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Surface and interfacial properties of clomethiazole

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Abstract

The surface and interfacial properties of clomethiazole (CMZ) were investigated by means of several techniques. Drop-volume measurements showed that CMZ is surface active, with an estimated area per molecule at the aqueous/air surface of about 50 Å^2. Surface balance experiments revealed that CMZ, originally in the subphase, mixes with spread monolayers of dimyristoylphophatidylcholine (DMPC) and dipalmitoylphophatidylcholine (DPPC). The interaction of CMZ with lipids was further illustrated using X-ray investigations of lamellar crystalline and liquid crystalline phases, where the repeat distance was shown to decrease with increasing amount of CMZ. The large decrease of the repeat distance was interpreted as an effect of the CMZ molecules residing in the lipid bilayer close to the interface. The hydrocarbon chains of the phospholipids fill the voids behind the small CMZ molecules, which in turn leads to a decrease in the bilayer thickness. This interpretation was also supported by the fact that large amounts (> 10% w/w) of CMZ could be incorporated into the bicontinuous cubic phase of monoolein and water. Furthermore, CMZ decreases the chain melting temperatures of DMPC and DPPC as revealed by X-ray and DSC.

Keywords: X-ray diffraction; Surface balance; Drop-volume method; Clomethiazole; Phospholipids; Liquid crystal

1. Introduction

Physico-chemical characterization of drugs is of vital importance for the formulator of drug delivery systems and includes, among others, the determination of the hydrophilic/lipophilic properties of the drug compound. These properties are often derived from a partition coefficient such as that between octanol and water. Although widely used, the octanol/water partition coefficient is of limited value, since it poorly mimics the lipid mono- and bilayers often encountered for drugs both in their formulation and in living systems.

In this work we have used surface balance and drop-volume methods to study the surface (i.e.

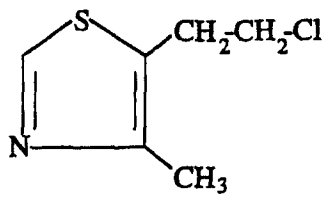

Fig. 1. The molecular structure of CMZ; pK_a for the ionisable nitrogen is 3.2

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air/aqueous) activity of the drug clomethiazole. These methods give valuable information on the drug's capability of forming stable monolayers alone or together with other substances (e.g., polar lipids), as well as the area occupied per molecule at the interface.

By mixing the drug with bilayer systems formed from phospholipids in water, one may study the drug's effect on the phase behaviour of such systems by means of the X-ray and differential scanning calorimetry (DSC) techniques. Such an investigation is highly relevant for the behaviour of the drug in emulsions and other formulations where polar lipids are present. It may also give some hints as to the behaviour of the drug in contact with the membranes of living systems.

The drug studied in this work, clomethiazole (Fig. 1), is derived from the thiazole moiety of vitamin B1. The drug has been widely used for more than 25 years for sedation as well as an anticonvulsant, tranquilizer and hypnotic. The active forms of the drug for clinical use are either the base or its edisylate salt. The base is an oily, colourless to yellow-brown liquid (Dollery, 1991), with an aqueous solubility of about 0.5% at 25°C and with partition coefficients of 132 and 45 for the octanol/water and Miglyol 812 (medium chain triglyceride)/water system, respectively. The melting point of the base is about -3°C.

2. Materials and methods

2.1. Materials

Clomethiazole, 4-methyl-5-(β-chloroethyl)-thiazole (CMZ), and its disulfonic acid salt, clomethiazole edisylate, were supplied by Astra Production Chemicals (Södertälje, Sweden). The phospholipids dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) were also supplied by Astra Production Chemicals. The water content of the DMPC was 1% w/w, the amount of lyso compounds < 0.1% w/w and the amount of free fatty acids 0.2% w/w (myristic). For DPPC the corresponding numbers were 0.6, < 0.1 and 1.4% (palmitic), respectively. Glycerol monooleate (GMO) was obtained from Grindsted (Brabrand, Denmark). The monoester content was 97% (w/w), the rest being 1.0% free glycerin, 1.0% free fatty acids, and 1.0% diglycerides. The fatty acid distribution was: 92.3% (w/w) oleic, 4.3% linoleic, 2.0% stearic, 0.5% arachidonic and 0.5% palmitic acid. All water used was doubly distilled (pH 6.5). Tristearin, sigma grade 99%, was purchased from Sigma Chemical Co., St Louis, USA, and α-aluminium oxide, for reference, was supplied by Seiko Instruments Inc., Japan.

2.2. Methods

2.2.1. Sample preparation

The samples were prepared in glass vials and sealed with rubber stoppers and aluminium seals. After preparation they were heated above the chain melting temperature (a few hours) to improve the mixing. When they appeared to be homogenous they were left standing at room temperature (≈ 20°C) until examined by X-ray or DSC. Prior to every sample withdrawal they were again kept at a temperature well above the chain melting, to ensure that the components were present according to the mixing ratio.

2.2.2. Drop-volume measurements

The surface tension measurements were performed using the drop-volume technique described previously (Tornberg, 1977) and later modified (Arnebrant and Nylander, 1985). The apparatus is designed to perform both static and dynamic surface tension measurements. All glassware was cleaned with concentrated HNO_3:H_2SO_4 (1:1 w/w) and thereafter rinsed with water. Water was also used for calibration. All measurements were performed at 25°C. The surface tension data presented are mean values of at least 15 single measurements. Two different drop-forming rates were used in the experiment: (i) 95% of the drop was formed at 4.25 μl/s and 5% at 0.177 μl/s; (ii) 85% at the highest speed and 15% at the lower speed.

2.2.3. Surface-balance measurements

The surface pressure/mean molecular area isotherms were monitored by the Wilhelmy plate

method using KSV 5000 Langmuir balance equipment (KSV Instruments, Helsinki, Finland). The equipment has been described previously (Mozaffary, 1991).

The phospholipid monolayers were spread from a 9:1 (v/v) mixture of hexane and ethanol at a concentration of 0.4 mg/ml ($\pm$ 0.01) by the use of a micro syringe (Hamilton, Switzerland). After spreading, the solvent was allowed to evaporate for 10 min. The monolayers were compressed at a rate of 20 mm/min until they collapsed or the subphase spilled over the edges of the trough. The data were collected by a computer every 2 s and the measurements were recorded at 25($\pm$ 1)°C. Prior to every measurement the air/water surface was cleaned by sweeping the Teflon barrier and aspirating any contaminants. This procedure was repeated at least three times in order to ensure an uncontaminated surface. Before dipping the platinum plate into the subphase, the plate was cleaned by heating in a flame.

When preparing the subphase containing CMZ, the drug was first dissolved in water and then subsequently filtered through a 0.2 mm presterilized Nalgene™ bottle top filter (Nalge Company, Rochester, NY, USA) to avoid contamination. All glassware was cleaned in a mixture of concentrated sulphuric and nitric acids and thereafter rinsed thoroughly with water.

2.2.4. X-ray diffraction measurements

The X-ray diffraction measurements were performed using a dual detector camera for simultaneous small- and wide-angle measurements (M Braun Graz Optical Systems, Graz, Austria) described previously (Laggner and Mio, 1992). The X-ray radiation was generated by a Philips PW 1830/40 generator with a Cu K_α anode (λ = 1.5418 Å, 1 Å = 0.1 nm). The samples were fixed and sealed between two mica windows. Temperature scans were taken between 5 and 50°C at an interval of 5°C. Each sample was allowed to equilibrate for 10 min at each temperature before measuring for at least 10 min (5 min at each detector). The diffraction measurements were repeated after 3 months on the same samples with no significant change in the diffraction patterns. The accuracy in the Peltier temperature control device (Anton Paar) was better than $\pm$ 1°C. To avoid air scattering, the camera tube was evacuated at 0.1 mbar. The *d*-spacings in the small-angle region were calibrated with tristearin.

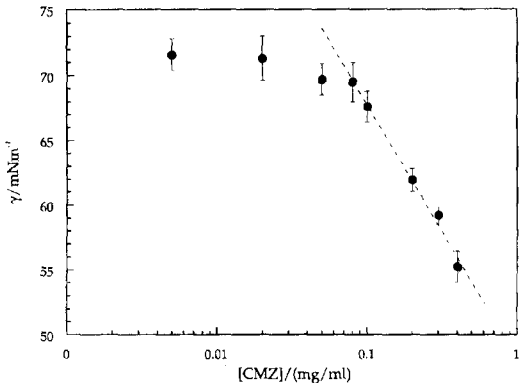

Fig. 2. Surface tension as a function of CMZ concentration (% w/w) measured at 25°C. The dashed line represents the linear least squares fit to Eq. (1) of the five highest concentrations.

2.2.5. DSC measurements

The DSC measurements were made using a Seiko DSC 220 C (Seiko Instruments, Japan). The samples were prepared in sealed aluminium pans and α-aluminium oxide was used as the reference. The amount of sample used for each measurement varied between 14 and 21 mg. The samples were equilibrated at 5°C until a stable base-line was obtained and then heated from 5 to 60°C at a rate of 5°C/min (DMPC) and 2°C/min (DPPC). After a hold of 0.2 min they were cooled to 5°C at the same rate. The computer data sampling was made every 0.5 s.

3. Results and discussion

3.1. Drop-volume measurements

The surface activity of CMZ in water as obtained by the drop-volume method is given in Fig. 2. It is seen that CMZ lowers the surface tension up to its solubility limit by approximately 20 mN/m. A comparison with the corresponding number for SDS at its critical micelle concentration, about 30 mN/m (Evans and Wennerström,

1994), reveals that CMZ has a substantial surface activity. Under the assumption that CMZ is the only molecule residing at the aqueous surface, the surface concentration Γ, and thus the area occupied per molecule, can be estimated by means of the Gibbs adsorption equation

$$\Gamma = \frac{1}{RT} \frac{d\gamma}{d \ln c} \quad (1)$$

where γ is the surface tension and c is the CMZ concentration. The surface concentration is estimated from the linear part of the curve γ vs. ln c.

From Fig. 2 it is seen that a constant negative slope is reached for CMZ concentrations above 0.08 mg/ml. The area per CMZ molecule (i.e. $1/\Gamma$) is determined to be about 50 Å^2. This number is about twice the size of the area occupied by a hydrocarbon chain in a fatty acid, and therefore comparable to the size of a phospholipid such as DPPC (see below). The determined area per CMZ molecule in relation to its molecular structure (Fig. 1) suggests that it lies more or less flat with its thiazole ring on the surface.

No significant change in surface tension was observed when using two different drop-forming rates (see above) in order to determine any time dependence of the surface activity of CMZ.

Drop-volume measurements were also performed at different pH in the range of 5.7 to 8.4, but no significant differences were observed. This is reasonable since pK_a for CMZ is 3.2. However, when lowering the pH to 2.8 by using the CMZ edisylate salt, only a slight decrease in surface tension, compared to pure water, was observed. Since CMZ is ionised at this pH the observed behaviour is what one could expect.

3.2. Surface balance measurements

One aim of this work is to show how CMZ interacts with phospholipids in mono- and bilayers. In order to study monolayers, the surface balance method was used. The two phospholipids, DMPC and DPPC, were spread on water and aqueous solutions of CMZ. The resulting surface pressures as a function of area per phospholipid are given in Figs. 3 and 4. A general observation from the figures is that at large areas per lipid, i.e. above 90 Å^2, the presence of CMZ in the subphase introduces a measurable surface pressure, below denoted the initial surface pressure, which is not the case for pure water. A second observation is that the initial surface pressure increases with increasing concentration of CMZ.

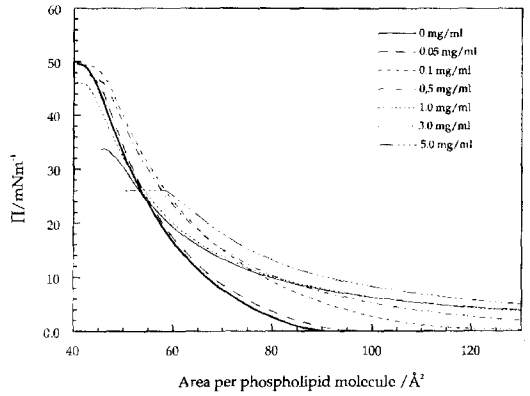

Fig. 3. Surface pressure/mean molecular area isotherms (T = 25°C) for DMPC spread on aqueous solutions of CMZ. Each curve represents the mean of three single measurements. The reproducibility was very good and the differences in the isotherms obtained for different concentrations of CMZ are significant.

Fig. 3 shows the isotherms obtained for DMPC above its chain melting temperature. The isotherms reveal a monolayer in the liquid expanded state since no sign of a phase transition is observed. The isotherms show three different types of behaviour: (i) for CMZ concentrations in

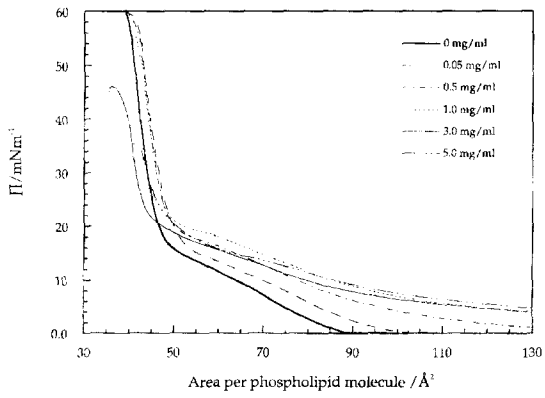

Fig. 4. Surface pressure/mean molecular area isotherms (T = 25°C) for DPPC spread on aqueous solutions of CMZ. The accuracy in the measurements is stated in Fig. 3.

the range 0–0.1 mg/ml, DMPC forms a well-defined monolayer on the subphase, and the film collapse occurs at approximately constant surface pressure with a slight increase in surface area per lipid for 0.1 mg/ml; (ii) in the range 0.5–3 mg CMZ/ml the isotherms start to cross over each other, and the film collapses at decreasing area per lipid and surface pressure; and (iii) for the highest CMZ concentration in the subphase, 5 mg/ml, the isotherm does not follow the trend observed for the lower concentrations, since the collapse is observed at a relatively high area per lipid and at an even lower surface pressure.

One reason for the initial surface pressure may be that the spread phospholipid molecules experience another (and perhaps better) solvent than water, and therefore are able to adopt more expanded configurations on the surface (cf. a polymer in a poor vs. a good solvent). A second reason may be that the spreading of the lipid monolayer leads to an increased accumulation of CMZ molecules at the phospholipid/aqueous interface. Several observations make the second explanation more plausible than the first (see below). Since the highest concentration, 5 mg CMZ/ml, is close to the solubility limit of CMZ in water, we cannot exclude that the phospholipid is spread on a pure CMZ layer. It should be noted that pure CMZ spreads on water, but does not form stable monolayers upon compression.

There are several arguments for an increased accumulation of CMZ at the interphase in the presence of a phospholipid monolayer since CMZ: (i) is surface active, (ii) has a higher affinity for octanol and Miglyol 812 than for water, and (iii) most probably prefers to be located within the phospholipid bilayers of a lamellar liquid crystalline phase (see below).

The crossing over of some isotherms may be an effect of dissolving some of the DMPC molecules in the subphase, since CMZ is a better solvent for the phospholipids than water. It seems, however, as if the CMZ molecules can be squeezed from the DMPC monolayer upon compression for subphase concentrations below 1 mg CMZ/ml. For the two highest concentrations of CMZ, it seems reasonable to assume that the surface film consists of phospholipid and islands of pure CMZ in which the phospholipid is more or less dissolved. The poor film forming capacity of CMZ becomes the dominating factor for the total compression behaviour, the higher the CMZ concentration.

Table 1
Small-angle X-ray diffraction data for DMPC/water 7:3 (w/w) with different amounts of CMZ (*d*-spacings in Å). Note the occurrence of a second lamellar repeat distance below the gel-to-liquid crystalline phase transition temperature for samples containing CMZ. Subscript 'G' indicates the presence of a gel-state as determined by wide-angle X-ray measurements

CMZ (%w/w)	10°C	20°C	30°C	40°C	50°C
0	60_G	62_G	55	53	52
2.5	59^a_G	57_G	53	51	50
5.0	55^a_G	54	51	50	49
10	52_G+43	49	48	47	46
15	52_G+43	47	47	46	45

[a]For samples containing 2.5 and 5% CMZ at 10°C, no obvious second lamellar repeat distance was observed.

For DPPC the situation is somewhat different (see Fig. 4), since the measurements were performed below DPPC's chain melting temperature, which is reflected by a transition between the liquid expanded and the liquid condensed states. Nevertheless, for larger surface areas there seems to be an interaction with the CMZ molecules, but as the area is decreased the CMZ molecules are squeezed from the interface. However, at high CMZ concentrations, the collapse pressure is significantly lowered. The interpretation of the behaviour of these isotherms follow that given for DMPC. It is, however, evident that the ability of DPPC to form closely packed hydrocarbon chains dominates the behaviour of these isotherms, except at the highest concentration.

Similar behaviour (i.e. an increase in initial surface pressure) has been observed for glycerol and trehalose in the subphase of phospholipid monolayers (Crowe et al., 1984). However, the monolayer expansion induced by CMZ is observed at much lower concentration compared to, for example, glycerol.

3.3. X-ray investigations

The data obtained from the small- and wide-angle X-ray investigations are summarized in Table

Table 2
Small-angle X-ray diffraction data for DPPC/water 7:3 (w/w) with different amounts of CMZ (d-spacings in Å). Note the occurrence of a second lamellar repeat distance below the gel-to-liquid crystalline phase transition temperature for samples containing CMZ. Subscript 'G' indicates the presence of a gel-state, as determined by wide-angle X-ray measurements

CMZ (% w/w)	10°C	20°C	30°C	40°C	50°C
0	65_G	65_G	65_G	66_G	57
2.5	65_G+48	65_G+48	64_G+48	$64_G+(58)$	56
5.0	63_G+47	63_G+46	61_G+46	55	53
10	63_G+46	63_G+45	61_G+45	53	51
15	62_G+45	62_G+44	60_G+44	49	48

1; Table 2 and Table 3. In the tables the spacing given by the primary reflection of the diffraction patterns is given. A typical example of small-angle diffraction patterns obtained are presented in Fig. 5, which shows the results obtained for DMPC/water (7/3 w/w) at 30°C with varying amounts of CMZ. In Fig. 5 the three diffraction peaks obtained in each spectrum reveals a lamellar structure since the positions of the peaks relate to each other approximately as 1:1/2:1/3:.... Wide-angle X-ray diffraction measurements showed that all samples were liquid crystalline at this temperature.

Fig. 6 shows typical small-angle diffraction patterns obtained for DPPC/water (7/3 w/w) at 10°C with and without CMZ, respectively. When CMZ was present two lamellar patterns were found (given as $a+b$ in Table 2), which merged into one lamellar pattern above the chain melting temperature. The existence of gel and/or crystalline phases, also indicated in Tables 1 and 2, was determined from wide-angle diffraction as illustrated in Fig. 7 and Table 3. The results are now discussed in more detail.

It was found that the lamellar repeat distance (d) decreases with increasing CMZ concentration, as shown by the DMPC data in Fig. 5. The decrease in the repeat distance for a lamellar liquid crystalline phase of DMPC/water 7:3 w/w when incorporating 15% CMZ is 8 and 7 Å at 30 and 50°C, respectively. The relatively large d-spacings obtained at 10 and 20°C for DMPC with CMZ concentrations below 5%, could be explained by the fact that the system is then in a gel state as revealed by the wide-angle diffraction patterns.

For the DPPC systems (Table 2), qualitatively similar changes in lamellar d-spacings were found. An amount of 15% CMZ decreases the lamellar repeat distance from about 57 to 48 Å when measured at 50°C, a temperature almost 10°C above the gel-to-liquid crystalline phase transition for DPPC (Small, 1986). Furthermore, large amounts of CMZ (25% w/w) induced a phase separation as revealed by visual inspection behind crossed polarizers.

In order to estimate the preferred location of CMZ in the lamellar liquid crystals we considered a simple model where CMZ is either treated as water or as phospholipid. If we assume that the phospholipid bilayer thickness is unaltered upon addition of CMZ, and that the phospholipid/water volume ratio is constant, the following expression, relating the measured repeat distance to the fraction of CMZ behaving as phospholipid, may be derived

$$d = d_0 \frac{1}{1-(1-(\alpha/\phi^0_{PC}))\phi_{CMZ}} \quad (2)$$

In Eq. (2), d and d_0 are the measured repeat distances with and without CMZ, respectively, ϕ^0_{PC} and ϕ_{CMZ} are the volume fractions of phospholipid (no drug added) and drug, respectively, and α the fraction of drug behaving as phospholipid ($1-\alpha$ is thus the fraction of CMZ behaving as water). It is evident from Eq. (2) that the repeat distance increases with CMZ concentration if $\alpha = 0$, i.e. when the drug behaves like water. A decrease of d is seen if $\alpha = 1$ since this has the same effect as increasing the phospholipid fraction.

In Fig. 8 the effect on d according to Eq. (2) is given for $\alpha = 0$ and $\alpha = 1$ respectively, together

Table 3
Gel-to-liquid crystalline phase transition temperature for DMPC and DPPC 30% water lamellar phases with different amounts of CMZ as obtained by wide-angle X-ray diffraction and DSC measurements

CMZ (% w/w)	DMPC			DPPC		
	X-ray	DSC		X-ray	DSC	
	T_c (°C)	T_{onset} (°C)	T_{peak} (°C)	T_c (°C)	T_{onset} (°C)	T_{peak} (°C)
0	20–25	24	26	40–45	40	42
2.5	20–25	14	20	40–45	32	39
5	15–20	11	18	35–40	31	33
10	10–15	11	15	30–35	29	32
15	10–15	11	15	30–35	29	31

with experimentally determined d-values for DMPC at 30°C. It is clearly seen from Fig. 8 that d decreases more than Eq. (2) allows for. Due to the small molecular volume of CMZ compared to that of the phospholipids it is reasonable to assume that the preferred location of CMZ is in the bilayer close to the interface. Further support for this interpretation is given below. The 'voids' created closer to the center of the bilayer are thus filled with the hydrocarbon chains of the phospholipids, which in turn leads to a decrease in bilayer thickness (which was assumed to be constant in Eq. (2)).

This behaviour is analogous to that assumed to describe how n-alkanols (with fewer than 10 carbons) interact with phospholipid lamellar phases (Lohner, 1991). It is known that small amphiphilic organic molecules, e.g. n-alkanols, are anchored at the lipid/water interface. As the length of these molecules is shorter than the length of the phospholipid hydrocarbon chains, their presence at the interface will result in the forming of voids within the lipid bilayer (Lohner, 1991). It is reasonable to assume that these voids will be filled by the hydrocarbon chains of the phospholipids which in turn will lead to a decrease in lamellar repeat distance.

CMZ was also added to the bicontinuous cubic phase of monoolein in 30% w/w water. The structure of this phase is that of a monoolein bilayer extending in three dimensions, separating two water channel systems (Hyde et al., 1984). The center of the bilayer forms a so-called infinite periodic minimal surface (IPMS). The phase has been extensively studied as a potential drug delivery system (e.g., Engström et al., 1995). If, for example, lidocaine base is added to the cubic phase, it transforms to a reversed hexagonal phase above approx. 3% w/w lidocaine base. If, on the other hand, the same amount of lidocaine HCl is added, a lamellar liquid crystalline phase is formed (Engström and Engström, 1992). Thus, the cubic structure is relatively sensitive to foreign molecules.

It was therefore surprising that the monoolein/water (7:3 w/w) cubic phase survived up to 12% w/w CMZ at room temperature ($\approx$ 20°C). The presence of a homogenous cubic phase was verified by both visual inspection behind crossed polarizers as well as X-ray diffraction measurements. This behaviour is similar to the effect on the cubic phase caused by the addition of dioleoylphosphatidylcholine, which can replace monoolein to a large extent in the cubic structure (Gutman et al., 1984). Also, a balanced mixture of lidocaine base and lidocaine HCl forms a cubic phase with monoolein and water due to a cancellation of the structural effects mentioned above (Engström and Engström, 1992).

In summary, large amounts of CMZ can be incorporated into the liquid crystals formed by the DMPC (DPPC)/water system and the monoolein/water system, without causing any phase transitions. The repeat distance of the lamellar phase is altered in such a way as to reveal the location of CMZ in the lipid bilayer close to

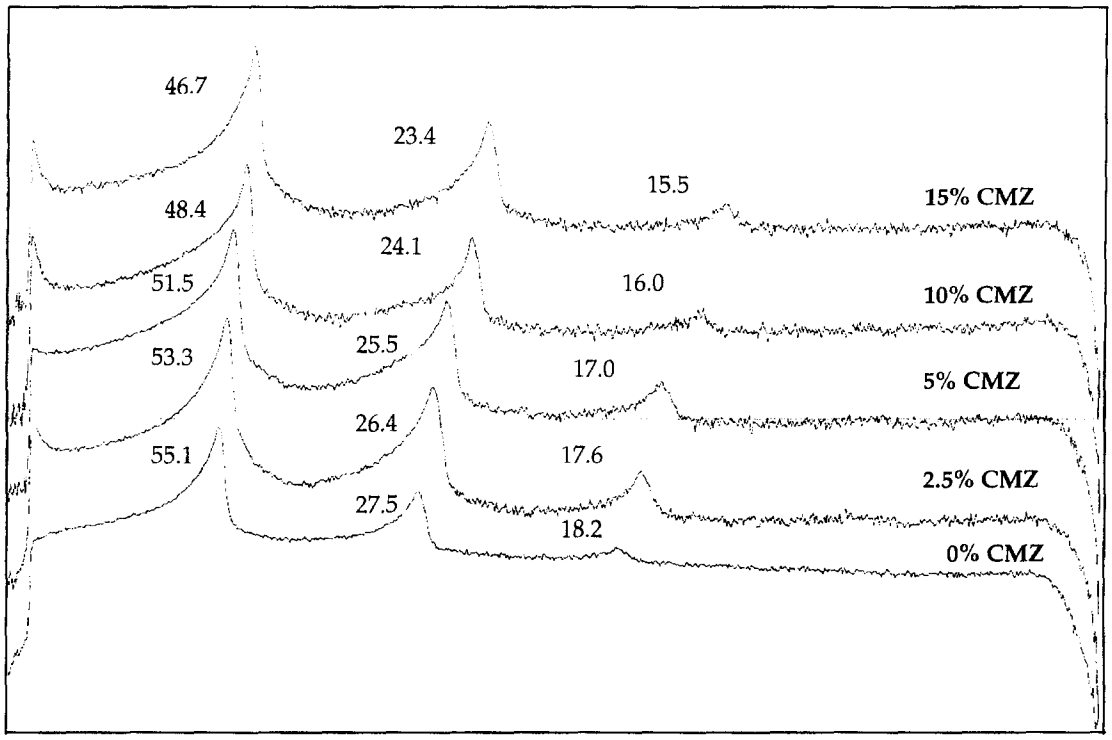

Fig. 5. Small-angle X-ray diffraction patterns for lamellar phases of DMPC/water 7:3 (w/w) with different CMZ concentrations % (w/w). The measurements were recorded at 30°C. The figures associated with each peak represent the *d*-spacing in Å.

the water/lipid interface. The effect on the unit cell dimensions of the cubic phase is currently under investigation.

So far we have focused the discussion on the interaction of CMZ with DMPC and DPPC at temperatures above the phospholipid chain melting temperature. Below this temperature, the phase behaviour becomes more complex. In some samples containing CMZ, small-angle X-ray diffraction measurements also reveal peaks from a second lamellar repeat distance (Tables 1 and 2). This observation is most obvious for DPPC. For instance, in DPPC samples containing 15% CMZ at 10°C, the pattern from a second lamellar repeat distance at 45 Å was detected (see Fig. 6). This repeat distance, only occurring in the gel state, is in the same range as that observed for lamellar liquid crystals rich in CMZ. Thus it seems plausible that CMZ promotes the formation of two coexisting lamellar phases, where one is similar to the gel state found without CMZ (long repeat distance) and the other a lamellar liquid crystal enriched in CMZ (short repeat distance). The decrease in repeat distance, upon addition of CMZ, shown in Tables 1 and 2 for the lower temperatures, is therefore probably a consequence of a decrease in water content of the gel state and an increased CMZ/DPPC ratio (leading to bilayer compression) in the liquid crystal. Another interpretation of this complex behaviour could be that both phases are in their gel state, which is perhaps more consistent with the DSC results (see below). Nevertheless, the observation of two coexisting lamellar repeat distances calls for further investigation, which is in progress.

Finally, the wide-angle X-ray diffraction data show that CMZ decreases the phospholipid gel-to-liquid crystalline phase transition temperature

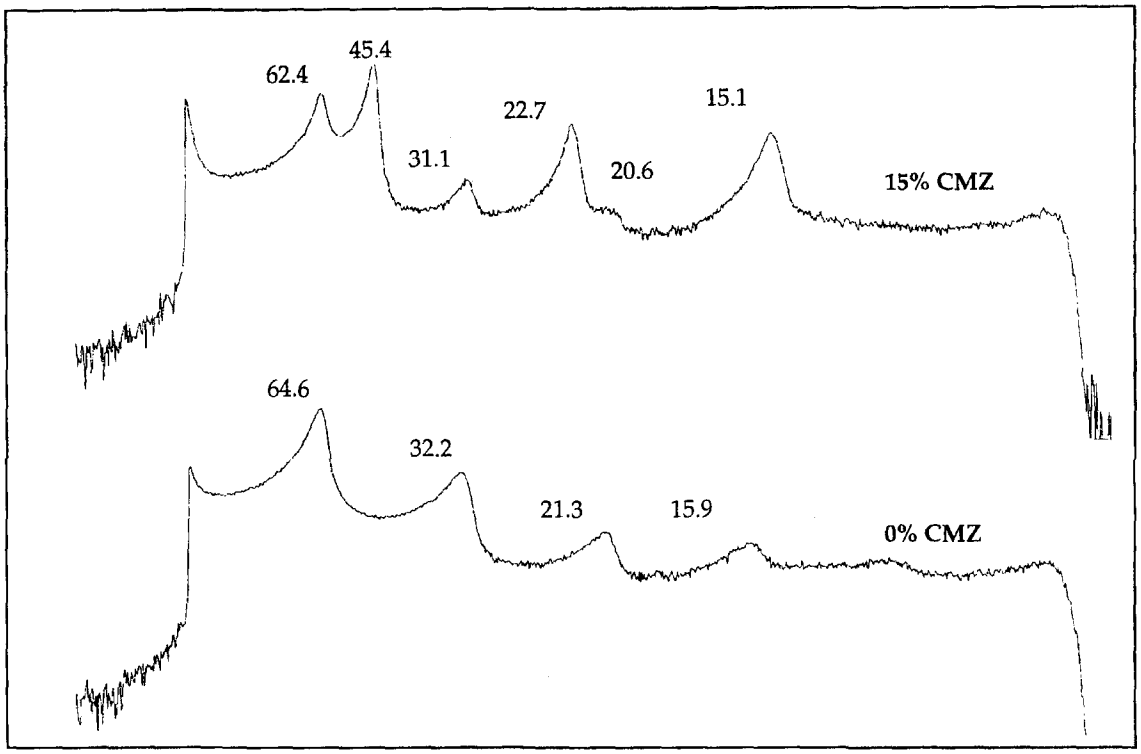

Fig. 6. Small-angle X-ray diffraction patterns for lamellar phases of DPPC/water 7:3 (w/w) with and without 15% (w/w) CMZ recorded at 10°C.

(Fig. 7 and Table 3). For DMPC the phase transition temperature is 23°C (Small, 1986) which agrees well with the X-ray results where T_c is determined to be 20–25°C. However, at a CMZ concentration of 10% the transition takes place already at 10–15°C. The same trend is observed for DPPC, for which T_c according to the X-ray experiments is lowered from 40–45 to 30–35°C by adding 10% CMZ to the lamellar phase (Table 3).

3.4. DSC investigations

Some DSC measurements were performed in order to determine the gel-to-liquid crystalline phase transition temperature. From the measurements on DMPC lamellar phases it is seen that CMZ depresses the gel-to-liquid crystalline phase transition, e.g. 5% of CMZ alters the T_c from 24 to 11°C. The same behaviour is also observed for lamellar phases made of DPPC, where T_c is lowered from 40 to about 31°C when 5% CMZ is present.

In thermograms of both DMPC and DPPC, free of CMZ, a small transition at 14 and 33°C, respectively, is observed. This transition, the so-called pretransition, most probably reflects the transition from a $L_{\beta'}$ phase to a $P_{\beta'}$ phase (Akiyama et al., 1982; Ruocco and Shipley, 1982). However, when CMZ is present the transition disappears. This behaviour is similar to that reported for cholesterol, where it is known that 5% (w/w) abolishes the pretransition of DPPC entirely (McMullen and McElhaney, 1995). It is also noteworthy that the addition of CMZ induces a change in the shape of the endothermic peak. As the CMZ concentration was increased the peak not only shifted to lower temperatures but also a significant broadening was observed. It seems as if the peak is the sum of at least two curves.

Nevertheless, the broadening of the endotherm is also consistent with results reported for the

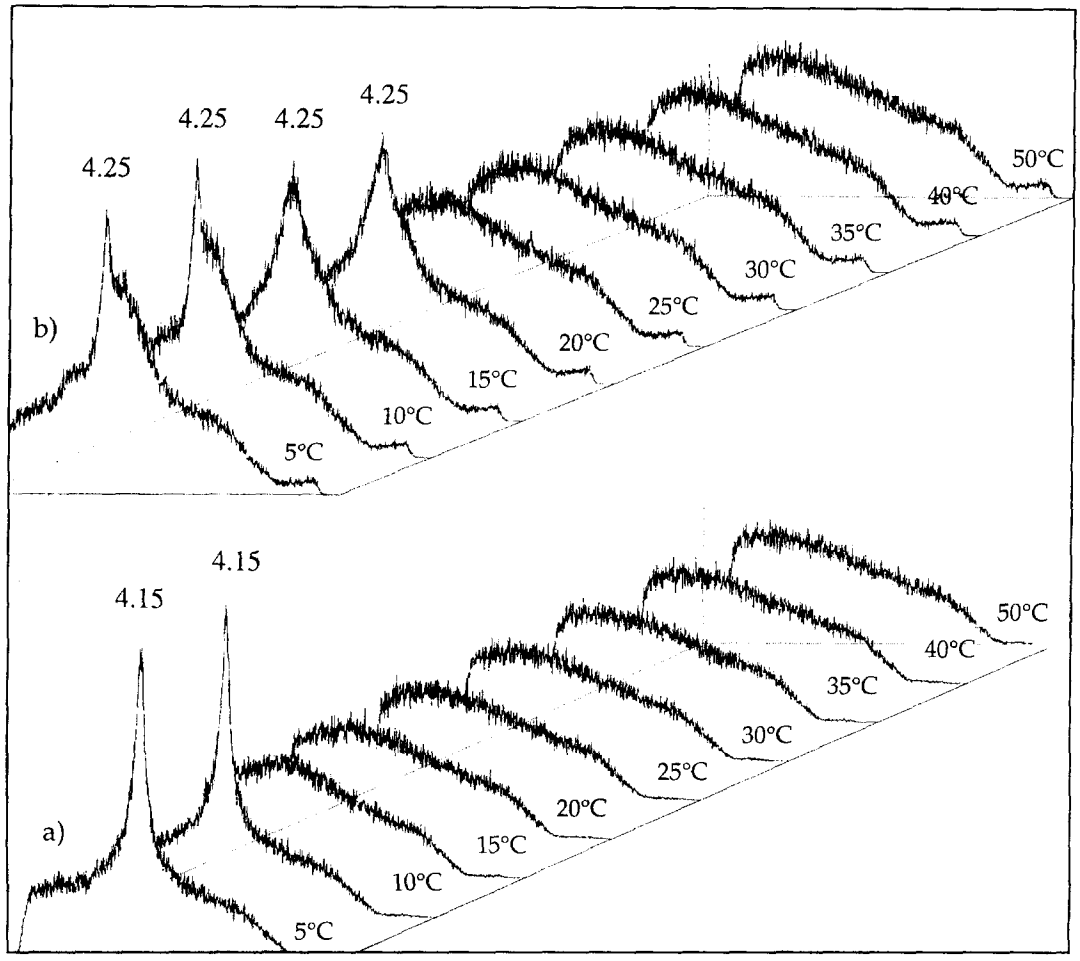

Fig. 7. Wide-angle X-ray diffraction patterns for lamellar phases of DMPC/water 7:3 (w/w) with (a) and without (b) 10% CMZ. The figures associated with each peak represent the *d*-spacing in Å. The detector range is 4.9–3.4 Å and the temperature scans were taken in the interval 5–50°C.

interaction of cholesterol with DPPC. In this case the asymmetry in the endothermic peak is thought to reflect the presence of a cholesterol-poor domain and a domain rich in cholesterol (McMullen and McElhaney, 1995). A broadening of the endothermic peak is also reported for DPPC model membranes containing capsaicin, where at high capsaicin concentrations two endothermic peaks were observed. A shift of the main transition to lower temperatures when the capsaicin concentration was increased, as well as the disappearance of the pretransition at mole fractions higher than 0.03 capsaicin, was also reported (Aranda et al., 1995). These findings were explained by the presence of two different capsaicin–DPPC domains, one richer in capsaicin than the other. Furthermore, these two domains were thought to be present in both the gel and the liquid crystalline state.

4. Conclusions

This work has shown that CMZ is surface active, and that it interacts with both mono- and bilayers of phospholipids. Drop-volume measurements showed that the surface tension of an aqueous solution of CMZ close to the solubility

limit is about 55 mN/m, with an estimated area per molecule at the aqueous/air surface of about 50 Å^2. The surface balance experiments revealed a complex situation when spreading DMPC and DPPC at the air/aqueous interface. The isotherms indicate an accumulation of CMZ at the interface which leads to a less well-defined monolayer at higher CMZ concentrations.

The accumulation of CMZ at the interface was further supported by X-ray investigations of liquid crystalline phases of DMPC and DPPC in water. It was found that the lamellar repeat distance decreased with increasing amount of CMZ. The large decrease of the repeat distance was interpreted as an effect of the CMZ molecules residing in the lipid bilayer close to the interface. The hydrocarbon chains of the phospholipids fill the voids behind the small CMZ molecules, which in turn leads to a decrease in the bilayer thickness. This interpretation was also supported by the fact that large amounts (> 10% w/w) of CMZ could be incorporated into the bicontinuous cubic phase of monoolein and water. CMZ was also shown to decrease the chain melting temperatures of DMPC and DPPC as revealed by both X-ray and DSC. X-ray diffraction patterns recorded below the chain melting temperatures of DMPC and DPPC revealed a complex situation with two or more coexisting structures, which are under investigation.

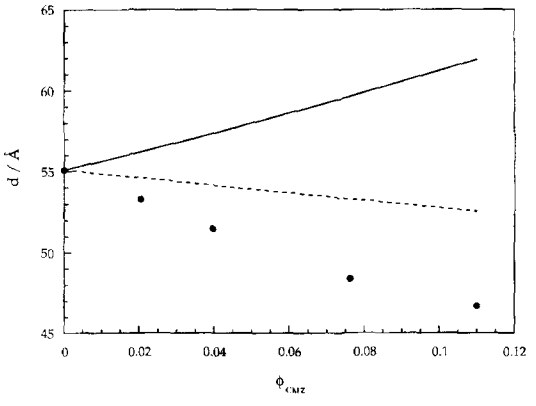

Fig. 8. *d*-Spacings obtained for the system DMPC/water 7/3 (w/w) at 30°C as a function of CMZ content (filled symbols). The solid and dashed lines are given by Eq. (2) for $\alpha = 0$ and 1, respectively. The volume fractions ϕ were determined from weight fractions assuming densities of 1.24, 1.03 and 1.00 g/ml for CMZ, DMPC and water, respectively.

The surface and interfacial properties studied for CMZ in the different systems used in this work, show that CMZ interacts with mono- and bilayers in a complicated way, which is not predictable from the octanol/water (or a similar) partition coefficient. Information of the kind obtained here is therefore of vital importance for the understanding of a drug delivery system involving lipids. Furthermore, since phospholipids are important constituents of cell membranes, the results should have some relevance for an understanding of the interaction between the drug/drug formulation and the biological system.

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