X-ray studies on polymorphism of lamellar lecithin/local anaesthetic/water mixtures

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Three mixtures with different compositions of 1,2-dipalmitoyl lecithin and the local anaesthetic heptacaine in dispersions with excess water, the so-called mixtures 36/1, 5/1 and 1/1, have been studied by X-ray diffraction techniques in the small and wide angle region as function of temperature. The polymorphism of mixture 36/1 and mixture 5/1 showed similarities. With increasing temperature the phase sequence $L_\beta \rightarrow L_{\beta_0} \rightarrow L_{\alpha_0}$ was observed. In all phases the hydrocarbon chains of the bilayer are arranged in an orthogonal lattice. The low temperature gel phase L_β is characterized by herringbone chain packing. In the gel phase, the distances between the lamellae in the mixture 5/1 are increased by Δd_L of 1·4 nm against mixture 36/1 and in the liquid-crystalline phase L_{α_0} by Δd_L of 0·5 nm. In the L_β phase alterations in the distances between the lamellae are observed during the hysteresis of the phase transition ice $\rightleftharpoons$ water. For the mixture 1/1 chain staggering was found in the low temperature phase, the gel phase and the liquid-crystalline phase.

1. Introduction

The preceding paper [1] dealt with results from calorimetric measurements on the influence of two local anaesthetics of the heptacaine type on the phase and miscibility behaviour of the ternary and quaternary dipalmitoyl lecithin/water systems. The thermodynamic analysis of the phase diagram in the ternary lecithin/anaesthetic/water mixtures indicates that the two local anaesthetics differ qualitatively in their miscibility properties in aqueous lecithin dispersions. These calorimetric investigations are now supplemented by X-ray diffraction measurements on three selected mixtures of heptacaine.

The X-ray diffraction investigations were carried out in aqueous dispersions with different mixtures of the following composition: mixture 36/1, this means 36 molecules of dipalmitoyl lecithin per molecule of heptacaine; mixture 5/1, has 5 molecules of dipalmitoyl lecithin per molecule of heptacaine and mixture 1/1, has 1 molecule dipalmitoyl lecithin per molecule of heptacaine ($x = 0.50$) and 50 wt% water in excess (see the phase diagram in [1]).

2. Methods

The X-ray diffraction measurements were carried out with a horizontal X-ray goniometer type HZG 4 (VEB Freiberger Präzisionsmechanik, G.D.R.) using the transmission technique and Ni-filtered CuK_α radiation ($\lambda = 1.54$ Å). The diffraction patterns were recorded within an angle range of $1.5° = 2\theta = 90°$ by means of a slit arrangement proposed by Schulze and Henke [2]. In this manner the scattering caused

by the slits was eliminated almost completely and both, small- and wide-angle reflections could be measured. The samples were contained in thin walled Mark tubes. The quantity of the samples used in our X-ray measurements were about 30–40 mg. The sample temperature controlled by the home made heating/cooling equipment [16] was better than ± 2 K in the temperature region from 250 K to 350 K. A proportional counter and auxiliary electronics (VEB Meßelektronik Dresden) recorded the diffraction patterns directly; Ni-filtered CuK_α-radiation was taken from a TUR M62 generator (VEB TUR Dresden).

Using the Bragg equation

$$n\lambda = 2d \sin \theta; \quad \frac{2 \sin \theta}{\lambda} = S\,nm^{-1}$$

the reciprocal spacings S were determined from the angle θ of the reflection maxima. From the structure-dependent positions of the reflections 2θ, information on the superstructure, chain packing, molecular lattice, and orientation of the molecules in the bilayers is obtained, as described in [3, 4]. Structural information about the phase type was derived from the low and wide angle diffraction patterns separately. The long spacings are in a ratio of $1/1 : 1/2 : 1/3 \ldots$ in lamellar phases. The structure parameters d_L, e.g. the repeat distance between the lamellae, were calculated with a least-squares method. The short spacings characterize the molecular lattice and/or the chain packing within a bilayer. The lattice constants were also calculated with least-squares methods [16].

3. Substances

The dipalmitoyl lecithin, 1,2-dipalmitoyl-DL-phosphatidylcholine, (di-(C16 : 0)-PC) Fluka AG (Switzerland) was used without further treatment. The TLC tests showed the absence of any impurities. The local anaesthetic heptacaine (HK), was the hydrochloride of 2-piperidino ethyl ester of the basic 2-heptyl-oxycarbanile acid with the chemical formula:
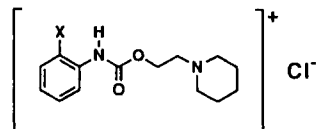
$X = O-C_7H_5$: hydrochloride of 2-piperidino ethyl ester of the basic 2-heptyloxy-carbanile acid, 'heptacaine', (HK). Lecithin and heptacaine were dissolved in a chloroform/methanol mixture (85 : 15 $=$ v : v) to prepare the sample. The dissolved mixture was evaporated under vacuum ($\sim 1 \cdot 33 \times 10^{-2}$ Pa) at 350 K for 2 hours. To the dried samples 50 wt % distilled water was added by means of a syringe. Homogenization was carried out by ultrasonics. Then the homogenized samples were put in Mark tubes (diameter $1 \cdot 5$ mm) which were closed by sealing and annealed for 2 hours above the main transition temperature of the lecithin/water mixtures (see [1]).

4. Results and discussion

4.1. *Polymorphism of the ternary system di-(C16 : 0)-PC/HK/H₂O: mixture 36/1*
4.1.1. *Analysis of diffraction patterns at various temperatures*

For our X-ray studies we used the mixture di-(C16 : 0)-PC/HK/H₂O with composition n_r of 36/1 with 50 wt % of water. Our measurements were carried out in the temperature range between 259 K and 332 K.
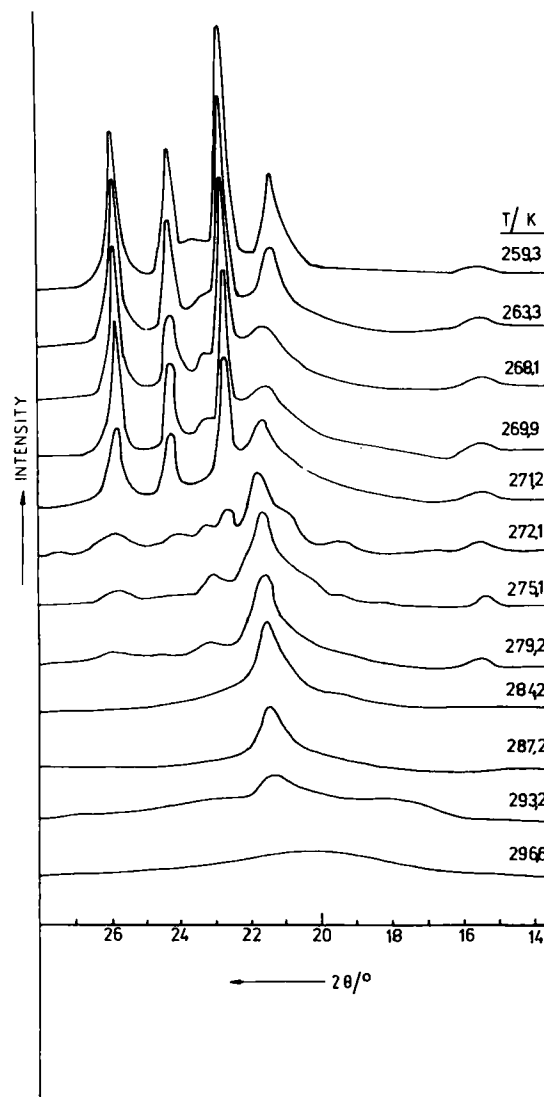
Figure 1. Typical small and wide angle powder diffraction patterns of the ternary system dipalmitoyl lecithin/heptacaine/water, mixture 36/1, at various temperatures.

Figure 1 shows the results of the X-ray diffraction patterns in wide angle region. The temperature decreases from the upper curves to the lower ones. At lower temperatures, five sharp reflections, characteristic for the hexagonal ice modification, are observed. The other two reflections in this angle range originate from chain packing within the bilayer. Above 273 K, melting ice → water causes the ice reflections to disappear. The wide angle peaks, distinctly separated at this temperature, are transformed into a single reflection of great intensity, when the transition into the gel phase takes place. At about 312 K chain melting was observed, indicated by the appearance of a broad peak in the wide angle region. Consequently with increasing temperature the following polymorphism is observed: $L_\rho \rightarrow L_{\beta_0} \rightarrow L_{\alpha_0}$ (for details see §4.1.6).

In the low angle region, the phase transitions occurring in the mixture effect a shift of the reflections towards greater angles. Further the intensities of the reflections are changed. From the ratios $S_1 : S_2 : S_3 = 1 : 2 : 3$ a lamellar superstructure in all phases

is observed. From the diffraction patterns in the small and wide angle, all reflection maxima are determined (compare the diffraction patterns in figure 1). Then the repeat distance of the lamellar phases and the area A, required by a single chain within the bilayer, were calculated [16].

4.1.2. *Analysis of the repeat distance of the lamellar at various temperatures*

In figure 2 the temperature dependence of the reciprocal spacings S of mixture 36/1 is shown. From these reciprocal spacings S two structural parameters, the average value of the distance between the lamellae d_L and the area A, required by a single chain within the bilayer, are determined [16]. The results of the calculations, the d_L/T plots, are shown in figure 3.
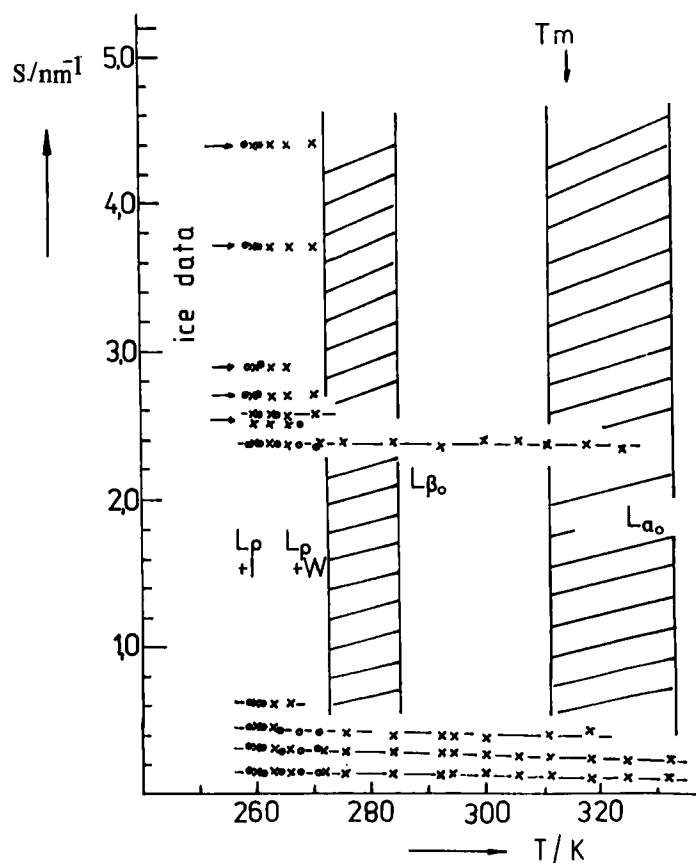
Figure 2. Reciprocal spacings S of the ternary system diplamitoyl lecithin/heptacaine/water, mixture 36/1, as function of temperature.

From this survey the following information results. There are phases transition regions where the structural parameters change step-wise. In addition the temperatures of the main transition T_m, obtained by calorimetry, are plotted in figure 3 and also data from the literature [7–11, 14] concerning the phases L_c, L_{β_i}, $L_{\beta'}$, $P_{\beta'}$, and L_α of lecithin/water mixtures. In the low temperature phase changes in the distances between the lamellae are clearly observed during hysteresis in the phase transition ice $\rightleftharpoons$ water. In the gel phase, the d_L values rise slowly. On passing into the
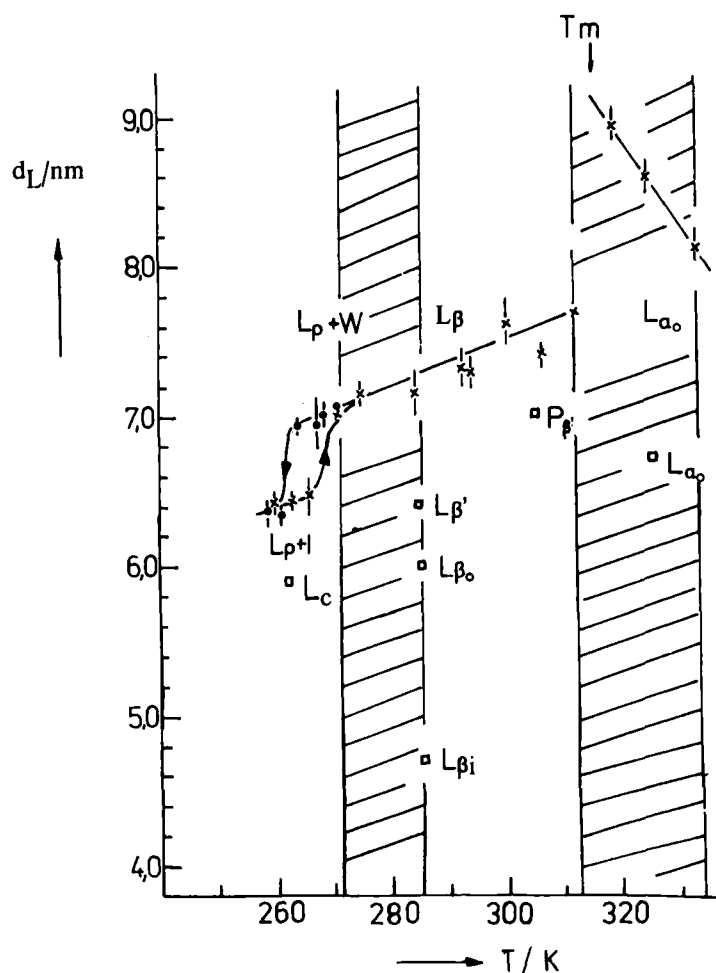
Figure 3.   Average values of the repeat distances of the lamellae d_L in the ternary system dipalmitoyl lecithin/heptacaine/water, mixture 36/1, at various temperatures. O, values from the literature [14]; ×, our values.

liquid-crystalline L_{α_0} phase, however, a change with a step-wise process where $\Delta d_L \sim$ 1·4 nm takes place. At the transitions $L_\alpha \rightarrow L_{\beta_0}$ and $L_{\beta_0} \rightarrow L_{\alpha_0}$ not only the distance between the lamellae changes but also the A values within the bilayer. In the table these parameters are summarized. In the low temperature phase the values of A are relatively small, but during chain melting the increase changes step-wise.

4.1.3. *Parameters for the L_p phase*

The X-ray diffraction pattern of the low temperature phase is characterized by two chain reflections; As for paraffins, they were indexed as an orthorhombic lattice and have the Miller indices (110) and (200). Based on this result, the parameters a and b for the chain packing in the molecule were calculated. The chains are arranged in an orthogonal lattice within the bilayer. The distance between the lamellae is for 6·36 nm at 258 K. Grünert *et al.* [5] discussed a value d_L of 5·8 nm for the low temperature phase L_c of the system dipalmitoyl lecithin/water. Since both d_L values are of the same

Structural parameters for the ternary system dipalmitoyl-lecithin/heptacaine/water with different compositions as a function of temperature.

Composition n_r	Phase	T/K	d_L/nm	a/nm	b/nm	A/nm^2
Mixture 36/1	L_p (heating)	259·9	6·432	0·839	0·448	0·188
		263·2	6·451	0·840	0·448	0·188
		266·2	6·472	0·842	0·453	0·190
		271·2	7·012	0·834	0·482	0·201
		275·2	7·146	0·830	0·479	0·199
	L_p (cooling)	271·2	7·071	0·836	0·483	0·202
		268·7	7·089	0·846	0·442	0·187
		267·7	6·956	0·844	0·441	0·186
		264·2	6·939	0·842	0·444	0·187
		261·2	6·345	0·839	0·449	0·188
Mixture 36/1	L_{β_0} (heating)	284·2	7·169	0·828	0·478	0·198
		292·5	7·315	0·851	0·491	0·209
		293·7	7·274	0·830	0·479	0·199
		300·7	7·623	0·835	0·482	0·201
		306·0	7·403	0·836	0·483	0·202
		311·9	7·701	0·856	0·494	0·207
Mixture 36/1	L_{α_0} (heating)	318·5	8·946	0·860	0·497	0·213
		324·2	8·550	0·890	0·514	0·228
		332·5	8·106	0·901	0·520	0·234
Mixture 5/1	L_p (heating)	262·1	6·387	0·838	0·445	0·187
		264·8	6·280	0·838	0·448	0·188
		267·9	6·238	0·830	0·479	0·199
		271·2	6·513	0·827	0·477	0·197
	L_p (cooling)	275·2	9·021	0·828	0·478	0·198
		271·2	9·552	0·825	0·476	0·196
		267·4	9·814	0·834	0·482	0·199
		266·4	6·314	0·834	0·482	0·201
		258·0	6·025	0·838	0·431	0·181
Mixture 5/1	L_{β_0} (heating)	286·2	7·886	0·808	0·466	0·188
		295·0	8·427	0·828	0·478	0·198
		297·5	8·834	0·830	0·479	0·199
		302·2	9·312	0·830	0·479	0·199
		306·5	9·816	0·842	0·486	0·205
Mixture 5/1	L_{α_0} (heating)	310·9	9·469	0·846	0·488	0·207
		316·0	9·382	0·875	0·505	0·221
		320·8	9·834	0·894	0·516	0·231
Mixture 1/1	L_{f_i} (heating)	263·3	4·430			
		266·1	4·536			
		267·8	4·574			
		269·9	4·820			
		271·2	7·463			
		272·7	8·202			
		274·4	8·035			
		277·3	7·902			
		279·2	7·682			
		281·2	7·302			
		282·2	7·575			

Table 1 (*continued*).

Composition n_r	Phase	T/K	d_L/nm	a/nm	b/nm	A/nm^2
	L_{f_i} (cooling)	271·2	6·720			
		266·8	6·889			
		263·1	6·801			
		259·3	4·382			
		257·2	4·242			
Mixture 1/1	L_{β_i} (heating)	284·2	6·544	0·825	0·476	0·196
		285·1	6·628	0.824	0·476	0·196
		287·2	6·240	0·827	0·477	0·197
		289·7	6·718	0·827	0·477	0·197
		290·2	6·587	0·825	0·477	0·197
		293·2	6·842	0·827	0·477	0·197
		294·1	7·267	0·879	0·507	0·223
		296·6	6·753	0·871	0·503	0·219
Mixture 1/1	$L_{\beta_i'}$ (heating)	280·1	6·165	0·823	0·475	0·195
		276·1	6·403	0·823	0·475	0·195
		271·9	6·666	0·823	0·475	0·195
		267·1	6·710	0·825	0·476	0·196
		263·1	6·801	0·830	0·442	0·183
Mixture 1/1	L_{α_i} (heating)	331·1	6·388	0·886	0·511	0·226
		324·4	6·588	0·888	0·513	0·228
		320·3	6·737			
		317·1	6·563	0·888	0·513	0·228
		306·2	6·588	0·884	0·511	0·226
		303·7	7·025	0·874	0·504	0·220
		302·5	6·593	0·888	0·513	0·227
		299·6	6·737	0·866	0·500	0·217

order of magnitude, we may conclude that in the bilayer the phospholipids are oriented opposite to each other.

On adding heptacaine, the distance between the lamellae is enlarged, due to the positive charge of the heptacaine. Two phase systems, L_ρ + ice and L_ρ + water, must be distinguished. Their interlamellae distances differ only by about 0·6 mm. But their A value of 0·188 nm² is independent of the state of the water and is typical of crystalline chain packing [6]. In the table the structure parameters of the mixture 36/1 in the L_ρ phase are summarized.

4.1.4. *Parameters for the L_{β_0} phase*

In the temperature region between 280 K and 315 K, we have observed the gel phase, L_{β_0}. The distance between the lamellae increases slightly from 7·28 nm to 7·68 nm (see figure 3). In contrast to data from the literature, the d_L values of this system are greater than those of the dipalmitoyl lecithin/water system without heptacaine [7, 8]; the difference is about 1·0 nm.

At $S = (0·42\,nm)^{-1}$ a sharp reflection appears which is typical of the wide angle region. According to [9] this reflection suggests a hexagonal arrangement of the chains which are packed in both an orthogonal and a hexagonal manner. The increase in distances between the lamellae is accompanied with an increase of the A values from

0·198 nm^2 to 0·207 nm^2 (compare the table). The structure parameters for the L_{β_0} phase are summarized in the table.

4.1.5. *Parameters for the L_{α_0} phase*

The L_{α_0} phase appears at temperatures above 312 K. Its diffraction pattern is characterized by a diffuse peak at $S = (0·44\,\text{nm})^{-1}$. As reported by Tardieu *et al.* [9], the hydrocarbon chains of lecithins have a fluid-like conformation. On raising the temperature within the melting phase from 318 K to 332·5 K, the distance between the lamellae decreases remarkably from 8·9 nm down to 8·1 nm. All of the values are distinctly increased in comparison with those given in the literature for the L_{α_0} phase of the dipalmitoyl lecithin/water system without added HK ($d_L = 6·7$ nm [8] and $d_L = 6·0$ nm [10]). The value A of the area per chain is also increased by 0·03 nm^2, when the amount of A in the gel phase is taken as reference. The table presents the structural parameters for the L_{α_0} phase.

4.1.6. *Oversimplified model for the polymorphism in mixture 36/1*

Figure 4 surveys the polymorphism in the mixture of dipalmitoyl lecithin/heptacaine/water on the basis of an oversimplified model. For each model the chain arrangements within the bilayer are illustrated. We selected a typical value from the temperature dependent distances between the lamellae in each phase. For the low temperature gel phase, we chose a value from the centre of the phase region; for the melting phase we use the value from 5 K above the starting temperature before the phase transition. The
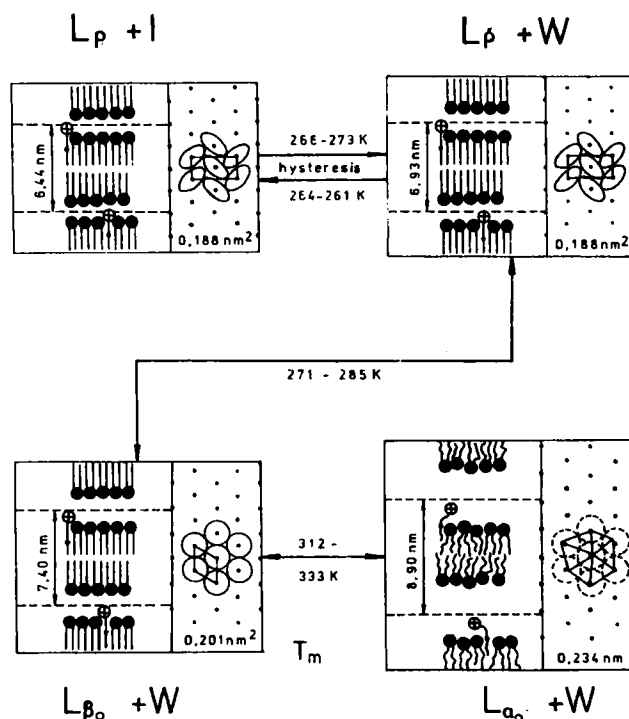

Figure 4. Oversimplified model for polymorphism in mixture 36/1 of the ternary system dipalmitoyl lecithin/heptacaine/water and schematic representation of the molecular packing in the bilayer.

orientation of molecules in the bilayer, resulting from these characteristic distances between the lamellae, is also shown in figure 4.

For each phase these structure models yield information on the chain packing with regard to the lattice arrangement and the A values characterizing the type of chain packing. Transition regions between the phases, polymorphism and the phase sequence are also shown. In accord with figure 4, the polymorphism in mixture 36/1 can be described in the following way.

The lamellar low temperature phase is represented by a herringbone chain packing in an orthorhombic lattice, whose chains are arranged perpendicular to each other. This results from an interpretation of the distances between the lamellae. Within error limits, their numerical values ($d_L = 6.4$ nm for the system L_p + ice and $d_L = 6.9$ nm for the system L_p + water) are of the same order of magnitude as those given by Stümpel [11] with $d_L = 5.8$ nm and by Tardieu *et al.* [9] with $d_L = 6.0$ nm. Simultaneously with the hysteresis in the ice $\rightleftharpoons$ water transition, the distance between the lamellae is changed slightly by about $\Delta d_L = 0.5$ nm. The amount of the area A, required by a lecithin chain, however, remains constant.

On passing into the gel phase L_{β_0}, the herringbone packing of the chains is transformed into a hexagonal chain lattice. In comparison with the d_L values in dipalmitoyl lecithin/water mixtures without added heptacaine, the distance between the lamellae in heptacaine/dipalmitoyl lecithin/water mixtures is increased by 3.0 nm as described in [9] or 1.0 nm as described in [12]. The area A per chain is increased by up to 0.013 nm^2.

On increasing the temperature melting occurs and the distance between the lamellae is increased by 1.5 nm. That means an increase of the distances between the lamellae by 2.1 nm or 2.8 nm, when referred to $d_L = 6.8$ nm, described in [8] and $d_L = 6.1$, described in [7] for dipalmitoyl lecithin/water mixtures without added heptacaine. The hexagonal lattice of fluid chains effects an increase in the A value by about 0.033 nm^2.

4.2. *Polymorphism of the ternary system di-$(C16:0)$-PC/HK-H_2O: mixture 5/1*

4.2.1. *Analysis of the diffraction patterns at various temperatures*

Diffraction measurements were performed on this mixed system within a temperature range from 258 K to 338.2 K, figure 5 shows selected curves in the wide angle range between 14.0° and 28°.

Comparing the diffraction patterns of figure 5 and figure 1, we observe pronounced similarities of X-ray diffraction between these two mixtures, which may originate in similar structures. The following information results from X-ray scattering in mixture 5/1 at various temperatures. The sharp reflections at the wide angle range of $2\theta < 21°$ indicate the existence of the hexagonal ice modification up to 271 K [13]. In addition two reflections are observed which indicate chain packing. At higher temperatures only one sharp reflection is found within the wide angle range; this is typical of the gel phase. Above 310 K, we observe a wide angle reflection with a broad and flat band, suggesting the existence of fluid chains.

The S values were determined from the diffraction patterns. Figure 6 gives a survey of the resulting S values at various temperatures. Reflections caused by the hexagonal ice modification are marked by arrows. The reciprocal spacings, derived from the small angle reflections, are related by $S_1 : S_2 : S_3 = 1 : 2 : 3$. This indicates bilayer formation in all phases.
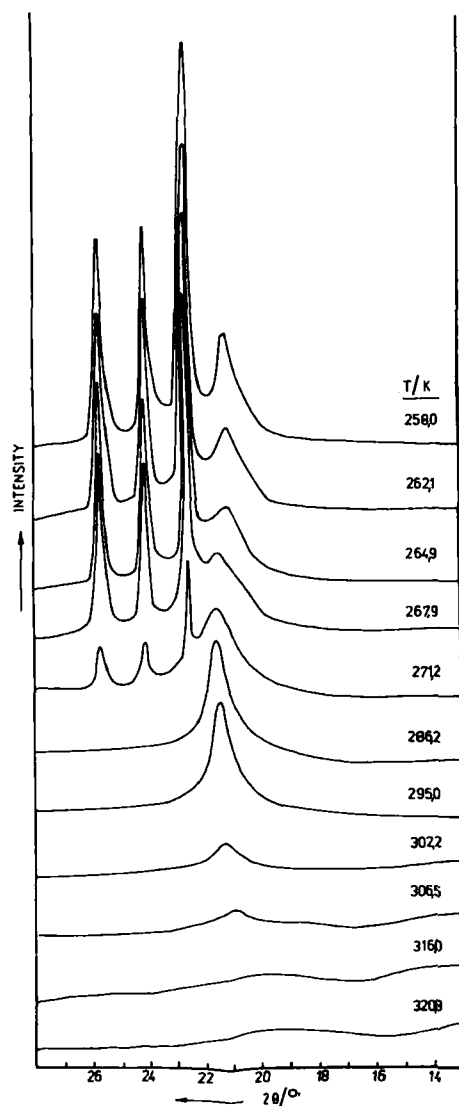
Figure 5. Typical small and wide angle powder diffraction patterns of the ternary system dipalmitoyl lecithin/heptacaine/water, mixture 5/1, at various temperatures.

The average distances between the lamellae d_L and the A values were calculated from the reciprocal spacings S. In figure 7 the d_L parameters are plotted as functions of temperature. From a comparison between X-ray diffraction of mixtures 5/1 and 36/1 we can generalized.

In the low temperature phase the difference between the distances d_L in the phase L_p + ice and those in the phase L_p + water is considerably greater. For mixture 5/1 we find $\Delta d_L = 3\cdot5$ nm, contrasting with $\Delta d_L = 0\cdot6$ nm for mixture 36/1. The portion of water, that is incorporated in the bilayer but can be frozen out below 273 K, is greater for mixture 5/1.

On passing the intermediate state toward the L_{β_0} phase, the area A per chain increases. The temperature dependence of the distance between the lamellae has its minimum with the L_{β_0} gel phase.
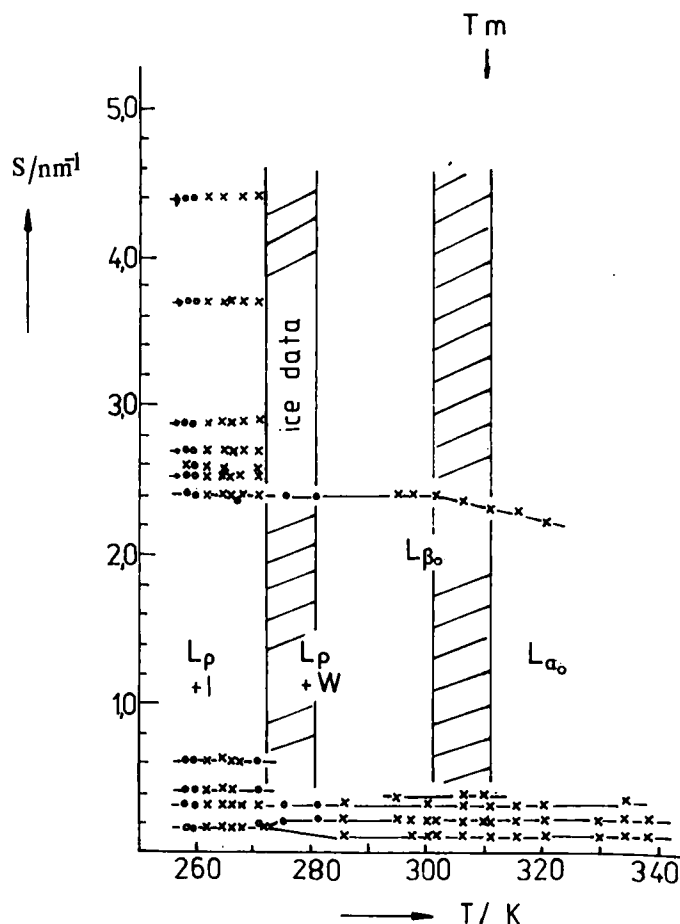
Figure 6.   Reciprocal spacings S of the ternary system dipalmitoyl lecithin/heptacaine/water, mixture 5/1, as a function of temperature.

In the melting phase L_{α_0} a remarkable decrease in the distances between the lamellae is observed on increasing temperature, also the results are seen for the X-ray investigations of mixture 36/1 (see §4.1.1).

In mixture 5/1 the distances between the lamellae are in all the phases L_ρ, L_{β_0}, and L_{α_0} considerably greater than in mixture 36/1.

4.2.2.   *Parameters for the L_ρ phase*

The low temperature phase L_ρ was studied at temperatures below 283 K. Alterations in the distances between the lamellae, caused by hysteresis during the phase transition ice $\rightleftarrows$ water, are more pronounced than for the mixture 36/1. In the wide angle region we observe two reflections at $S_1 = (0\cdot415\,\mathrm{nm})^{-1}$ and $S_2 = (0\cdot384\,\mathrm{nm})^{-1}$ which are referred to chains and indicate an orthorhombic chain packing. The area A, occupied by a chain, is about $0\cdot185\,\mathrm{nm}^2$. Structural parameters of the L_ρ phase obtained at various temperatures by heating or cooling are summarized in the table.

4.2.3.   *Parameters for the L_{β_0} phase*

The low temperature gel phase exists at temperatures between 275 K and 307 K. At 259 K, a sharp reflection at $S = (0\cdot417\,\mathrm{nm})^{-1}$, typical of hexagonal packing,
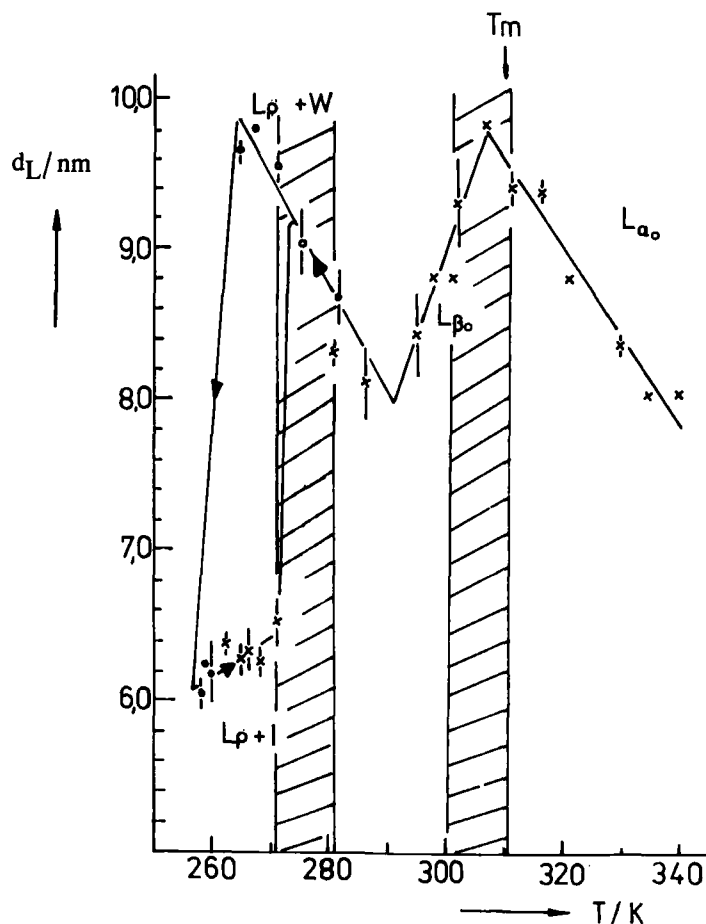
Figure 7. Average values of the repeat distances of the lamellae d_L in the ternary system dipalmitoyl lecithin/heptacaine/water, mixture 5/1, as a function of temperature.

appears in the wide angle region. The minimum distance between the lamellae is observed at 290 K (see figure 7). This minimum is interpreted as caused by the phase transition $L_\rho \to L_{\beta_0}$, a structural transformation recognized by a broadening of the chain reflection before this reflection begins to split. Because of difficulties in separating these two reflections the boundaries of this intermediate state cannot be determined exactly.

Typical of the L_{β_0} phase is a step increase of the distances between the lamellae ($\Delta d_L \sim 2\cdot 0$ nm). The A value is about $0\cdot 198$ nm^2. All structural parameters of the L_{β_0} phase are given in the table.

4.2.4. Parameters for the L_{α_0} phase

Between 306 K and 338 K the mixture is melting. Its wide angle scattering is characterized by a broad reflection at $S = (0\cdot 421$ nm$)^{-1}$; this is typical of fluid chains and the liquid-crystalline state. On raising the temperature from 306 K to 338 K, the distances between the lamellae decrease from $9\cdot 8$ nm down to $8\cdot 4$ nm. A similar change in these distances was also observed in the mixture 36/1. The area A, required

per chain, however, is enlarged from $0.207 \, nm^2$ to $0.231 \, nm^2$. The table lists the structural parameters for the L_{α_0} phase.

4.2.5. *Oversimplified model for polymorphism in the mixture 5/1*

Figure 8 illustrates the oversimplified structural model resulting from the structural parameters given in the table. Evidently, mixture 5/1 and mixture 36/1 are identical in their polymorphism. In all phases the chains are arranged orthogonally within the bilayer.
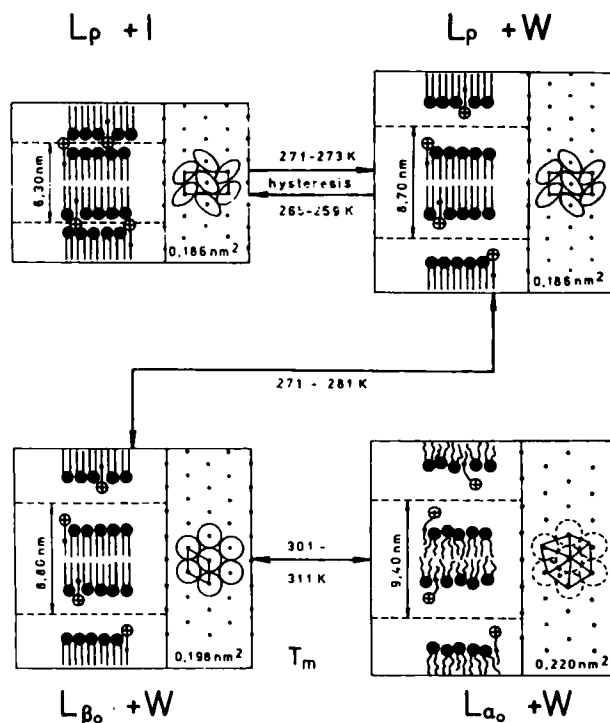

Figure 8. Oversimplified model for polymorphism in mixture 5/1 of the ternary system dipalmitoyl lecithin/heptacaine/water and schematic representation of the molecular packing in the bilayer.

The low temperature phase is again characterized by herringbone chain packing in an orthorhmobic lattice. But the distance between the lamellae in the phase L_ρ + water are greater than that of mixture 36/1 by $1.8 \, nm$.

In the mixture 5/1 the distance between the lamellae is distinctly increased ($\Delta d_L = 2.4 \, nm$) during hysteresis in the phase transition ice $\rightleftharpoons$ water. In mixture 36/1, however, the difference in the d_L values is only $0.5 \, nm$.

In the gel phase the distance between the lamellae d_L in mixture 5/1 is increased by $1.4 \, nm$ against mixture 36/1, and in the liquid-crystalline phase L_{α_0} by $0.5 \, nm$.

The greater distance between the lamellae in mixture 5/1 may be interpreted by a greater amount of water incorporated in the bilayer as discussed in [1] together with the incorporation of HK into the lamellae.

4.3. *Polymorphism of the ternary di-(C16 : 0)-PC/HK/H₂O; mixture 1/1*

4.3.1. *Analysis of diffraction patterns at various temperatures*

Our X-ray diffraction measurements were carried out in the temperature range from 257 K to 330 K. Figure 9 summarizes the results. As also observed for the mixtures 36/1 and 5/1, reflections due to a hexagonal ice modification are seen at low temperatures. However, in contrast to these mixtures, six reflections are observed; their appearance suggests a higher degree of order within the bilayer. These additional diffraction peaks are observed in a more distinct manner at temperatures above 273 K, when the reflections from ice have vanished. On entering the gel phase, only one reflection is observed, which is caused by a hexagonal lattice of rigid chains.
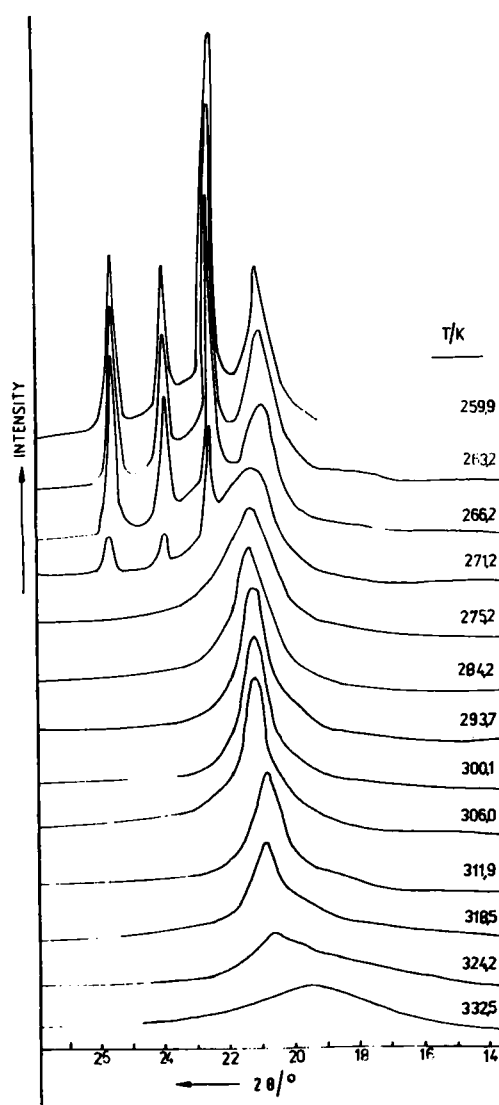

Figure 9. Typical small and wide angle powder diffraction patterns of the ternary system dipalmitoyl lecithin/heptacaine/water, mixture 1/1, at various temperatures.
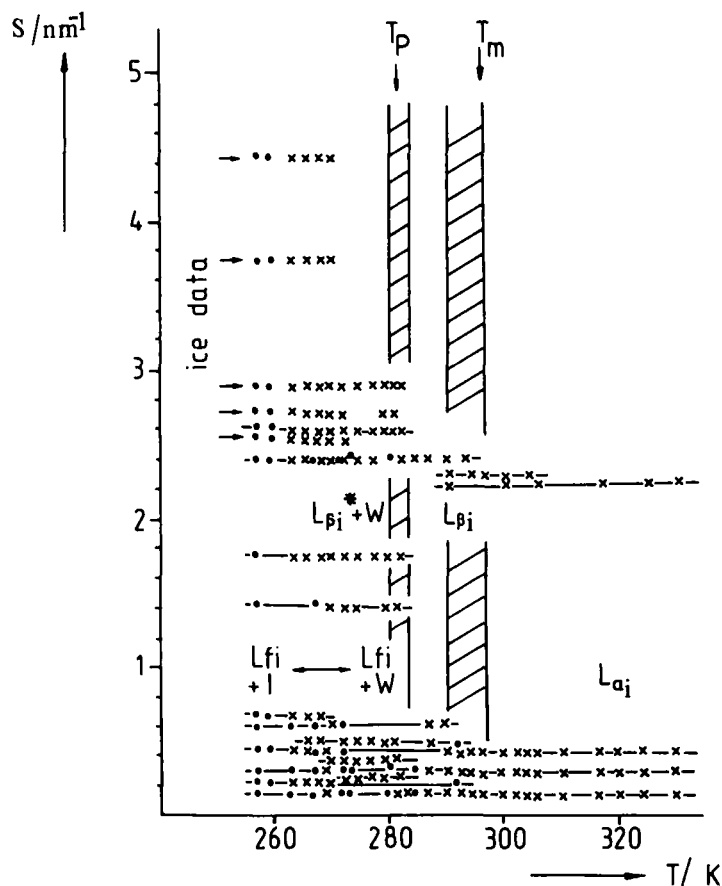
Figure 10.   Reciprocal spacings S of the ternary system dipalmitoyl lecithin/heptacaine/water, mixture 1/1, as a function of temperature.

At 295 K, a diffuse scattering peak appears, which indicates chain melting and the formation of the liquid-crystalline phase. The ratios $S_1:S_2:S_3 = 1:2:3$ of the reciprocal spacings, recorded in the small angle region, indicate a lamellar superstructure for all phases. In figure 10 the reciprocal interplanar spacings S are plotted against temperature. From the S values the distances between the lamellae d_L and the areas A per chain were calculated. Figure 11 and the table give graphical and numerical representations of the results. There are step-like changes of the d_L-values which originate in phase transitions. Obviously, the d_L values of mixture 1/1 are in all phases less than those of the mixtures 36/1 and 5/1. At 263·3 K, the distance between the lamellae 4·43 nm is in the accord with the values given in literature for the L_{β_i} phase of the system dipalmitoyl lecithin/water.

From the system 1,3-di-(C16:0)-PC/H_2O, Serrallach *et al.* [15] obtained $d_L = 4·7$ nm. For the system 1,2-di-(C16:0)-PC/C_2H_5OH/H_2O Simon and McIntosh [14] discussed a d_L value of 4·8 nm. In both cases the authors interpreted the bilayer structures in terms of chain staggering, this means that the chains in the bilayer are interdigitated. Since the distances between the lamellae differ only slightly, we may also apply this model. On increasing the temperature, mixture 1/1 shows the following sequence of phase transitions: $L_{f_i} \rightarrow L_{\beta_i} \rightarrow L_{\alpha_i}$ (for details see §4.3.5).
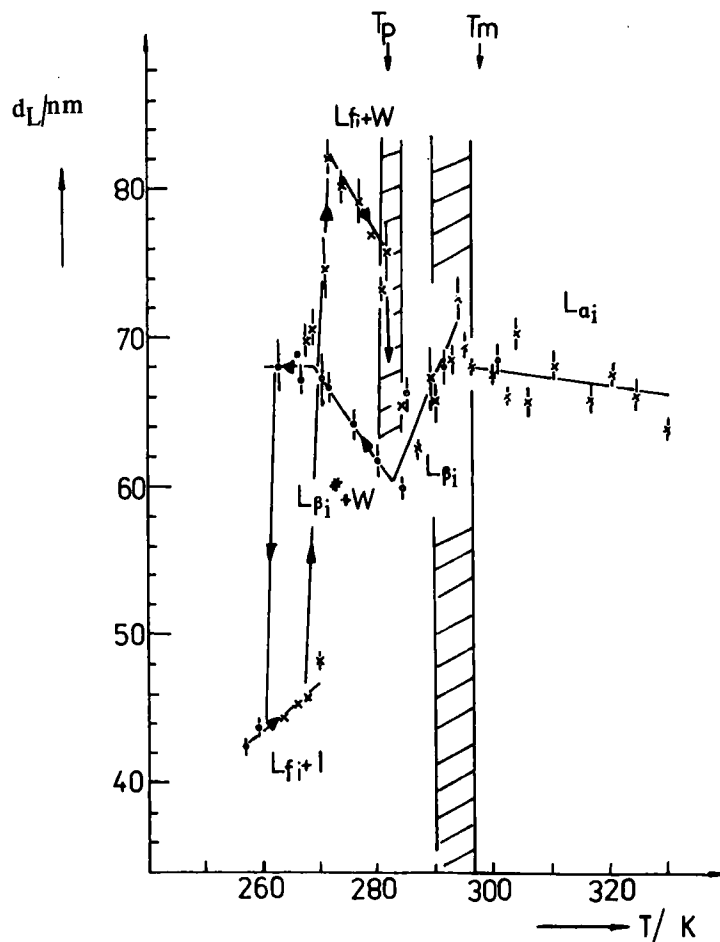
Figure 11.   Average values of the repeat distances of the lamellae d_L in the ternary system dipalmitoyl lecithin/heptacaine/water, mixture 1/1, at various temperatures. ×, values during heating; O, values during cooling.

4.3.2. *Parameters for the L_{f_i} phase*

Within the low temperature phase between 259 K and 283 K, the X-ray diffraction patterns of the mixture 1/1 differs from that in the mixtures 36/1 and 5/1, which was discussed already. In addition to reflections typical for chains, additional reflections are observed at 4°. In analogous cases [9, 11], such diffraction patterns have been indexed as a two dimensional molecular lattice within the bilayer. With regard to the molecular lattices (a, b, c, d, e), observed hitherto [16], we have denoted this structure with the symbol f and consequently called it the L_{f_i} phase. The number of reflections suggests the existence of an ordered phase, in which the lecithin molecules may be surrounded with a definite number of heptacaine molecules, forming a two dimensional lattice. These six reflections, obtained at 263 K, have positions nearly independent of temperature.

On passing from the phase systems L_{f_i} + water to L_{β_i} + water, a decrease in the distance between the lamellae ($\Delta d_L = 1\cdot04$ nm) is observed for the first time. During this structural transformation, the melting of the molecular lattice is typical of a sub-transition. If a molecular lattice exists, ice melting is accompanied with an

extreme alteration in the distance between the lamellae ($\Delta d_L \sim 4 \cdot 0$ nm). In the table the structural parameters of the L_{f_i} phase are summarized.

Analysis of these data gives the distances between the lamellae in the low temperature phase of the mixture 1/1 smaller than the corresponding values for mixtures 36/1 or 5/1 (L_{f_i} + ice; $d_L = 4 \cdot 4$ nm; L_{f_i} + water; $d_L = 7 \cdot 7$ nm).

4.3.3. *Parameters for the L_{β_i} phase*

The gel phase L_{β_i} exists within the temperature range between 293 K and 294 K. It is characterized by a considerable increase of the distance between the lamellae ($\Delta d_L = 0 \cdot 8$ nm) and the A value ($A \sim 0 \cdot 20$ nm^2, compare data in the table), which is typical for gel phases. On cooling from the L_{α} phase, the L_{β_i} phase could be supercooled to 262 K. This $L_{\beta'}$ phase represents a metastable state, extending over the whole temperature range of the L_{β_i} phase. At 262 K the distance between the lamellae has reached its minimum value of $6 \cdot 0$ nm. As for mixture 5/1, it increases remarkably when the temperature is decreased further. Under our experimental conditions, we did not observe any tendency for recrystallization (transition $L_{\beta_i} \rightarrow L_{f_i}$). When the water, incorporated in the head group region, is frozen out (transition L_{β_i} + water $\rightarrow$ L_{f_i} + ice $\rightarrow$ L_{f_i} + water), the L_{f_i} phase is reformed without delay.

The structure parameters of the $L_{\beta'}$ and the L_{β_i} phases are summarized in the table. Since also in the L_{β_i} phase the distances between the lamellae are considerably smaller than those in the gel phases of mixtures 36/1 and 5/1, chain staggering is assumed to be preserved on increasing the temperature.

4.3.4. *Parameters for the L_{α_i} phase*

Chain melting which is typical of the main transition, starts at about 295 K. The distances between the lamellae are about $6 \cdot 8$ nm and almost independent of temperature, but remarkably smaller than those in mixtures 36/1 and 5/1. Therefore, also in the high temperature phase L_{α_i} chain, staggering may be assumed. The structural parameters of the L_{α_i} phase are summarized in the table.

4.3.5. *Oversimplified model for polymorphism in mixture 1/1*

Based on the evaluation of the structural parameters, figure 12 illustrates the oversimplified model for polymorphism in mixture 1/1. With increasing temperature the following changes in the structures can be discussed.

In the low temperature phase, a molecular lattice exists which differs in its X-ray scattering from the types known until now. Since we have not yet succeeded in indexing it, the A values cannot therefore be calculated.

The distances between the lamellae in the L_{f_i} phase is reduced by $1 \cdot 9$ nm (L_{f_i} + ice) or $1 \cdot 0$ nm (L_{f_i} + water) respectively, when referred to the values of mixture 5/1. Its extremely small value may be explained by staggering of the hydrocarbon chains in the bilayer.

Hysteresis in the phase transition ice $\rightleftharpoons$ water effects an extreme alteration in the distances between the lamellae ($\Delta d_L = 3 \cdot 3$ nm). But from the value, obtained for mixture 5/1 it differs only by $\Delta d_L = 2 \cdot 4$ nm.

In the gel phase, a hexagonal chain packing is observed. The distance between the lamellae being very small, interdigitated hydrocarbon chains may be preserved. The
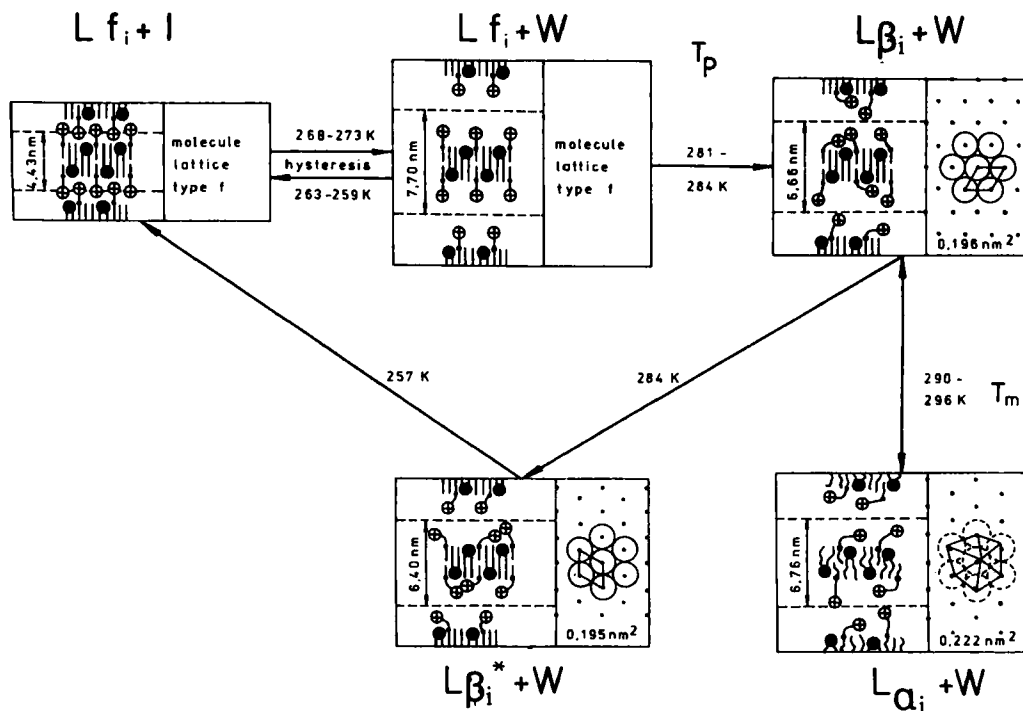
Figure 12. Oversimplified model for polymorphism in mixture 1/1 of the ternary system dipalmitoyl lecithin/heptacaine/water and schematic representation of the molecular packing in the bilayer.

transition into the gel phase is accompanied by a sub-transition; this means a decomposition of the molecular lattice.

On supercooling the L_{β_i} phase, the metastable L_{β_i*} phase is formed. Under the experimental conditions, the transition into the L_{f_i} phase could be brought about only by freezing out the ice.

In comparison with mixture 5/1, the distances between the lamellae in the liquid-crystalline phase of mixture 1/1 is reduced by 2·6 nm. This also suggests a staggering of hydrocarbon chains. The arrangement of the fluid chains may be represented with sufficient accuracy by a hexagonal lattice. The area A required per chain is extended by 0·026 nm², when the gel phase L_{β_i} is taken as a reference.

Though the distances between the lamellae in mixture 1/1 are much smaller than those in mixtures 36/1 and 5/1, water incorporation into the bilayers is made easier by chain staggering as demonstrated by considering the spatial arrangements.

5. Conclusions

On presenting data and structural changes for the three mixtures we have tried to draw some conclusions. In their dependence on the mixing ratios, HK mixtures have identical or different structures. At a mixing ratio of 36/1 or 5/1, all phases of the mixtures had orthogonal chain packing within the bilayers. In contrast, at a molar ratio of 1/1, chain staggering may be assumed for the low temperature phase, the gel phase and the melting phase. The added heptacaine causes a pronounced increase in

the distances between the lamellae (see the table). This becomes evident when these values are compared with those from dipalmitoyl lecithin/water mixtures without added HK. Thus the bilayer is enlarged and the local anaesthetics are incorporated. This may be interpreted by an increased hydration capacity which is discussed in [1]. Consequently the results from the calorimetric measurements are not in contradiction with the results from the X-ray diffraction.

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