μ l of 0.185 M borate buffer (pH 7.4) by heating at 60–65°C (well above the T_i of the PC) for 20 min and shaking it in a Super-Mixer (Lab-Line Instruments, Inc., Melrose Park, Illinois) for 10 min immediately after removing from the oven. The dispersions were incubated at 37°C for 40–72 h prior to running on the differential scanning calorimeter (DSC).

A second set of samples was prepared using the same series of C-PC physical mixtures and coprecipitates in a similar way, but the powders contained 10% (w/w) BSA.

Another set of samples was prepared from C-PC coprecipitates, but without heating at 60–65°C. Instead, these samples were incubated at 37°C throughout for 40–72 h.

The samples were kept at room temperature for at least half an hour before analyses on the DSC.

Differential Scanning Calorimetry A Perkin-Elmer DSC 7, equipped with a Thermal Analyser (TAC 7/DX) and computer for process controlling, data collection and evaluation was used. The instrument was calibrated with indium at the same scanning rate as the samples. The onset temperatures, taken as the points of intersections of the tangents to the leading edges of the endotherms or exotherms and to the baseline, of the heating thermograms were considered as the temperatures of the peaks. The thermograms are not normalised and hence, comparisons among the areas under the peaks are not valid.

Approximately one drop (4–12 mg) of the dispersions was loaded into Perkin-Elmer 40 μ l aluminium 'non-volatile' sample pans (2 bar int. press.), sealed, heated at a rate of 8°C/min and thermograms recorded over the temperature range of 20 to 90°C.

Binary mixtures of C and PC (1.5–2.5 mg) were loaded into Perkin Elmer 50 μ l aluminium 'non-volatile' sample pans (1 bar int. press.), sealed and heated at a rate of 12°C/min. The thermograms were recorded between 20 and 120°C.

All the samples were cooled at a rate of 200°C/min and subsequently rerun.

Erosion Mechanism of C-PC Pellets Pellets consisting of three C-PC ratios (2:1, 1:1, 1:2, w/w) with and without BSA (10%, w/w) were prepared from physical mixtures and coprecipitates of C and PC as described elsewhere.²⁸⁾ Three pellets from each type were placed into 12.5 ml plastic tubes containing 10 ml of 0.185 M borate buffer with 0.1% sodium azide (w/v) as preservative. The tubes were tumbled (end over end) at 25 rpm in a water bath at 37°C for 0.5–3 h depending on the C-PC ratio and type of pellets, and the dissolution media were separated and stored at 37°C for 40–72 h. Single drops from the dissolution media were examined under light microscope, the remainder centrifuged and the sediment loaded into sample pans for scanning on DSC as described for aqueous dispersions of C and PC.

Results

The thermograms for first and repeat runs of a series of C-PC coprecipitate dispersions prepared by heating above the phase transition temperature of the PC are shown in Figs. 1 and 2, respectively. PC showed a gel to liquid crystalline phase transition endothermic peak at 46°C with a width of the transition over 41.5 to 51.5°C. Addition of C up to 1:15 (C-PC, w/w) caused slight

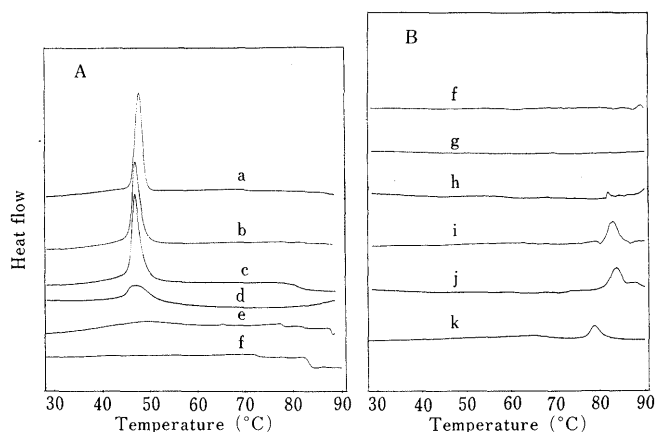


Fig. 1. DSC Thermograms of the First Run of Aqueous Dispersions of Coprecipitates of C and PC at pH 7.4 Containing C:PC (w/w): (A) a, 0:1; b, 1:20; c, 1:15; d, 1:8; e, 1:4; f, 1:2; (B) f, 1:2 (Duplicate); g, 1:1; h, 2:1; i, 4:1; j, 8:1; and k, 1:0

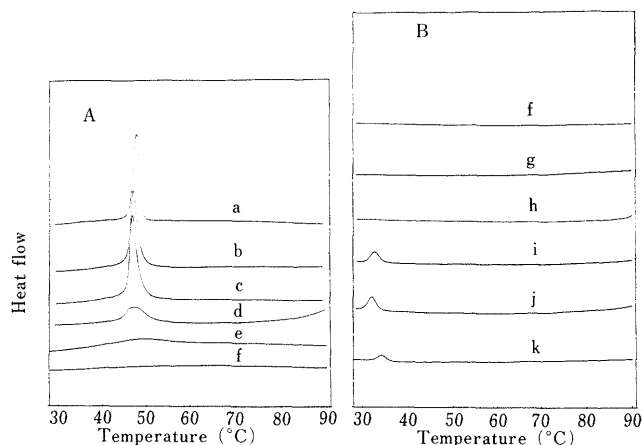


Fig. 2. DSC Thermograms of the Repeat Run of Aqueous Dispersions of Coprecipitates of C and PC at pH 7.4 Containing C:PC (w/w): (A) a, 0:1; b, 1:20; c, 1:15; d, 1:8; e, 1:4; f, 1:2; (B) f, 1:2 (Duplicate); g, 1:1; h, 2:1; i, 4:1; j, 8:1; and k, 1:0

broadening of the peak and reduced the T_i of the PC by 0.5°C. At C-PC level 1:8 (w/w) the peak was broadened further over the temperature range of 33 to 59°C. At 1:4 (w/w) ratio a broad peak over 28 to 64°C was just detectable. When the C-PC ratio was increased to 1:2 (approx. 1:1 molar) the peak vanished. The first runs of the samples with high C contents (C:PC > 2:1) produced a small endothermic peak at 81–82°C which disappeared on the second run with the appearance of a new peak at about 31°C. Dispersions of C alone (precipitated from chloroform) behaved similarly giving the small peak at 76–78°C on the first run and at 32–34°C on subsequent runs. The second and subsequent runs were reproducible.

The incorporation of BSA at the level of 10% (w/w) of the total lipid-protein did not alter the phase transition behaviour at the T_i at any C-PC level (data not shown). The first run of the samples prepared from the same series of C-PC coprecipitates without heating above T_i but incubating at 37°C for 40–72 h produced the same type of thermograms as those prepared by heating at above T_i (Fig. 3).

The dispersions prepared from C-PC physical mixtures by heating above the T_i gave endothermic peaks at the same T_i as dispersion of precipitated PC. Addition of C to

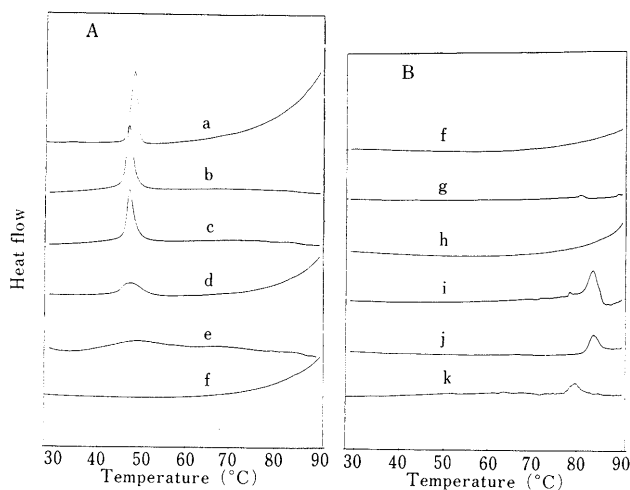


Fig. 3. DSC Thermograms of the First Run of Aqueous Dispersions (pH 7.4) of C-PC Coprecipitates Heated at 37°C (Well Below T_i) Containing C:PC (w/w): (A) a, 0:1; b, 1:20; c, 1:15; d, 1:8; e, 1:4; f, 1:2; (B) f, 1:2 (Duplicate); g, 1:1; h, 2:1; i, 4:1; j, 8:1; and k, 1:0

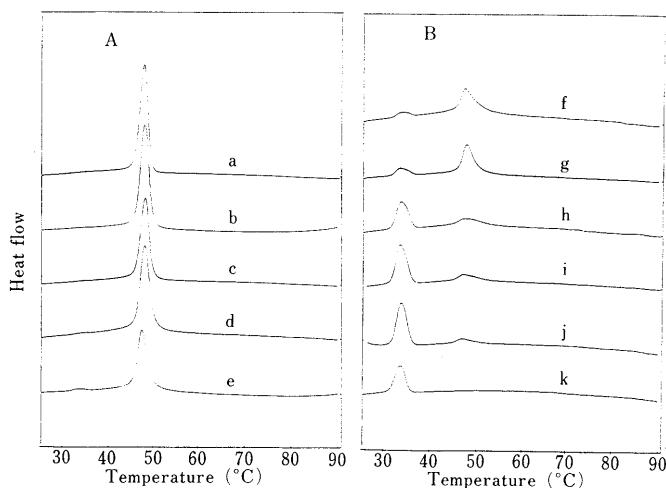


Fig. 4. DSC Traces of the Repeat Run of Aqueous Dispersions of C-PC Physical Mixtures Containing C:PC (w/w): (A) a, 0:1; b, 1:20; c, 1:15; d, 1:8; e, 1:4; (B) f, 1:2; g, 1:1; h, 2:1; i, 4:1; j, 8:1; and k, 1:0

PC at any level did not change the shape of the peaks (data for the first runs are not provided). During the reruns of these samples the extra small peaks (at 31–32°C) characteristic of pure C were detectable at and above C-PC ratio 1:8 (Fig. 4). Samples containing BSA (10%, w/w of the total lipid-protein) showed similar phase transition endothermic peaks (data not shown).

Powdered PC, both precipitated from chloroform and nonprecipitated (as received from the supplier) underwent solid to mesophase transitions at wide ranges of temperature with broad tailings at the leading edges of the endotherms and sharp endings. Nonprecipitated PC gave a peak over 34.5 to 77.5°C with the main transition temperature at 62°C. A minor peak appeared at 97°C. Physical mixing of C to PC caused shouldering at the leading edges of the peaks with the appearance of multi-component peaks at higher levels. At 1:2 ratio the early peak characteristic of C (data not shown) appeared at 32–34°C and disappeared on rerunning. Thermograms of the first runs are presented here (Fig. 5).

PC precipitated from chloroform had the solid to

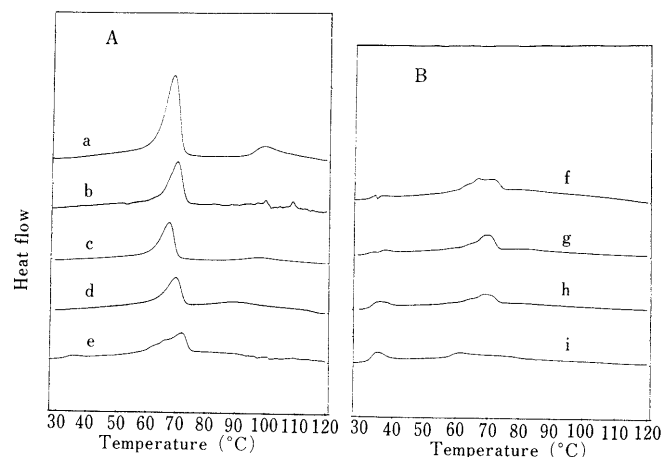


Fig. 5. DSC Thermograms of the First Run of Powdered C-PC Physical Mixtures Containing C:PC (w/w): (A) a, 0:1; b, 1:20; c, 1:15; d, 1:8; e, 1:2; (B) f, 1:1; g, 2:1; h, 4:1; and i, 8:1

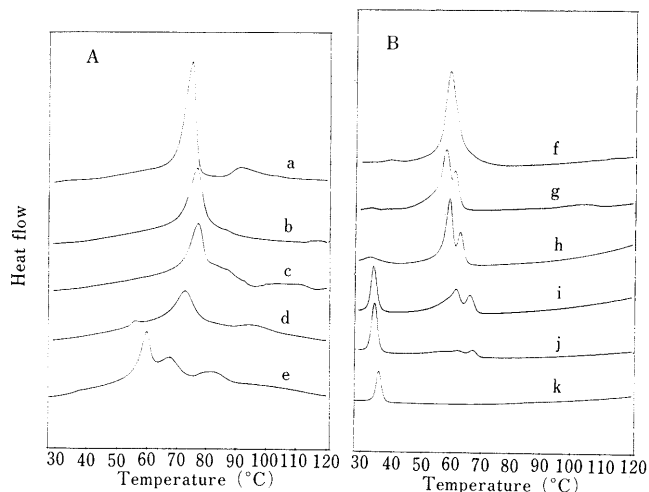


Fig. 6. DSC Thermograms of the First Run of Anhydrous C-PC Coprecipitates Containing C:PC (w/w): (A) a, 0:1; b, 1:20; c, 1:15; d, 1:8; e, 1:4; (B) f, 1:2; g, 1:1; h, 2:1; i, 4:1; j, 8:1; and k, 1:0

mesophase transition at 69.5°C. The second minor peak was detectable at about 87°C. With the addition of C these two peaks merged together giving multicomponent peaks with broader bases. At C-PC ratio 1:8, a small shouldering appeared on the lower temperature side which became prominent with increase in C contents. At high C levels (C:PC > 1:2) the early peaks appeared at 32–33°C (Fig. 6) and disappeared while rerunning (data not shown). Powdered C precipitated from chloroform showed a single endothermic peak at 34°C on the first run (Fig. 6) which disappeared on rerunning (data not shown).

Myelin bodies were observed under light microscope in the dissolution media separated from the eroded pellets prepared from both physical mixes of C and PC (Fig. 7A) and from their chloroform coprecipitates (Fig. 7B). But the DSC thermograms of the myelin bodies were different for the physical mixture and coprecipitate type of pellets; namely: the thermograms of the myelin bodies from coprecipitate type of pellets had no endothermic peaks, whereas the myelin bodies from physical mixture type of pellets produced endotherms at the T_i of the PC with the peaks characteristic of C during the first and repeat runs.

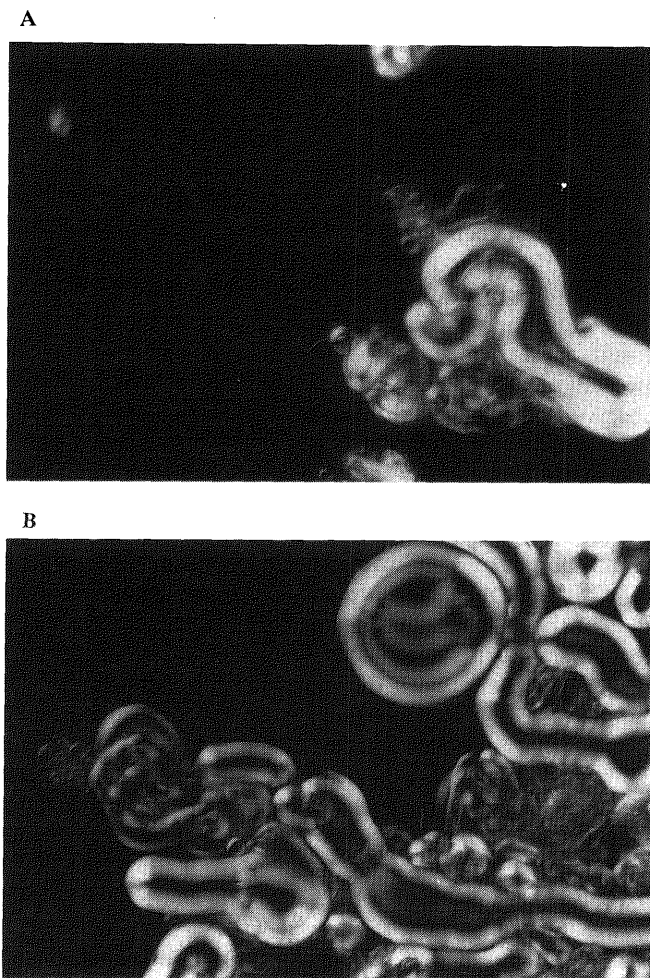


Fig. 7. Myelin Bodies Observed under Microscope Produced as a Result of Erosion (at 37°C) of C-PC (1:2, w/w) Pellets Prepared from Their Physical Mixtures (A) and Chloroform Coprecipitates (B)

The pellets contained BSA (10% of the total lipid-protein) and the dissolution medium was 0.185M borate buffer (pH 7.4).

Thermograms of the first runs are presented in Fig. 8.

Discussion

In the presence of water the observed T_i of the PC at 46°C with considerable broad base (10°C) is within the expected range considering its chemically heterogeneous nature with saturated fatty acids (iodine value=0) of various lengths (C_{14} , C_{16} , C_{18} , C_{20} , and C_{22}). Fatty acids of chain lengths C_{16} and C_{18} constituted about 35% and 52%, respectively, of the total fatty acids in the molecules of the PC used, with C_{14} representing only 0.2%. Saturated homogeneous lecithin like 1,2-dipalmitoyl-L-lecithin was reported to give the gel to liquid crystalline phase transition at 41°C^{1,16}; lecithins with longer chains are likely to have higher T_i .¹³

The endothermic peak of anhydrous C at 34°C is not surprising since there have been a number of reports of a polymorphic change in the structure of C at about this temperature.³⁴⁻³⁹ These authors differed in their observations of the temperature at which the polymorphic change in C structure occurred with reports of 40°C,³⁴ 35–40°C,³⁵ 37°C,³⁶ 36–37°C,³⁷ 39°C³⁸ and 25–40°C.³⁹ Petropavlov and Kostin³⁷ emphasised that even for the

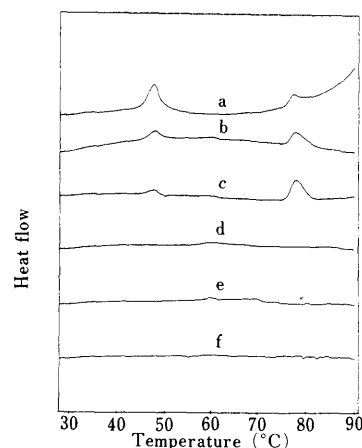


Fig. 8. Effect of C-PC Mixing Method in Preparing the Pellets on Their Erosion

DSC thermograms of the first run of myelin bodies produced as a result of erosion in 0.185M borate buffer (pH 7.4) of pellets prepared from physical mixtures containing C:PC (w/w): a, 1:2; b, 1:1; and c, 2:1; and from coprecipitates containing C:PC (w/w): d, 1:2; e, 1:1; and f, 2:1. The pellets contained BSA at 10% of the total lipid-protein, and the dissolution medium was 0.185M borate buffer (pH 7.4).

same crystal, several transition temperatures can exist depending on the perfection of the sample, its previous history and thermal conditions. We were unable to detect this endothermic peak in the thermograms of the repeat runs of powdered C perhaps because there was insufficient time for reorganising the C molecules to the initial polymorphic structure.

In the presence of water, the C dispersions did not show the peak at 34°C during the first runs because these samples were prepared by heating them above 34°C and incubating for as long as 40–72 h. Equilibration of the low-temperature state was reported to be a slow process and required supercooling.³⁵ Therefore, equilibrating the incubated samples for about half an hour at room temperature was not enough to revert to the low temperature state. However, the peak reappeared at 32–34°C during the repeat runs due to supercooling of the samples in the presence of water which improved the thermal conductivity inside the sample pans. The peak detected at 76–78°C during the first runs of these samples corresponds to another transition of C reported by van Putte *et al.*³⁵ as a result of their dilatometric study on a commercial grade of C. The transition was observed during a heating run between 70 and 110°C, and the authors did not mention whether it was a reversible endothermic transition. Loomis *et al.*³⁸ reported an endotherm centred at 86°C for C monohydrate during a first heating run on DSC (Perkin-Elmer DSC-II), which disappeared during the second run. They were unable to detect this transition in anhydrous C, and concluded that C monohydrate loses its water of hydration at this temperature forming the high temperature polymorph of anhydrous C. These reports and the absence of the peak in our anhydrous samples suggest that in our dispersions C existed, at least in part, in its monohydrate form in the first run. C molecules reverted to their anhydrous form did not show this transition during repeat runs due to kinetics of hydration. The mode of calculation of transition temperatures and the different models of DSC apparatus used by Loomis *et al.*³⁸ and us probably account for the discrepancies (± 6 –8°C)

observed for the transition temperature since the same authors also reported the early transition for anhydrous C at 39°C compared with 34°C as we discussed. The consistency of the slight changes (± 2 –3°C) in the onset temperatures of the endotherms characteristic of C in C-PC (coprecipitates) samples with high C contents both in powder form and in the presence of water suggests an influence of PC on these polymorphic changes.

The gel to liquid crystalline phase transition peak in the thermograms of the dispersions prepared from C-PC physical mixtures were unaffected by the addition of C at any level, indicating no C-PC interaction and C acting like an inert diluent. The extra small peaks whose positions on the temperature axis were variable depending on the thermal history of the samples were characteristic of C.

The thermograms obtained from dispersions of C-PC coprecipitates are in broad agreement with other reports of similar DSC studies on influence of C on phase transitions of various lecithins.^{1,14,16} The disappearance of the peak at about 1:1 molar ratio (1:2, w/w) has been explained as being due to the ability of C to hinder the phospholipid acyl chains in achieving the quasi-crystalline order below T_i that characterises the gel state, and above T_i it interferes with the lateral motions of the acyl chains that characterise the liquid crystalline state. C creates a condition of intermediate fluidity between gel and liquid crystalline states.^{1,13} This 1:1 stoichiometry of C-lecithin association in the lamellar phase was proposed by Bourguès *et al.*⁶ from X-ray diffraction studies. These authors suggested the creation of a paracrystalline phase (neat phase) of concentric bilayers of C and lecithin in water. The 1:1 stoichiometry was later supported by other investigators,^{10,12,18} although controversial reports and various interpretations of data exist.^{8,15–17,19}

The lipid-protein association in biological systems is known to occur primarily due to electrostatic forces between the charged ionic groups of the lipid bilayers and the protein, and the combination is an adsorption complex rather than a chemical compound.^{40–42} It is therefore crucial that the pH of the system be maintained such that both the lipid and the protein exist in ionised form in order to get the lipid-protein association. The pH of the studied system reported here was not maintained at well below the isoelectric point of the BSA (*ca.* 4.8), and hence, formation of BSA-PC (or BSA-PC-C) complex was unlikely. Hanai *et al.*⁴³ studied the effect of albumin on lecithin bilayers and concluded similarly. Sweet and Zull⁴⁴ found that at low pH BSA associated with multilayered vesicles formed from a mixture of lecithin, C and dicetyl phosphate. The association was increased with an increase in dicetyl phosphate concentration which provided negative charges to attract the positively charged BSA.

It is generally accepted that for a particular phospholipid to hydrate in water, form myelin bodies and interact with C, there is a minimum temperature below which there is no mixing with water and no liquid crystalline state exists.²¹ Ladbroke *et al.*¹ and Chapman and Fluck⁴⁵ described this minimum temperature as the gel to liquid crystalline phase transition temperature ($-T_i$). Bourguès *et al.*⁶ emphasised that swelling of lecithin does not occur and the paraffinic chains are not in a melted

state below T_i , and therefore, C-lecithin association cannot occur below T_i . The myelin bodies observed in the dissolution media of the eroded C-PC pellets at 37°C indicates that hydration of PC and formation of myelin bodies occurred below the T_i of the PC (46°C). The disagreement of our observation with the previous reports is attributable to the heterogeneous nature of the PC used. There have been reports about the coexistence of a number of phases in an egg lecithin-water system below the T_i of the lecithin. It was noted that below T_i at least a small amount of the egg lecithin molecules exist in the liquid crystalline state (melted chains) while the other molecules are in the gel state.⁴⁶ The myelin bodies observed in the dissolution media of the eroded C-PC physically mixed type pellets at 37°C were perhaps due to the hydration of the molecules existing in the liquid crystalline state at this temperature. In the dissolution media of the coprecipitated C-PC pellets formation of myelin bodies at 37°C was expected because, as a result of C-PC interaction, the presence of equimolar amounts of C in the C-phospholipid mixtures causes the system to be dispersible in water over a much wider temperature range than occurs with the individual phospholipid.¹ The different DSC traces obtained from the myelin bodies of C-PC pellets prepared differently are in agreement with the results described above for C-PC dispersions suggesting C-PC interaction in the myelin bodies formed from coprecipitate type pellets, whereas the physical mixture type pellets produced myelin bodies from PC alone and C crystals separated out from PC gave its characteristic peak at about 76°C in the presence of water.

The state of hydrocarbon chains is considered one of the most important aspects of C-PC interaction. The dispersions prepared from physical mixtures of C-PC were heated at well above the T_i in order to have the paraffinic chains in melted state, yet no C-PC interaction was observed. On the other hand, the first runs of the dispersions prepared from C-PC coprecipitates without heating at $>T_i$ showed the C-PC interaction, suggesting that C-PC molecules can associate below the T_i . This fact suggests that for C-PC interaction the melting of the paraffinic chains is not as essential as the intimacy of the C and PC molecules when they are mixed.

The broad phase transition with multiple peaks of PC and C-PC powders, both from physical mixtures and coprecipitates are not surprising given the chemical composition of this PC. This is related to the heterogeneous nature of the PC itself, and the addition of C to PC worsened the situation. PC is highly hygroscopic, and on storage might have absorbed some moisture. This explains why the PC used as received from the supplier underwent solid to mesophase transition at a lower temperature (62°C) than the PC precipitated from chloroform (69.5°C), which was dried under vacuum.² Addition of water (in excess) reduced the phase transition temperature and caused heterogeneous molecules of PC to act like a single component.^{1,6} Furthermore, in such a multicomponent heterogeneous solid systems the poor thermal conductivity may have resulted in the sample undergoing transition into discrete areas within the pan, rather than a uniform transformation. The inhomogeneities in samples of polyethylene glycols were reported to broaden the endotherm

and create multiple peaks in the melting process.⁴⁷⁾

From the pharmaceutical viewpoint the formation of C-PC myelin bodies from coprecipitate C-PC pellets in aqueous media at 37°C is encouraging. Firstly, it supports the use of C and PC combinations as biodegradable and implantable drug carriers. C provides adequate physical properties for preparation of the dosage form, egg lecithin controls the penetration of fluid into the dosage form and solubilizes C to make it biodegradable. Secondly, these liposome precursors from subcutaneously implanted solid pellets might replace, at least in part, liposome preparations with stability problems. Thirdly, incorporation of C molecules in the bilayer structures of these liposome precursors might reduce the permeability of the resultant liposomes. Finally, it is suggested that hydration of heterogeneous phospholipids can occur below their gel to liquid crystalline phase transition temperature. The majority of saturated lecithins undergo this phase transition above the body temperature, and the ability of saturated heterogeneous lecithins to hydrate at 37°C makes them suitable for use in preparation of solid dosage forms.

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