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Phase Equilibria and Phase Properties in Systems Containing Lecithins, Triglycerides, and Water

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The phase behaviour and molecular interaction in systems of lecithins (egg lecithin, dipalmitoyl lecithin) and triglycerides (triolein, tristearin) with and without water were studied with differential thermal analysis, X-ray diffraction, IR spectrometry, and polarizing microscopy.

A maximum of 15 % (w/w) triolein can be incorporated into the lamellar liquid crystalline phase of hydrated lecithin. Low-angle X-ray diffraction measurements of this phase indicate that the triolein is mostly located at the lipid-water interface whereas a minor part is dissolved in the apolar region. Triolein induced a broadening of the gel-liquid crystalline transition of dipalmitoyl lecithin, but no change in the transition temperature. The solubilities of the lecithins in triolein were found to be less than 1 %.

No pronounced molecular interaction between tristearin and the lecithins was recorded, neither with differential thermal analysis nor X-ray diffraction. On heating, pure tristearin shows a complex phase behaviour which is preserved also in mixtures with lecithins, but the α_L form is less stable.

From a practical point of view, IR spectrometry appears to be a convenient method to recognize different polymorphic forms of triglycerides also in mixtures with other lipids.

Lecithins and triglycerides have great biological and food-technological importance. Lecithins are major constituents of cell membranes and serum lipoproteins. In food technology lecithins are used as dispersants for water-insoluble components. Triglycerides constitute a major form of energy storage for both plants and animals. The triglycerides have also attracted attention as a risk factor for atherosclerosis.

From the physicochemical point of view [1] the lecithins belong to the swelling lipids. They exhibit thermotropic and lyotropic mesomorphism and can solubilize water-insoluble lipids. They also spread to form monolayers on water surfaces. The triglycerides are classified as insoluble, slightly polar lipids. They have extremely low solubility in water, but spread on water surfaces forming stable monolayers.

Despite its obvious importance, the interaction between lecithins and triglycerides has not been much studied. The interaction between mixtures of natural triglycerides and phospholipids has been examined in monolayers on water [2] and in bulk systems.[3] Surface balance studies of mixed monolayers with well-characterized triglycerides and egg lecithin showed large differences between the surface properties of different triglycerides in mixed films.[4] In this study the bulk phase interactions between some triglycerides and lecithins in both non-aqueous and aqueous environment have been investigated.

MATERIALS AND METHODS

The egg lecithin was isolated in this laboratory.[5] It was proved to be pure by TLC and IR spectroscopy. The dipalmitoyl lecithin was purchased from Sigma, Missouri, U.S.A. and used without further purification. Tristearin and triolein were purchased from Fluka AG, Buchs, Switzerland. ^{14}C Phosphatidylcholine was purchased from New England Nuclear, Dreieichenhain, W. Germany and ^{3}H triolein from Radiochemical Centre, Amersham, England. The tristearin was purified by recrystallization from acetone and the triolein by Florisil column chromatography. The organic solvents used were Merck *p.a.*

The lipid mixtures were prepared by dissolving the lipid components in chloroform whereafter the solvent was removed in a stream of

nitrogen. The last traces of solvent were removed *in vacuo*. When water was added the lipid-water sample was centrifuged and allowed to equilibrate for several days at 20 °C.

The differential thermal analysis (DTA) was conducted with a Fisher Model 370 Differential Thermal Analyzer. The samples (5 – 15 mg) were weighed into aluminium pans which were then sealed with an aluminium lid. The heating rate used was mostly 10 °C min^{-1} and the cooling rate 5 °C min^{-1}. Usually a second and a third heating/cooling cycle was carried out immediately after the first. The temperature range investigated was −50 °C to +100 °C. The cooling was carried out with tap water or liquid nitrogen.

The X-ray diffraction examinations were performed with nickel-filtered Cu$K\alpha$-radiation.

For exposures in the wide-angle range a Debye-Scherrer powder camera with a diameter of 114.6 mm was used. The samples were placed into glass capillary tubes which were sealed in both ends.

The low angle X-ray diffraction measurements were conducted with an automatically registering Rigaku-Denki camera. The incident X-ray beam was collimated by a slit system. All X-ray measurements were performed at a temperature of 25 °C.

The IR spectra were recorded with a Perkin-Elmer Model 700 Spectrophotometer. The samples were dried from chloroform to a thin film on a KBr-tablet. Another tablet was pressed upon the first and the transmission measured at once. During heating and cooling of the samples precautions were taken to avoid air moisture.

The phase behaviour of the systems was studied with a combination of different methods. The solubilization capacities were measured by separation of the phases by centrifugation and analysis of the phases by liquid scintillation counting or thin layer chromatography. With DTA the numbers and temperatures of phase transitions were recorded. A polarizing microscope equipped with a thermostated stage was used to document the number of phases and their textures. X-Ray and IR measurements gave information about the molecular arrangements and interactions in the different phases.

RESULTS AND DISCUSSION

Solubilization. The ternary phase diagram of egg lecithin (EL) – triolein (TO) – water (W) at 20 °C is given in Fig. 1. Hydrated EL with between 6 and 45 % W forms a lamellar mesophase, which can solubilize 15 % TO per dry weight lipid (zone I). If the W and TO concentrations exceed 45 and 15 %, respectively, two and three phase regions are formed with excess W (zone II) and/or TO (zones III and IV). The solubility of EL in TO is very low ~1 % (zone V). The solubilizing capacity of EL for tristearin (TS) is much less than for TO, only ~1 %.

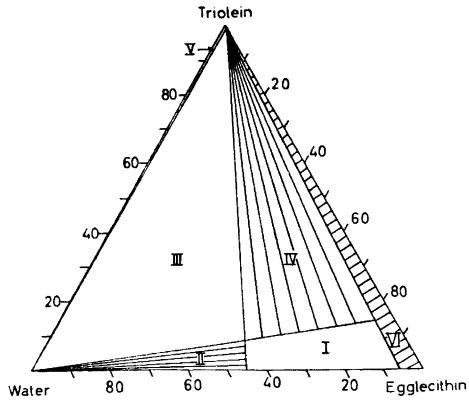

Fig. 1. Ternary phase diagram of egg lecithin – triolein – water at 20 °C. Zone I, single phase region of lamellar mesophase. Zone II contains two phases: lamellar mesophase and water. Zone III contains three phases: lamellar mesophase, triolein, and water. Zone IV contains two phases: lamellar mesophase and triolein. Zone V contains two phases: triolein (with a small amount of dissolved lecithin) and water. Zone VI contains several partially ordered phases of lecithin and varying amounts of excess triolein.

The phase behaviour of the ternary system dipalmitoyl lecithin (DPL) – TO – W was also studied in some detail. Solubilization experiments showed that DPL like EL can incorporate 15 % triolein. The solubility of DPL in TO was determined to be less than 1 %.

The solubilizing capacity of EL for TO is less than for the polar lipid cholesterol [6] but higher than for the apolar cholesteryl ester.[7] Thus the triglycerides take up a midposition between the polar and apolar lipids as surface film studies have already indicated.[4]

Differential thermal analysis. The phase transitions of the systems: EL – TS and DPL – TS with and without water were determined. The results are presented in Fig. 2 a, b and 3 a, b. The diagrams show no pronounced interaction between tristearin and the lecithins, only some depressing effect on the melting point of TS and on the gel-liquid crystalline transition of DPL. The systems

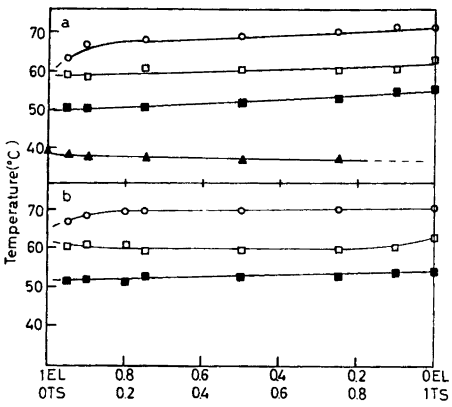

Fig. 2. Condensed binary phase diagrams for the systems (a) egg lecithin (EL) – tristearin (TS) and (b) egg lecithin – tristearin – water (30 %). Melting of tristearin β_L form ○, the $\alpha_L - \beta_L'$ transition □, melting of tristearin α_L form ■, and the solid – liquid crystalline transition of egg lecithin ▲.

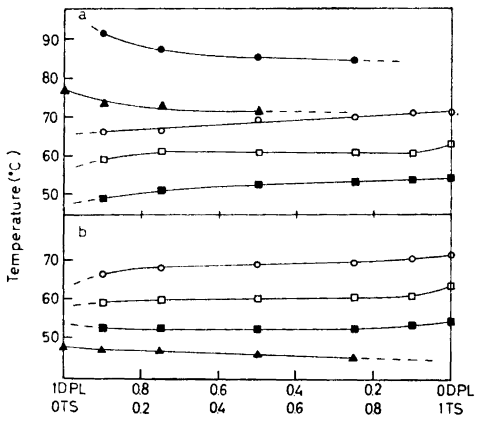

Fig. 3. Condensed binary phase diagrams for the systems (a) dipalmitoyl lecithin (DPL) – tristearin (TS), and dipalmitoyl lecithin – tristearin – water (30 %). The solid – liquid crystalline transitions of dipalmitoyl lecithin (anhydrous) ●, and (monohydrate in a, and fully hydrated in b) ▲, melting of tristearin β_L form ○, the $\alpha_L \rightarrow \beta_L$ transition □, and melting of tristearin α_L form ■.

EL – TO and DPL – TO were also studied in some detail. Special interest was taken in elucidating the effect of the lecithins and of water on the polymorphism of triglycerides.

DTA studies show that TS has a complex phase behaviour on heating. The DTA curves

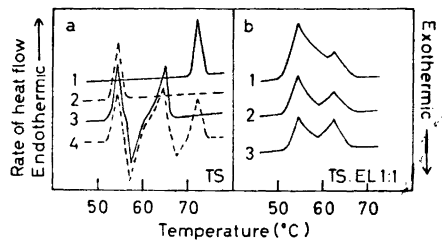

Fig. 4. DTA curves of tristearin (TS), and a 1:1 mixture of tristearin and egg lecithin (EL). (a) In curve 1 the melting endotherm of tristearin β_L is shown, curve 2 represents the melting of the α_L modification, curve 3 shows the melting of α_L, the crystallization exotherm and melting endotherm of β_L', curve 4 shows the same transitions as curve 3 but additionally the crystallization exotherm and melting endotherm of β_L. (b) The curves 1, 2, and 3 show the relative amounts of tristearin β_L, and β_L' at 0, 1, and 3 h after first heating in a 1:1 mixture of tristearin and egg lecithin.

of pure TS are shown in Fig. 4a. TS crystallized from chloroform reveals a single endotherm at 72 °C representing the melting of the β_L modification (Curve 1). After crystallization by cooling and by heating at a rate of 10 °C min^{-1} the melting takes place at 54.5 °C, the melting point of the α_L modification (Curve 2). If the second heating run is performed at a rate of 2 °C min^{-1} the DTA curve shows the melting peak of the α_L form at 54.5 °C, then the crystallization exotherm at 57 °C of the β_L' form, which then melts at 65 °C (Curve 3). At a heating rate of 1 °C min^{-1} the $\beta_L' \rightarrow \beta_L$ transition appears with the β_L form crystallizing at 68 °C and melting at 72 °C (Curve 4). At a heating rate of 0.5 °C min^{-1} a small pretransition can be observed before the melting endotherm of each polymorphic form.

The α_L form of TS is rather stable at room temperature and no β_L' form was found within 3 h with a heating rate of 10 °C min^{-1}. In mixtures with EL the α_L form of TS is less stable, which is illustrated in Fig. 4b for the system EL:TS 1:1. After only 1 h a good amount of β_L' form was found (Curve 2) and after 3 h there was even more (Curve 3). This enhancement of the $\alpha_L \rightarrow \beta_L'$ transition was found also in systems with EL replaced by DPL and even in the presence of water.

In systems with DPL and TO a broadening of the DPL gel-liquid crystalline transition was

Table 1. X-Ray diffraction bands of egg lecithin (EL) and tristearin (TS) and mixtures of these without and with water and crystallized from chloroform (chl) or from melt. The relative intensities of the bands are denoted by: strong (s), medium (m), weak (w), and diffuse (d).

TS chl	EL chl	1:3 EL:TS chl	1:1 EL:TS chl	3:1 EL:TS chl	1:3 EL:TS melt	1:1 EL:TS melt	3:1 EL:TS melt	EL melt	TS melt
5.20 m	4.7 d	4.7 d	4.7 d	4.7 d	4.7 d		4.7 d	4.7 d	
4.72 w	4.71 m								
4.56 s		4.56 s	4.55 s	4.56 s					
	4.23 s	4.10 m		4.25 m	4.15 s	4.15 s	4.15 s		4.25 s
							4.05 s		
3.86 s		3.86 s	3.84 s	3.86 s					
3.70 s		3.70 s	3.70 s	3.70 s					
As above + 25 % water									
		4.7 d	4.7 d	4.7 d	4.7 d	4.7 d	4.7 d		
		4.54 s	4.55 s	4.59 s					
		4.15 w	4.15 w		4.15 s	4.15 s	4.15 s		
		3.85 s	3.86 s	3.85 s					
					3.78 s	3.82 s	3.81 s		
		3.71 s	3.71 s	3.68 s					

found, but no difference in the transition temperature compared with pure DPL. This result was unexpected when regarding the pronounced effect of cholesterol on the same transition.[8]

X-ray diffraction. Pure TS and EL and mixtures of these with and without water and crystallized from chloroform or from melt were examined at 25 °C in the wide-angle region. The results are presented in Table 1.

The chloroform-crystallized pure TS shows three characteristic reflections at 4.56, 3.86 and 3.70 Å. This is in accordance with the triclinic $\beta_{L'}$ form of TS. The TS sample crystallized from melt gave the strong, broad reflection of the $\alpha_{L'}$ form at ~4.15 Å. No trace of $\beta_{L'}'$ was detected even after one week at room temperature. Apparently the contact with the glass walls of the capillary tube prevented the $\alpha_{L'} \rightarrow \beta_{L'}'$ transition.

Pure EL crystallized from chloroform shows two sharp reflections; a strong one at 4.23 Å and a weaker one at 4.71 Å. The reflection pattern indicates the presence of two different phases; a crystalline one (C) and one with rectangular lattice and partly ordered hydrocarbon chains (Pσ).[9]

EL-TS mixtures crystallized from chloroform show two bands deriving from EL; a very broad band at 4.7 Å related to the lamellar mesophase (L_α) and one of medium strength at *ca.* 4.2 Å probably implying the presence of the crystalline phase (C), although these findings also can indicate presence of traces of a lamellar phase with partially stiff chains (L_β). Readers wanting a more detailed discussion about the conformational transitions of EL are referred to Refs. 9, 10 and 11. The strong diffuse reflection of the $\alpha_{L'}$ form of TS at ~4.15 partly overlaps the reflections of the L_β and/or C phases of EL. TS gives the same characteristic reflections as in pure form indicating little effect of EL on the crystallization of TS. In chloroform crystallized mixtures TS shows the same characteristic reflections as pure TS in $\beta_{L'}$ form.

In EL–TS mixtures with 25 % W the X-ray spacings show that EL is in L_α phase and chloroform-crystallized TS in its $\beta_{L'}$ modification. Further there is an unexpected, weak reflection at 4.15 Å, alternatively indicating cocrystallization of TS and EL or traces of an $\alpha_{L'}$ form of TS. In mixtures crystallized from melt and with 25 % W, TS has its strong reflection at ~4.15 Å and a new one of medium strength at ~3.8 Å. These reflections may originate from a mixture of $\alpha_{L'}$ and $\beta_{L'}'$ form of TS where the broad 4.15 Å reflection is common for both the forms. Thus in hydrated

samples the $\alpha_L \rightarrow \beta_L'$ transition occurs also in capillary tubes.

Both the DTA and X-ray results show that there is no marked interaction between TS and EL. This is probably explained mainly by stearic reasons. The molecular surface areas of EL and TS are very similar ~58 Å. [4] But TS does, however, exist in so called "tuning fork" structures with the hydrocarbon chain in the 2-position pointing in opposite direction from those in 1- and 3-positions while in EL the bulky phosphatidylcholine group in 3-position opposes the hydrocarbon chains.

The lamellar mesophase in the EL – TO – W system was subjected to low angle X-ray diffraction measurements. In order to clarify the effect of triolein incorporation on the parameters of the lamellar mesophase the concentration of triolein was varied at three constant EL/W ratios (9:1, 7.5:2.5, 6.7:3.3). The variations in long spacings, d, with triolein for the three constant EL/W ratios are shown in Fig. 5. Starting with the d values and the corresponding EL, TO, and W percentages one can calculate some structural parameters. Partial specific volumes used were: 0.982 ($cm^3\ g^{-1}$) for EL, [5] 1.112 for TO, [10] and 1.002 for W.[10]

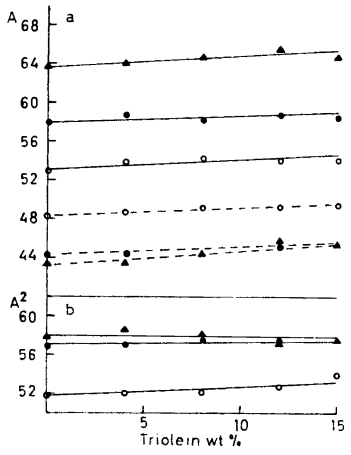

Fig. 5. (a) The long spacings (d), continuous lines, and the thickness (d_L) in Å of the lipid layer, broken lines, and (b) the mean molecular surface area (S_M), as a function of the dry weight percentage of triolein at three fixed egg lecithin to water ratios of 9:1 (○), 7.5:2.5 (●), and 6.7:3.3 (▲).

(1) The mean molecular surface area, S_M, in Å², $S_M = V_{L,T}/(d/2 \times \emptyset_{L,T})$, where $V_{L,T}$ is the mean molecular volume for mixtures of EL and TO, and $\emptyset_{L,T}$ equals volume fraction of EL and TO.

(2) The thickness, d_L, in Å of the lipid layer. $d_L = 2V_{L,T}/S_M$.

The calculated parameters are given in Fig. 5. A small increase in S_M is noted for 9 EL:1 W with increasing TO concentration while the values are rather constant for the lower EL/W ratios. Assuming a constant value at each of the different EL/W ratios for the molecular surface area of EL, a calculation of the molecular surface area of TO gives ~60 Å² for all mixtures. This value is somewhat low even for condensed TO indicating that not all of the TO is located at the interface. This conclusion agrees with the increase in d_L with the TO concentration indicating that some TO is immersed in the hydrocarbon region. These results are in

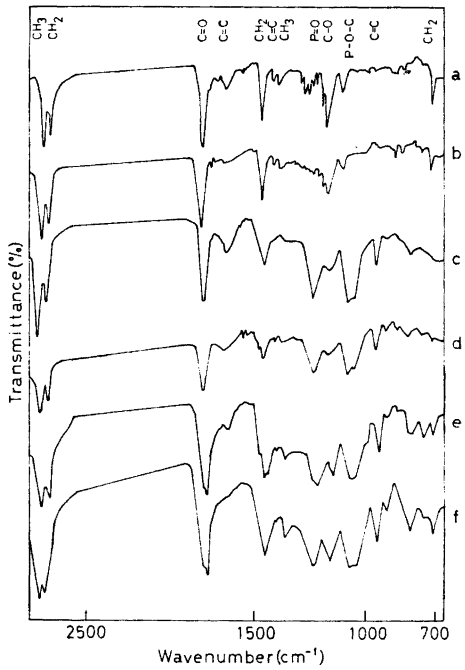

Fig. 6. IR spectra of tristearin and egg lecithin and mixtures of these. Tristearin crystallized from chloroform (a), and from melt (b), egg lecithin crystallized from chloroform (c), and from melt (d), 1:3 mixtures of tristearin and egg lecithin crystallized from chloroform (e), and from melt (f).

disagreement with surface film studies showing that TO is ejected from a TO – EL mixed film at its collapse pressure and that TO has low solubility in EL.[4]

IR analysis. The IR spectra of pure TS and EL and mixtures of these in the frequency range 650 – 3 000 cm^{-1} are shown in Fig. 6. The curves a,c, and e show the spectra of samples crystallized from chloroform (TS is in its β_L form) and b, d, and f show the spectra after crystallization from melt (TS is in its α_L form). No change in the spectra of the samples was noted within 24 h at room temperature.

In order to distinguish between different polymorphic forms of triglycerides the bands in the 720 cm^{-1} and 1 190 – 1 350 cm^{-1} regions are of special interest. In the latter region there is a great difference in number and intensity of the bands of the different forms, the β_L form showing eight characteristic bands, the α_L form just one clearly discernible band. Also in the regions; 1 060 – 1 120, 1 380 – 1 430, and 1 640 – 1 680 cm^{-1} the β_L form gives more sharp bands than the α_L form. The chloroform-crystallized 1 TS:3 EL mixture has a band at 1 760 cm^{-1} not found in the other samples indicating that the carbonyl group is involved in hydrogen bonding lowering the frequency band of the group by 50 cm^{-1}. The spectra and thus the crystalline structure of the EL crystallized from melt and from chloroform are very similar.

IR spectrometry seems, from a practical point of view, to be a convenient method to recognize the various polymorphic forms of triglycerides, also in mixtures with other lipids. The most useful absorption bands being those in the 700 – 750 and 1 190 – 1 350 cm^{-1} spectral regions.

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