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Review

Mixed micelles and other structures in the solubilization of bilayer lipid membranes by surfactants

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Abstract

The solubilization of lipid bilayers by surfactants is accompanied by morphological changes of the bilayer and the emergence of mixed micelles. From a phase equilibrium perspective, the lipid/surfactant/water system is in a two-phase area during the solubilization: a phase containing mixed micelles is in equilibrium with bilayer structures of the lamellar phase. In some cases three phases are present, the single micelle phase replaced by a concentrated and a dilute solution phase. In the case of non-ionic surfactants, the lipid bilayers reach saturation when mixed micelles, often flexible rod-like or thread-like, start to form in the aqueous solution, at a constant chemical potential of the surfactant. The composition of the bilayers also remains fixed during the dissolution. The phase behavior encountered with many charged surfactants is different. The lamellar phase becomes destabilized at a certain content of surfactant in the membrane, and then disintegrates, forming mixed micelles, or a hexagonal phase, or an intermediate phase. Defective bilayer intermediates, such as perforated vesicles, have been found in several systems, mainly with charged surfactants. The perforated membranes, in some systems, go over into thread-like micelles via lace-like structures, often without a clear two-phase region. Intermediates in the form of disks, either micelles or bilayer fragments, have been observed in several cases. Most noteworthy are the planar and circular disks found in systems containing a large fraction of cholesterol in the bilayer. Bile salts are a special class of surfactants that seem to break down the bilayer at low additions. Originally, disk-like mixed micelles were conjectured, with polar membrane lipids building the disk, and the bile salts covering the hydrophobic rim. Later work has shown that flexible cylinders are the dominant intermediates also in these systems, even if the disk-like structures have been re-established as transients in the transformation from mixed micelles to vesicles.

Keywords: Solubilization; Lipid bilayer; Mixed micelle; Phase diagram; Liposome; Perforated bilayer; Intermediate phase; Mesh phase; Disk

Abbreviations: C_xE_y, $CH_3(CH_2)_{x-1}(OCH_2)_yOH$; C_xTAB(C), alkyltrimethylammonium bromide(chloride) x C in alkyl chain; cmc, critical micellar concentration; cTEM, cryotransmission electron microscopy; DG, dodecylglucoside; DMPC, dimyristoylphosphatidylcholine; DM, dodecylmaltoside; DOPE, dioctadecylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; EPC, egg phosphatidylcholine; GMO, glyceryl monooleate; HG, heptylglucoside; H_1, hexagonal phase; L_1, L_2, L_3, isotropic liquid phases (micelle phase, reversed micelle phase, sponge phase); L_α, lamellar liquid crystalline phase; OG, octylglucoside; PEG, polyethylene glycol; Q_1, isotropic cubic phase; R_{sol}, R_{sat}, surfactant to lipid molar ratio at complete solubilization of the bilayer, and at saturation of the bilayer by surfactant, respectively; SDS, sodium dodecylsulfate; SANS, small angle neutron scattering; SAXS, small angle X-ray scattering; Triton X-100, [*p*-(1,1,3,3-tetramethylbutyl)phenyl]poly(oxyethylene)

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1. Introduction

The dissolution of a bilayer lipid membrane by addition of surfactants finally results in mixed micelles containing both polar lipids and surfactants. By removing surfactant from the mixed structures, for instance by adsorption, dialysis or, if the surfactant is sufficiently soluble, just by dilution, the lipid bilayer may be reconstituted and closed into a liposome. The equilibrium and transient structures encountered in lipid–surfactant mixtures, and the kinetics of the dissolution and of the closure to vesicles are important issues, and have attracted considerable interest [1,2]. In this review we will focus on the structures and discuss them in relation to the equilibrium properties and phase relations. These aspects of the equilibrium state of the systems are contained in the phase diagrams. Unfortunately, only few systems comprising polar lipids and surfactants in water have been studied in sufficient detail. We shall review the information available, and let that knowledge guide the discussion of the structures in the systems.

The dissolution of bilayer membranes is usually studied in an excess of solvent, either water or an electrolyte solution, starting from an aqueous dispersion of the lamellar phase in the form of liposomes (or vesicles), and ending with mixed micelles belonging to the L_1 phase. A variety of structural transitions may occur in the process. Often non-equilibrium structures, such as liposomes, are kinetically trapped, and have a size and morphology depending on the history of the sample. Sometimes slow changes can be followed over hours or weeks, but in other cases no changes are observed even after years of storage, at least nothing that can be distinguished from transformations due to a slow chemical breakdown.

2. Systems of polar lipid, surfactant, and water

A phase diagram summarizes extensive information about the equilibrium state of a system. The (quasi) three-component systems considered here are usually presented in the form of Gibbs triangles, at a constant temperature. A complete diagram should contain tie lines in the two-phase areas, showing the equilibrium compositions of the two phases involved, but few systems have as yet been studied in such detail.

2.1. Phospholipids and non-ionic surfactants

The solubilization of bilayer membranes by various non-ionic surfactants has attracted much attention, in particular using Triton X-100 ([*p*-(1,1,3,3-tetramethylbutyl)phenyl]poly(oxyethylene) with an average of 9.5 oxyethylene groups), octylglucoside (OG), and $C_{12}E_8$ and related polyethylene glycol (PEG) surfactants. Such surfactants are commonly employed in membrane research, e.g. in the extraction and reconstitution of membrane proteins. In spite of that, fundamental phase studies of such systems are sparse. In fact, except for a partial diagram of the system egg phosphatidylcholine (EPC)/OG/water presented in a PhD thesis [3], soybean PC/Triton X-100/water seems to be the only system of a non-ionic surfactant and phospholipid for which a detailed study of the phase behavior has been published [4]. Although neither the polar lipid nor the surfactant are single pure substances, we have to rely on this system for general considerations.

2.1.1. Phospholipid/Triton X-100/water

The surfactant forms a normal sequence of phases in water: the micelle phase L_1 is followed by a hexagonal phase, and at high concentrations of the surfactant, which is fluid at room temperature, there is a L_2 phase. Each of these phases can solubilize lecithin to some extent, at most about 10% in the L_1 phase (Fig. 1). The lamellar phase, L_α, of the phospholipid readily takes up Triton X-100, and then swells in water to a maximum of 50% water. At lower temperatures (278 K) the lamellar phase extends to the surfactant–water axis, i.e. the surfactant itself forms a lamellar phase in water, which is connected to the lamellar phase of the lecithin.

Solubilization of bilayers is usually studied in dilute liposomal dispersions. We start then at a point, say A, on the lecithin–water axis, where the lamellar phase is dispersed in excess water. On addition of surfactant the composition follows the line AB in the two-phase area, and enters the mixed micelle region at point B. In the two-phase area the concentration of surfactant increases in both phases until

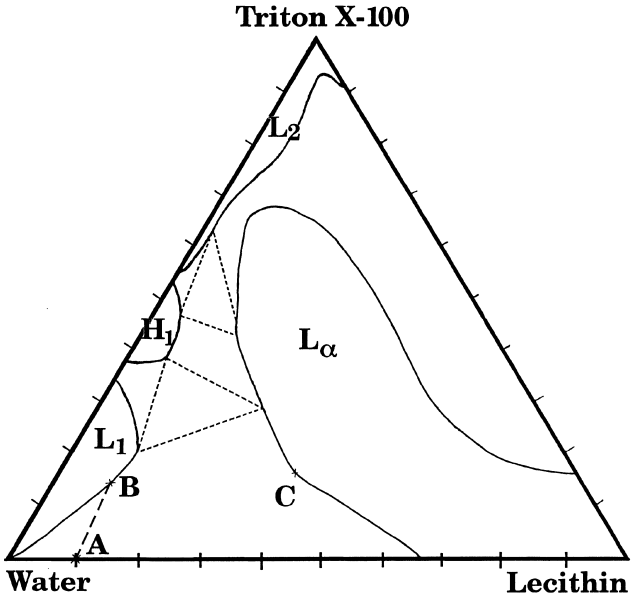

Fig. 1. Phase diagram of Triton X-100/soybean lecithin/water at 23°C. Four one-phase areas are shown: L_1 (micelle solution), H_1 (hexagonal phase), L_2 (reversed micelles) and L_α (lamellar phase), and two three-phase triangles. The line AB indicates total compositions followed on addition of surfactant to a sample of lecithin in water at point A. Point C indicates a possible composition of the bilayer in equilibrium with mixed micelles. Adapted from [4] with permission. Copyright 1989 Academic Press.

the critical micellar concentration (cmc) for formation of mixed micelles is reached. This concentration is necessarily lower than the cmc of the pure surfactant. In the system of Fig. 1 the cmc is close to the water corner and not marked. At higher concentrations of surfactant, saturated mixed micelles are in equilibrium with the mixed lamellar phase. The saturation limit of the L_1 phase is almost a straight line extending to the water corner (or more precisely to the cmc), implying that the composition of the micelles in equilibrium with the lamellar phase is almost constant (37% lecithin by weight is obtained from the 'slope' of the line, or about 32 mol%, corresponding to a molar ratio of surfactant to lipid of 2.1). The concentration of surfactant in the intermicellar aqueous solution is then also almost constant and so is the composition of the bilayer. Although the tie lines in the two-phase area have not been determined, it is a fair guess that when micelles have formed the tie lines all end at a bilayer composition close to point C on the L_α border, and start on the straight line part of the L_1 saturation curve. The change of the bilayer composition up to point C occurs as the surfactant concentration in the aqueous phase, on the first additions, increases from nothing to the mixed cmc.

The solubilization process can then be described as follows. The surfactant is distributed between solution and membrane up to a point (close to C) where the membrane is 'saturated' with surfactant, at a surfactant/lipid molar ratio, R_{sat}. Further addition of surfactant results in the formation of mixed micelles of an almost constant composition, given by a ratio R_{sol}. At point B, the last piece of membrane is dissolved when the system enters the L_1 phase. This description of the process is close to that of the 'three-stage model' usually assumed, where the constant compositions of the mixed micelles and the 'saturated' bilayers are taken for granted [2,5–7]. From the three-component phase diagram, we conclude that constant compositions of bilayer and mixed micelles during the dissolution require that the tie lines in the L_α/L_1 two-phase area connect a single point on the limit of the lamellar phase, with points on the linear part of the L_1 border that extends to a point close to the water corner. Furthermore, the diagram shows that in the case of Triton X-100, the composition of the bilayer during the solubilization is not determined by a saturation of the bilayer – the bilayer can accept much more of the surfactant – but by the onset of micelle formation.

Many detailed studies of the dissolution have been made by measurement of turbidity or scattered light intensity. The changes often follow a general pattern, and it is possible to identify a few characteristic features of the solubilization curve. By measuring the surfactant concentration required to reach these features at different lipid concentrations, both the concentration of free surfactant and the composition of the aggregate can be determined. These parameters are assumed constant. Referring to the phase diagram in Fig. 1, it is evident that characteristic points that can be identified in this way correspond to compositions on the tie line connecting the 'mixed cmc' and the point C, and the compositions defining the saturation boundary of the L_1 phase.

Paternostre et al. [8] studied the solubilization of EPC liposomes by Triton X-100, OG, and sodium cholate, using both turbidity monitoring and NMR methods. They found for Triton X-100 a molar ratio of surfactant to lipid of $R_{sol} = 0.64$ in the saturated

bilayer, and $R_{sat} = 2.5$ in the saturated mixed micelles. Dennis [5], using direct chemical analysis of the phases separated by centrifugation, obtained very similar results, 0.63 and 2.4, respectively, at higher concentrations of lipid (2.2%) and surfactant. It can be noted that Paternostre et al. and Dennis prepared the samples in very different ways. The former made liposomes by reversed phase separation, whereas the latter normally used a gentle homogenizer to mix the samples. Point C in Fig. 1 corresponds to a surfactant/lipid ratio of 0.54, and the slope of the L_1 saturation line to 2.2. These latter values refer to soybean lecithin, which may explain the deviation from the solubilization studies using EPC.

The morphologies of the bilayers and the micelles during the solubilization process were studied in cryotransmission electron microscopy (cTEM) investigations of the same system [9]. The bilayers were originally present as small sonicated vesicles, which grew after a certain amount of surfactant had been added. The large vesicles opened and turned partly into irregular and curved bilayer flakes at higher concentrations of surfactant, and at the same composition long cylindrical micelles started to appear. Similar results have been reported with other detergents [10,11]. A typical series of micelle and bilayer morphologies is shown in Fig. 2. If the three-stage model were perfectly valid, one would expect that when mixed micelles and saturated bilayers are simultaneously present, they would have morphologies that were independent of the total composition. The cTEM investigations, however, and also the detailed analysis of binding isotherms [1,12], suggest a more gradual transition. Several reasons for such a behavior may be envisioned. (i) Small changes in the composition of the mixed micelles and of the bilayer may occur that can give large morphological effects. (ii) Interactions between the bilayer structures, or between micelles, or between micelles and bilayers, may give appreciable differences in the morphologies of both bilayer structures and micelles. (iii) During

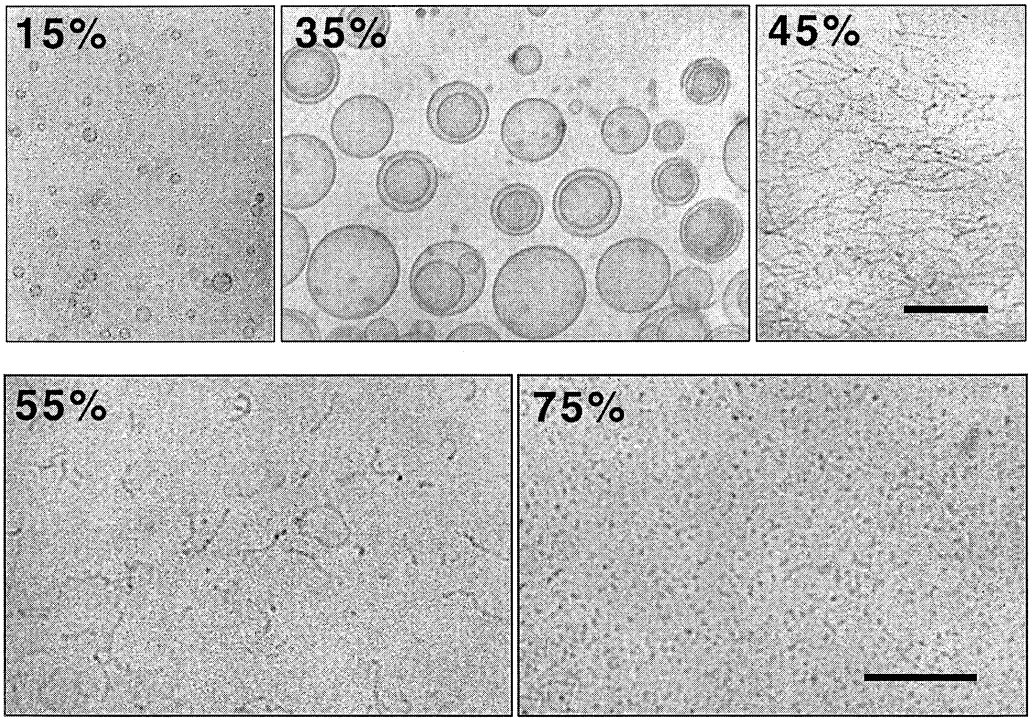

Fig. 2. Structures observed after addition of a non-ionic surfactant, $C_{12}E_8$, to small, sonicated EPC vesicles. The mol% surfactant in the dry sample is indicated; the EPC concentration was 1.2 mM. At 35% the vesicles have grown to their maximum radius. With larger addition of surfactant long thread-like micelles are first formed, followed at higher surfactant concentrations by shorter threads and finally globular micelles. Reproduced and adapted with permission from [11]. Copyright 1991 Academic Press.

solubilization the system may become trapped in different non-equilibrium states.

Points (ii) and (iii) need some comments. (ii) When solubilization curves are recorded at different lipid concentrations an increased turbidity is often observed at the higher concentrations (which in itself is a source of error), due to attractive interactions between the liposomes that give rise to clustering, and sometimes to a secondary transformation into large and multilamellar structures. Ultimately, macroscopic phase separation may occur. The attraction may partly be an effect of depletion forces from the mixed micelles. (iii) With respect to the equilibration time, there are several stages. The redistribution of surfactants between the aqueous solution and the bilayers can be expected to be fast (at least to the outer half of the bilayer, the flip-flop or other penetration of the membrane may be very slow, in particular for charged surfactants [2]). The equilibration of the mixed micelles and the solubilization of a bilayer in excess surfactant have also been found to proceed relatively fast in studies of micelle relaxation processes [13], as well as in direct measurements of the solubilization times of bilayers [2,9,11]. The morphology of the bilayer structures, however, can be expected to evolve very slowly towards the most stable ones – probably often represented by a small chunk of lamellar phase. As a secondary effect from the slow change of the bilayer arrangements a redistribution of surfactant may occur, possibly in turn having a small effect also on the composition of the mixed micelles.

Normally, however, we expect that the micelles observed represent equilibrium at the solubilization limit of the L_1 phase, and that the composition of the bilayers is close to the equilibrium composition, even if the bilayers may take on a variety of non-equilibrated structures depending on how the samples were prepared.

2.1.2. Alkylglucosides and other non-ionic surfactants

OG has been utilized extensively in biochemical membrane research to solubilize bilayer membranes and for reconstitution experiments, and the interactions of OG with bilayers have been studied thoroughly [1–3,6–8,14–17]. A range of other sugar surfactants have also been examined, with a variation of both alkyl tail length and headgroup [18–23], for example dodecylmaltoside (DM). Phase separation and secondary aggregation phenomena during the solubilization are often noticed for this class of surfactants [14]. Such complicating phenomena were examined in detail recently, in a series of papers from Ollivon and his group [15,21,22]. The main objective of these studies was to investigate an enzymatic procedure for the formation of vesicles from mixed micelles of polar lipids and DM. By enzymatic hydrolysis of the maltoside group, first to glucoside, DG, and eventually to dodecanol and glucose, the large polar headgroup of the maltoside is converted into a much smaller headgroup, so that the surfactant packing parameter, $v/a_{head}l_0$, approaches unity, or in the case of the alcohol exceeds unity. A transition from the mixed micelles with positive spontaneous curvature to the balanced state of the bilayer is the result.

To clarify the details of the process, dipalmitoylphosphatidylcholine (DPPC)–DG mixtures were investigated in excess buffer, at temperatures above the gel-to-liquid crystalline transition of the mixtures, by several methods, and found to form closed bilayer vesicles at DG/DPPC molar ratios up to 1.8; above this concentration discoid structures also appeared (but not thread-like micelles) [21]. Solubilization of the mixed bilayers with DM gave complex turbidity curves with a number of characteristic points. Measurement at different total concentrations resulted in the pseudo-ternary phase diagram, in excess buffer, that is reproduced in Fig. 3 [21]. The morphologies in the different areas were determined by X-ray studies and freeze-fracture electron microscopy. In the absence of DG, DM solubilizes DPPC bilayers as shown on the *y*-axis in the figure. The lamellar phase can take up DM to a molar ratio of 0.5 before micelles appear (step III), and the last bilayers are solubilized at a molar ratio of 1.5 (step VII). With DG added, DM/DPPC increases slightly for step III, and strongly for step VII, indicating that DG is incorporated into the bilayer without much destabilization.

The complexity of the turbidity curve and the phase diagram is due to secondary aggregation and phase separation. Two types of processes seem to be involved: liquid–liquid phase separation of the mixed micellar solutions, similar to the well known clouding of non-ionic surfactants, and attractive interactions between the vesicles in dispersion when the

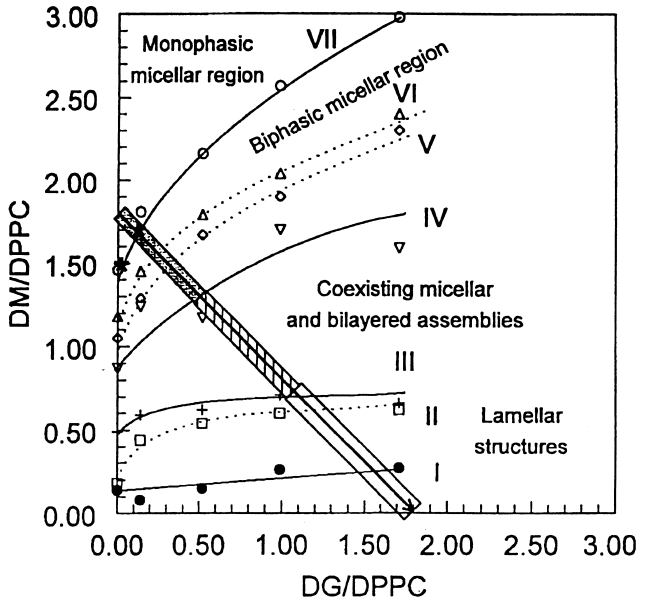

Fig. 3. Phase behavior in excess buffer (145 mM NaCl, 10 mM HEPES) of DPPC/DG/DM at 37°C. The solid lines delimit main phase transitions (observed by SAXS) and the dotted lines secondary aggregation transitions. The crossing arrow indicates the path with a constant sugar/lipid ratio of 1.8, from DM/DPPC mixed micelles, over two-phase regions with micelles, and micelle water, to the three-phase area with two micellar and a lamellar phase, and then lamellar phases in equilibrium with dilute L_1; the final composition without DM and 1.8 DG/DPPC is a pure lamellar phase in buffer. Reproduced and adapted with permission from [21]. Copyright 1998 American Chemical Society.

surfactant is added, leading to agglomeration and at high lipid concentrations to macroscopic phase separation [21]. The latter process has been observed also with Triton X-100, as remarked above. From the results and discussion presented in [21] and from private communication with Lesieur and Ollivon, the following picture emerges.

Up to line III in Fig. 3, there are different lamellar phases in equilibrium with a dilute L_1 phase, denoted excess water in the reports. Between lines III and V there is a coexistence region of mixed micelle and bilayer, which in reality is a three-phase area of lamellar phase and two L_1 phases, one mixed micellar, the other very dilute. After line V no lamellar phase is present, and lines V–VII delimit a region with the two micellar phases in equilibrium, and beyond line VII there are only mixed micelles.

In the PhD thesis of Beugin-Deroo [3] a similar phase behavior of heptylglucoside (HG) and OG/EPC/water was demonstrated. In this system, it was shown by centrifugation of samples and subsequent X-ray examination of the separated phases that at EPC concentrations close to saturation of the mixed micelles, a liquid–liquid phase separation occurred into a dilute phase containing no or few micelles, and a more concentrated mixed micellar solution. In the phase diagram, the first part of the solubility limit of the L_1 phase was thus replaced by a narrow two-phase area. At higher lipid-to-surfactant ratios, the samples contained three phases. In addition to the concentrated and dilute micelle phases, there was a new phase of lamellar structure, according to the small angle X-ray scattering (SAXS) study. A turbidity peak in the solubilization curve corresponds to compositions within the three-phase triangle. Solubilization of the bilayer with these surfactants thus brings the system into a three-phase triangle, with one corner fixed on the lamellar phase border, at the appropriate R_{sat}, and the other two being the endpoints of the narrow two-phase area, one close to the mixed cmc, and the other at a higher concentration of lipid. The difference of this case compared to the Triton X-100 described above is that the narrow two-phase strip has closed to the L_1 saturation border in the Triton case; a two-phase system is replaced by a pseudo-two-phase system of mixed micelles in aqueous solution.

PEG surfactants like Triton X-100 have a cloud point, an upper consolute temperature, that is expected to be lowered by mixing in polar lipids like EPC. It would be interesting, therefore, to examine the Triton X-100 phase behavior, with EPC added, at higher temperature to see if clouding would occur, and if it would give rise to a phase behavior similar to that of OG (or HG) and EPC. OG itself, according to the studies of Kameyama and Takagi [24], does not have an upper consolute temperature; on the contrary, the light scattering studies show that the virial coefficient is negative at low temperature and positive at high; the zero point of the virial coefficient is close to 40°C, so that if anything, the attractive interactions favor phase separation at low temperatures. The issue is not yet sufficiently clarified.

In a study using higher lipid concentrations a gelling of the solutions was reported [20] at a ratio of

DM to phospholipid around 1.4–1.5, close to the end of the bilayer-to-micelle transition region ($R_{sol} \approx 1.6$ in this case). (Similar findings were discussed earlier for EPC/OG [14].) cTEM micrographs reveal that very long thread-like micelles coexist with a few vesicles and other bilayer structures in this region. The micrographs also show that substantial changes of the bilayer morphologies occur at lower surfactant concentrations, before the thread-like micelles appear, and it is possible that the turbidity starts to decrease due to such morphological changes, already before the micelles form.

In summary, the fact that the results from DM, as well as some other non-ionic surfactants, are not fully accounted for by the simple three-stage model is probably mainly due to secondary aggregation phenomena and phase separation. There is also evidence, however, from cTEM micrographs and the rheology of the solution [20], that the micelle morphology changes within the transition region, before all bilayers have disappeared. The maximum viscosity and the so-called Weissenberger effect indicate that the thread-like micelles are particularly long and entangled below R_{sol}. It is possible that an interaction between bilayers and micelles is important for the solution properties, or that non-equilibrium effects can play an important role.

The effect of OG on different polar lipids was recently investigated [15]. DPPC, DOPE, and glyceryl monooleate (GMO) in excess water form a L_α phase, a reversed hexagonal, H_2 phase, and a reversed cubic bicontinuous phase, C_D, respectively. It was nicely demonstrated that the addition of OG increases the curvature of the structures somewhat, so that the reversed structures of GMO and DOPE were transformed into L_α bilayers, and that DPPC remained in that state (with a reduction of the gel-to-liquid-crystal transition temperature).

2.2. Polar lipids and ionic surfactants

The phase behavior of polar lipids and ionic surfactants, in water or in salt solution, has been better studied than that of non-ionic surfactants. It is mainly phospholipids and glycerol monoalkanoates, together with bile salts or cationic surfactants, that have attracted attention; much less is known of normal anionic surfactants. We consider first alkyltrimethylammonium surfactants and polar lipids, and discuss the bile salts in a separate section.

2.2.1. Dimyristoylphosphatidylcholine (DMPC)/C_{16}TAB/water

This was for a long time the only system of this type that had been thoroughly studied [25]. The most conspicuous feature of the diagram (Fig. 4) is the enormous swelling of the lamellar phase that accompanies charging of the bilayer. Already at an addition of 1% of C_{16}TAB the lamellar phase swells out to more than 80% water. The maximum swelling, which is not easy to quantify experimentally but exceeds 99% water, is obtained at molar ratios of surfactant to lipid in the range 0.8–1.2. When the surfactant/lipid ratio exceeds $R_{sat} \approx 1.9$ mixed micelles form, with a composition corresponding to a surfactant/lipid ratio of $R_{sol} \approx 2.8$, both the saturation limit of the L_1 phase and the lamellar phase border are linear, defining constant surfactant/lipid ratios. In this system, therefore, it seems to be the bilayer composition that limits the stability.

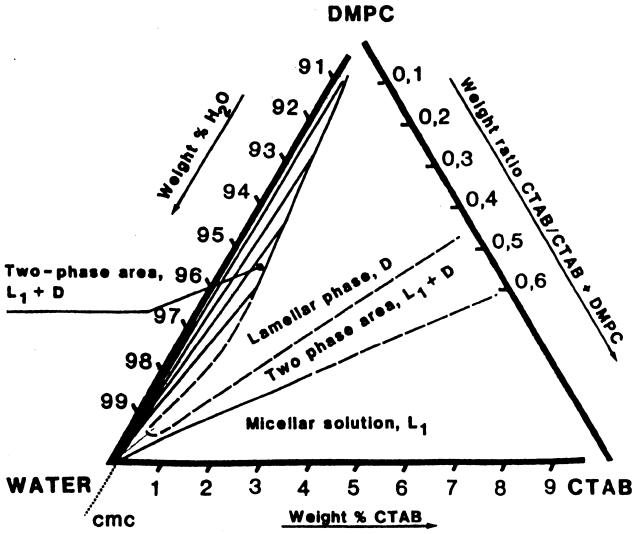

Fig. 4. Phase diagram of C_{16}TAB/DMPC/water at 30°C, the water corner. The lamellar phase of DPPC swells from about 40% water without C_{16}TAB, to a maximum of 99%. On the surfactant-rich side, C_{16}TAB micelles can solubilize DMPC as mixed micelles to a weight ratio 0.67 lipid to surfactant, independent of the total concentration, whereas stable vesicle dispersions are formed on surfactant poor side. Note that the surfactant is at the right-hand corner in this diagram, instead of the top corner. Reproduced with permission from [25]. Copyright 1982 Elsevier Science.

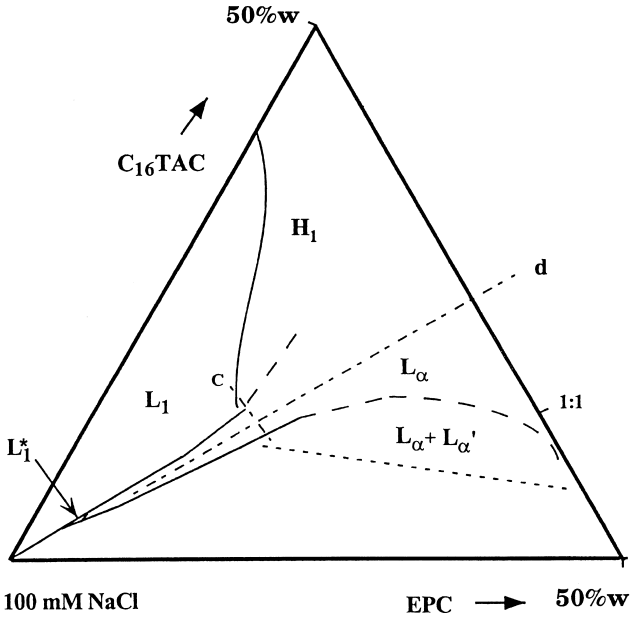

Fig. 5. The system C_{16}TAC/EPC/0.100 M NaCl at 25°C. The swelling of the lamellar phase is retained in a narrow tongue, compare Fig. 4. A new flow-birefringent phase L_1* appears. The two-phase area between L_1 and L_α, and between the hexagonal phase H_1 and L_α, is seemingly suppressed. Low addition of surfactant yields two lamellar phases in equilibrium, L_α and L_α'. Reproduced and adapted with permission from [27]. Copyright 1997 American Chemical Society.

On the surfactant-rich side of the lamellar phase, the two phases at equilibrium are the saturated lamellar phase and the mixed micelles. In the surfactant-poor region, a less strongly swollen lamellar phase is in equilibrium with a surfactant solution at a concentration below the cmc (1.3 mM). Vesicle dispersions form easily from the swollen lamellar phase, and the vesicles are stabilized electrostatically towards fusion [26]; charged lipids promote vesicle formation. The maximum swelling limit of the lamellar phase could not be determined precisely. Reliable X-ray results were obtained only at water contents below 80%, which is a level reached already on addition of about 1% of C_{16}TAB. It is difficult determine the transition point from a strongly swollen, lamellar phase, with an average distance of maybe 400 nm of water between the undulating bilayers, to a dispersion containing very large bilayer vesicles [25].

When solubilization studies are performed with ionic surfactants, it is not only the charged surfactant that is added, but also its counterion. The electrostatic interactions are most important on the first additions of the surfactant, and are then gradually screened. It is of interest to study the phase behavior with the electrostatic screening kept more nearly constant. Fig. 5 presents a phase diagram of EPC/C_{16}TAB/0.100 M NaCl [27]. The swelling of the lamellar phase is reduced on the surfactant-poor side, so that more surfactant has to be added to get an effect. The maximum swelling is reached in a narrow tongue, which extends to about 94% water, centered around a molar ratio of surfactant to lipid of 2.1. With more surfactant the lamellar phase goes over into the micellar phase, without any discernible two-phase area, at a surfactant/lipid ratio of 2.5. A new liquid phase, denoted L_1* in Fig. 5, occupies the tip of the tongue. L_1* is an isotropic (but flow-birefringent) phase, which was not studied in detail. On the other side of the tongue, two lamellar phases are in equilibrium, the one is swollen and surfactant-rich, the other with less surfactant and limited swelling. Similar phase equilibria between two lamellar phases were observed and discussed for other systems containing a mixture of a zwitterionic lipid and a charged surfactant [28,29]. In the EPC/C_{16}TAC case the swollen tongue was shown by extensive SAXS and NMR studies to contain a lamellar phase with water-filled defects [27,30] that on dispersion produces perforated vesicles. In fact, it was just the discovery of such vesicles that prompted the closer inspection of the phase behavior in this system [31].

The turbidity changes observed during the solubilization process, starting with a dilute sample of small unilamellar lecithin vesicles and adding surfactant, is distinctly different with C_{16}TAC or C_{16}TAB than with non-ionic surfactants. Without salt, no changes were observed before the final dissolution into mixed micelles, not even when very small sonicated vesicles were employed at the starting point. In 0.100 M NaCl, on the other hand, the turbidity change indicates a growth similar to that observed with e.g. $C_{12}E_8$, but in this case the change is slow and continues for about 24 h. Micrographs from a cTEM investigation are shown in Fig. 6, where the most unusual feature is the perforated bilayers, which emerge slowly, and are found both closed to vesicles and as open flakes [31].

The cTEM investigations are normally made on very dilute systems, and all micrographs shown

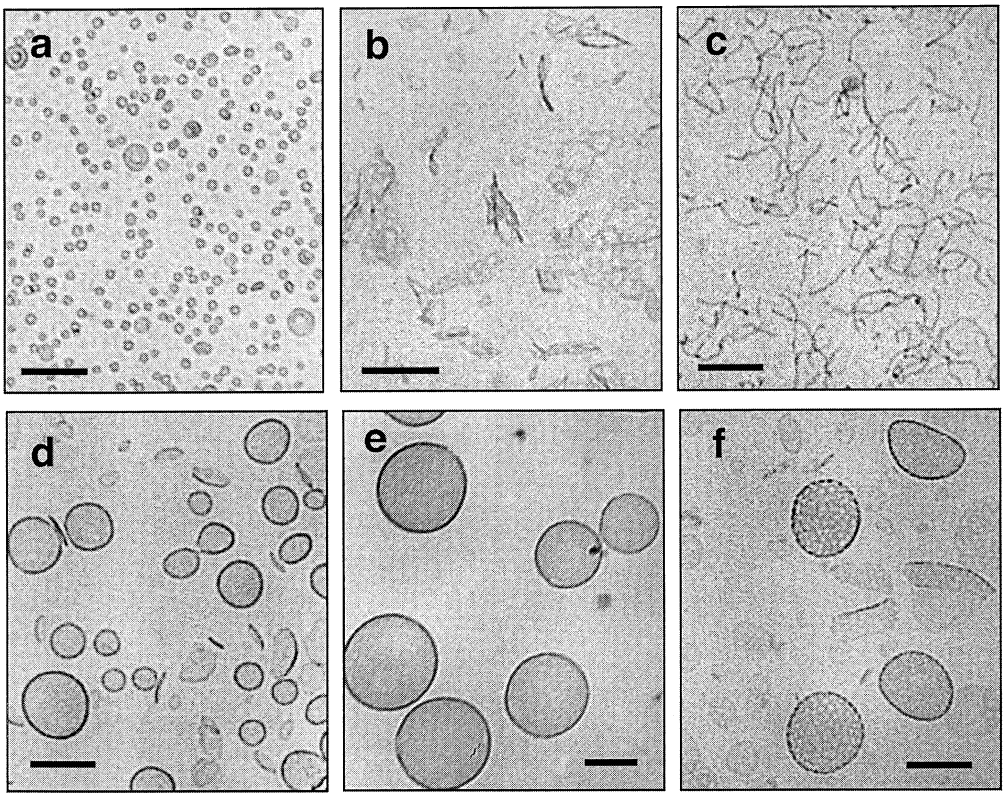

Fig. 6. A sequence of cTEM micrographs from addition of C_{16}TAB to sonicated and centrifuged EPC vesicles in 0.100 M NaCl. (a) The initial preparation. (b) Lace-like nets and entangled thread-like micelles are found 24 h after addition of 75% C_{16}TAC. (c) Thread-like micelles, 80% C_{16}TAC, 24 h. The sequence d–f shows structures found 15 min, 3 h, and 24 h, respectively, after addition of 60% C_{16}TAC. Note in f that even the rim of the vesicles looks perforated. Bar: 100 nm. Reproduced and adapted with permission from [31]. Copyright 1993 Academic Press.

here belong to samples that contain only about 1% of lipids and surfactants. The lamellar phase was imaged only in dispersions, therefore. Micrographs of freshly diluted samples prepared from the lamellar phase and the adjoining L_1 phase are also shown in Fig. 6 [30]. There is no clear two-phase area separating L_α and L_1, and the micrographs likewise seem to indicate a gradual transition with increasing surfactant concentration, from perforated bilayers via more loosely woven lace-like structures, to thread-like entangled micelles, and finally shorter rod-like, eventually globular, mixed micelles. No change of the vesicles was observed at low surfactant additions, until the concentration where growth starts. Since growth was slow in this case, it was possible to capture some intermediates during the growth process. It appears that the vesicles first open to discoid structures, which presumably grow by fusion, and finally close again to large vesicles, all this within 1 h. Thereafter the perforated texture slowly emerges and is fully evolved after some 10 h [31].

This process deviates from the three-stage model where there is a clear transition region from bilayers to mixed micelles. Investigations where the tail length and headgroup of the ionic surfactant and the type of polar lipid have been varied, will be discussed next.

2.2.2. *EPC/C_{12}TAC/0.100 M NaCl*

The phase behavior and solubilization characteristics described for the C_{16}TAC system change dramatically when the chain length of the surfactant is reduced (Fig. 7) [30]. The tongue towards the aqueous corner is reduced giving a maximum swelling of about 70%. Now two-phase regions are present between the lamellar phase and L_1 and the hexagonal phase, H_1. The lamellar phase was free from water-filled defects, as evidenced from the SAXS and NMR

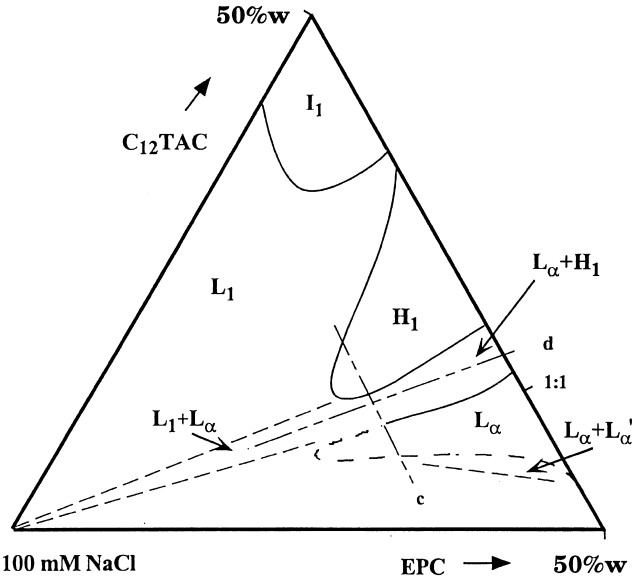

Fig. 7. C_{12}TAC/EPC/0.100 M NaCl at 25°C. Compared to the corresponding C_{16}TAC system in Fig. 5, the lamellar phase swells much less, and there are now clear two-phase regions between L_α and L_1 or H_1. Reproduced and adapted with permission from [30]. Copyright 1997 American Chemical Society.

measurements. The extensive swelling in the EPC/C_{16}TAC system, even in the presence of salt, indicates that relatively long-range repulsive forces are present, other than electrostatic. Helfrich undulation forces [32] are the probable source of the repulsion. The reason for the large swelling with C_{16}TAC as surfactant, compared to C_{12}TAC, would thus be that the perforated bilayers are much more flexible than the compact bilayers, and give large amplitude undulations.

The solubilization process is illustrated by the micrographs in Fig. 8, and is seen to occur in a typical three-stage sequence: incorporation of surfactant in the bilayers gives vesicles of varying size and shape, mixed micelles start to form, in this system thread-like micelles, that coexist with vesicles until all bilayers are solubilized. As more surfactant is added, the micelles finally go over into globular form (not shown). Most of the vesicles found in the two-phase area, together with thread-like micelles, had disrupted bilayers [30].

2.2.3. *GMO/C_{16}TAC/water or brine*

GMO is probably best known for the fact that it exposes a cubic phase to excess water or brine. The cubic phase is of the reversed type, i.e. the mean curvature of the lipid monolayers is slightly negative. When a charged surfactant is added the spontaneous curvature first gets balanced at zero, forming planar bilayers in a lamellar phase that on further addition of surfactant decomposes into mixed micelles, sometimes via defect bilayers. As shown in the phase diagrams of Fig. 9, for the system GMO/C_{16}TAC/water (brine), the charging of the bilayers also gives rise to an strong swelling of both the lamellar phase and the

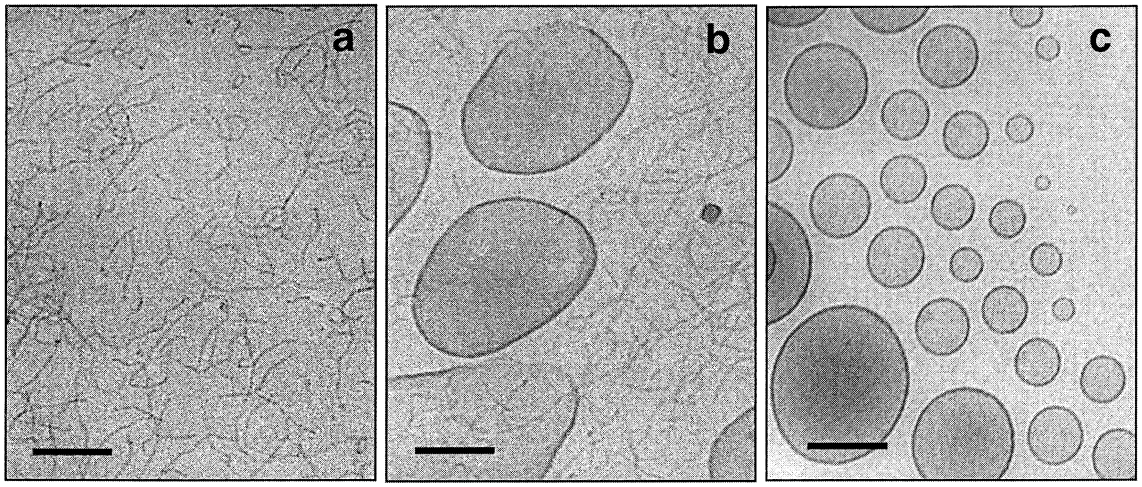

Fig. 8. In the solubilization of EPC by C_{12}TAC, large vesicles (c) dissolve into threads, coexisting (b) with vesicles having disrupted bilayers, and finally make thread-like micelles (a). Reproduced and adapted with permission from [30]. Copyright 1997 American Chemical Society.

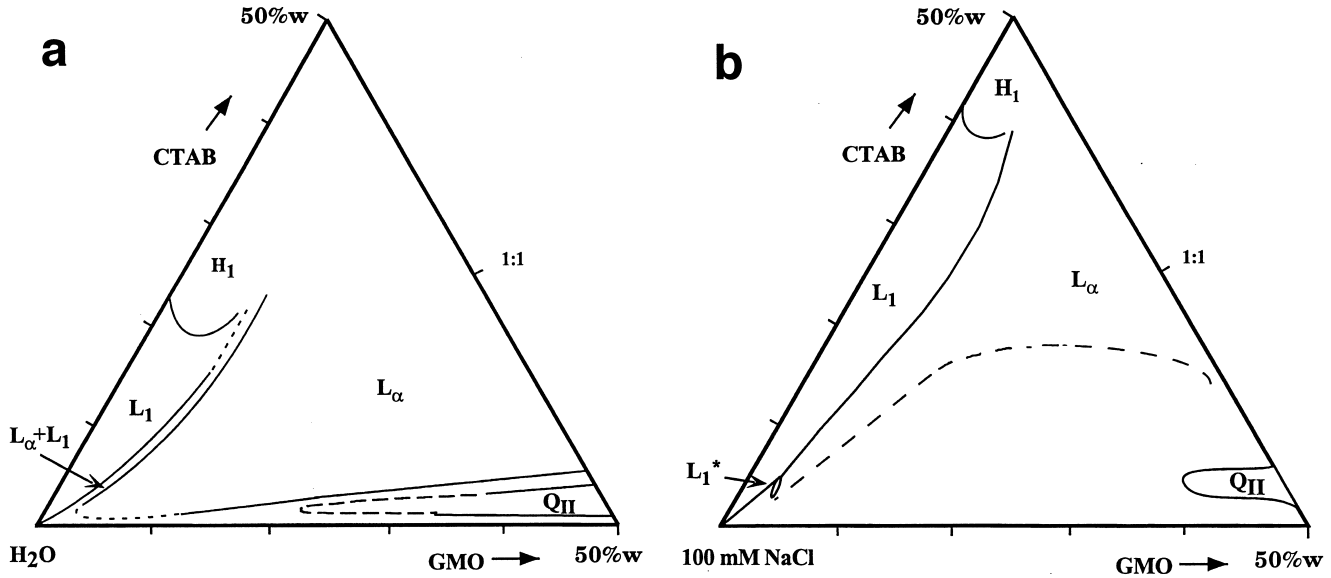

Fig. 9. Gibbs phase triangles for the systems C_{16}TAB/GMO/water and C_{16}TAB/GMO/0.100 M NaCl. The diagrams are similar in gross features with those for the lecithins, Figs. 4 and 5. Note, however, the cubic phase Q_{II} which swells from about 40% of water without surfactant (not shown) to 75% in water and 60% in brine. Reproduced and adapted with permission from [33]. Copyright 1998 American Chemical Society.

cubic phase. The lamellar phase, which is of prime interest here, is strikingly similar to that of EPC (Fig. 4), both with and without salt in the aqueous solvent. In particular, the narrow tongue that remains swollen in 0.100 M NaCl, also in the GMO system, seems to be stabilized by undulation forces, which are strengthened by a reduction of the rigidity of the bilayers from water-filled defects [33]. Just as in the phospholipid systems the formation of vesicles is facilitated when the bilayers become charged, and vesicles and open bilayer flakes with defects in the membranes are formed when salt is present. The perforated structures are solubilized without a clear two-phase region when more surfactant is added, forming lace-like structures and long thread-like or band-like micelles. In the absence of added salt the vesicles are without perforations, and no lace-like structures are found. The solubilization is more distinct with a two-phase region in the phase diagram (Fig. 9), but now both small globular micelles and long micelles formed as twisted ribbons are found together with defect free vesicles (Fig. 10) [33].

2.2.4. *EPC/$C_xSO_4^-Na^+$/water or brine*

The solubilization of sonicated EPC liposomes by anionic surfactants of the sodium dodecylsulfate (SDS) family has been investigated by turbidity and cTEM studies [34]. The solubilization processes are similar to those observed with cationic surfactants. Without salt there is just a dissolution of the small vesicles into mixed micelles, without any intermediate increase of the turbidity, whereas with 0.100 or 0.150 M NaCl growth occurs before the solubilization. The cTEM investigations reveal an interesting variation of the morphology, strongly depending upon the alkyl chain length. The surfactant with the shortest chain (C_{10}) at 0.150 M salt gave a normal three-stage course: large vesicles and open bilayers dissolve into thread-like micelles, which then gradually shorten in the L_1 area. The behavior is very different with SDS and the C_{14} homologue. Now the large intermediate vesicles have perforated or disrupted membranes, and are found together with open perforated bilayer flakes. With a further increase of the surfactant concentration, these structures turn directly into globular micelles without any thread-like micelles as intermediates. Just as when C_{16}TAC in 0.100 M salt is used to solubilize EPC, it is not clear where the lamellar phase stops and the micellar phase starts. It is possible that the regions where small perforated disks are seen together with micelles (in the case of SDS), or lace-like structures and entangled threads (in the

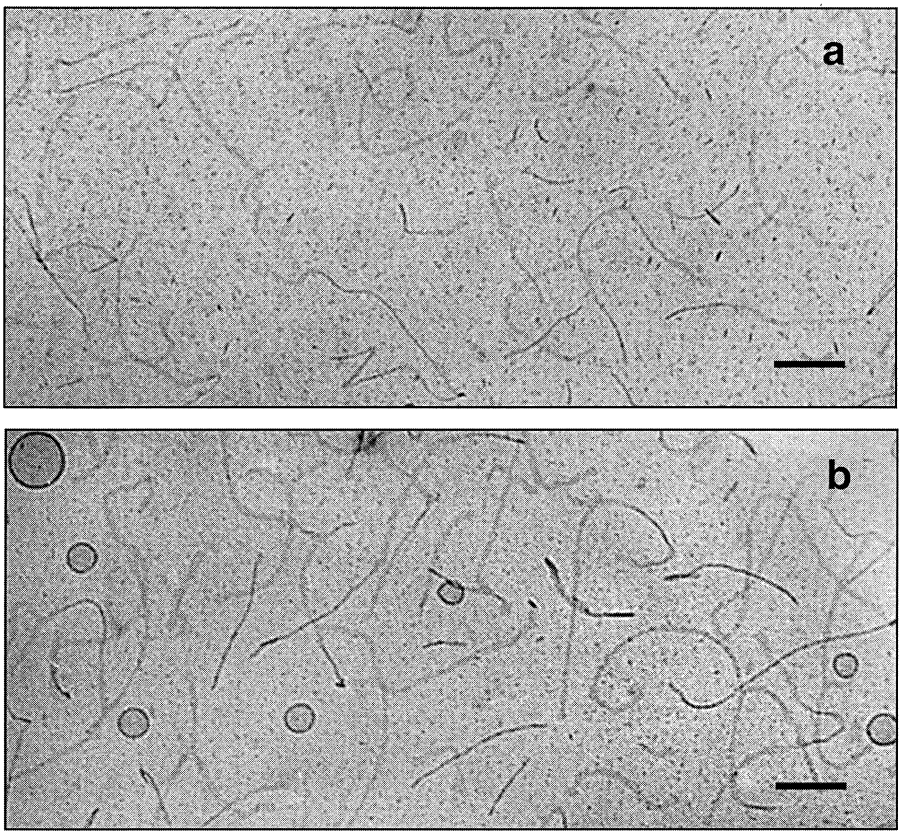

Fig. 10. cTEM micrographs showing long thread-like mixed micelles, apparently formed as twisted bands, in C_{16}TAB/GMO mixtures in 99.7% water. The weight fraction of surfactant to lipid was (a) 0.67 and (b) 0.47. In addition there are small micelles present in both, and in b also vesicles with intact bilayers. Reproduced with permission from [33]. Copyright 1998 American Chemical Society.

case of C_{16}TAC), belong to the two-phase area, although the solution appears as an L_1 phase, without birefringence or other indications of a lamellar phase. The lace-like structures and the perforated flakes would then be dispersed bilayers, and not equilibrium structures formed reversibly in the L_1 phase [34].

For SDS, when the salt concentration was reduced to 0.100 M, the perforated intermediates disappeared, and disk-like structures were observed, turning into globular micelles at higher surfactant concentrations. Thread-like micelles were only found in systems without salt, at high SDS concentrations, still with discoid structures appearing at lower surfactant concentration [34].

2.3. EPC or GMO/Na-cholate/water or brine

The interactions of bile salts with polar lipids are of particular interest because of the physiological significance of the bile salts. They effectively solubilize bilayer-forming lipids such as phospholipids and monoglycerides. A detailed phase diagram of lecithin and sodium cholate in water was published in 1966 by Small et al. [35,64], and molecular models of the structures of the mixed micelles and bilayers were proposed in 1967 [36,37]. These models have been very influential, and for a long time it was accepted as a fact that the mixed micelles were circular disk-like structures, comprising a bilayer of lecithins coated on the perimeter by bile salt molecules, exposing their hydrophilic surfaces to the surrounding aqueous solution, and their hydrophobic surfaces to the hydrophobic tails of the lipids. The long cylindrical micelles, which assembled into the hexagonal phase at high concentrations, were supposed to be built by stacking of discrete disk units. From static and dynamic light scattering results, Mazer et al. [38,39] proposed a modified model, retaining the disks, but now assuming that the bilayer contained

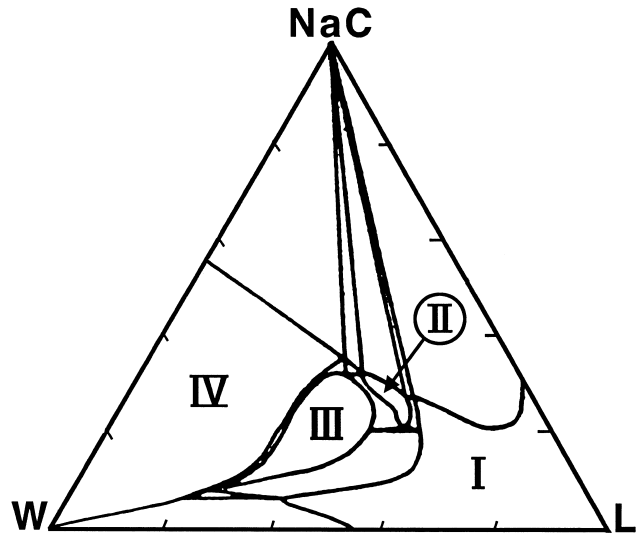

Fig. 11. The three-component phase diagram for sodium cholate/EPC/water as determined by Small et al. [35,64]. I to IV represent lamellar, bicontinuous cubic, hexagonal, and micellar phases, respectively. Note the three-phase triangle IV–III–I which guarantees that no hexagonal phase appears when the bile salt is added to the lecithins in excess water, but only lamellar phase and mixed micelles. Reproduced and adapted with permission from [35]. Copyright 1966 Elsevier Science.

some inserted bile salt molecules, possibly as hydrogen-bonded dimers.

Subsequently, results from small angle neutron scattering (SANS) were found to disagree with the disk-like micelle model, and indicated instead that rod-like structures were predominant [40,41]. A combination of careful static and dynamic light scattering measurements [42] and SANS experiments [43] showed that long flexible micelles were formed; such structures were also directly imaged by cTEM [10,44]. NMR relaxation and diffusion studies of the hexagonal phase [45] had already shown that the lipid diffusion in the cylinders of the hexagonal phase was about as facile as in a neat liquid, and showed clearly that the cylinders had a continuous hydrophobic core that was liquid-like. This result refuted both the idea of the rod as a stack of disks, and in my mind also any model that prescribes a very precise molecular packing of the lipids and bile salts in the structures – we are dealing with mixed micelles, not crystalline material. Anyway, now it seems to be settled that rod-like micelles are the important intermediates in the solubilization of lecithin bilayers by bile salts. However, by time-resolved SANS studies [46], it has recently been shown that the postulated [47,65] disk-like intermediates really are present as

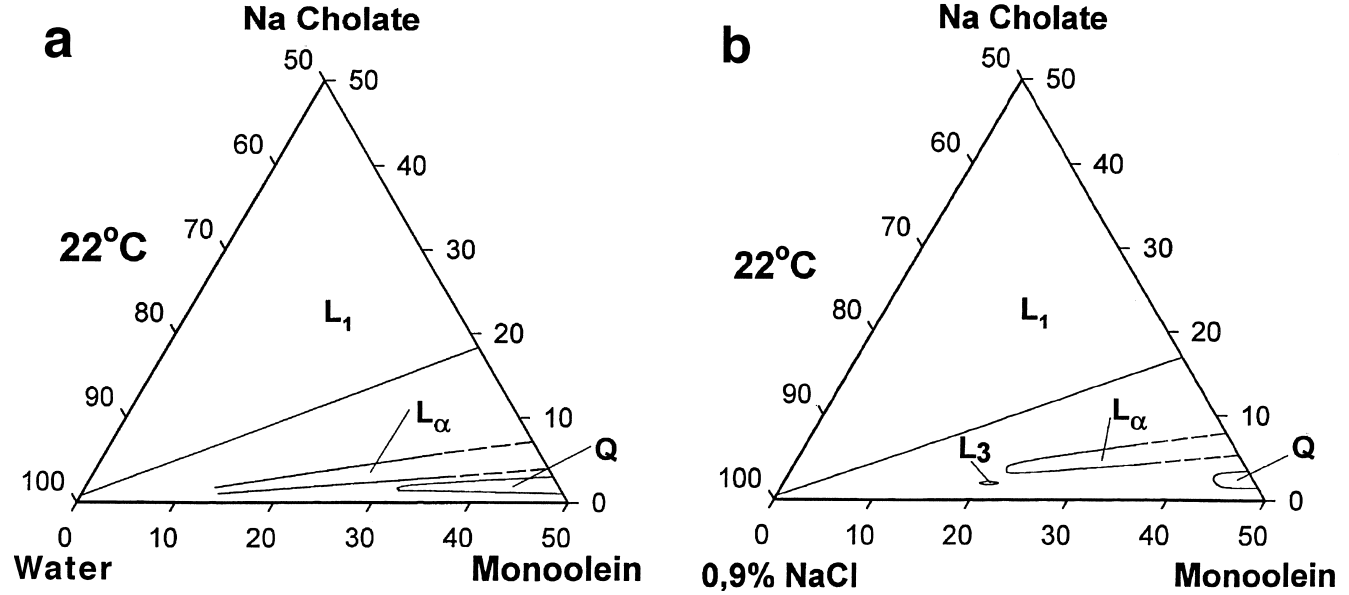

Fig. 12. The systems sodium cholate/GMO/water (a) and 0.9 wt% NaCl (b) at 22°C. Both in water and in brine the lamellar and cubic phases swell strongly, in water to a limit hard to determine exactly. Note the appearance of a L_3 phase, a so-called sponge phase or 'molten cubic' phase in the brine system, and that the micellar phase is very dominant in both systems, with almost the same solubility limit. Reproduced and adapted with permission from [48]. Copyright 1999 Academic Press.

short-lived transient species in the reversed process where vesicles are formed from mixed micelles by dilution (which due to the comparatively high solubility of the bile salt reduces the concentration of bile salt in the micelle, so that bilayers form).

Phase diagrams are presented in Figs. 11 and 12, for EPC/Na-cholate/water [35,64], and GMO/Na-cholate/water or brine (0.150 M NaCl) [48]. In all cases we see that the micellar L_1 phase is very prominent, extending to a molar ratio of lipid to cholate of about 2 for both lecithin and GMO. The bile salts are thus very effective in breaking bilayers and solubilizing polar lipids. The GMO bilayer can take up only 0.14 cholate molecules per lipid, whereas the EPC bilayers can accommodate up to 0.3 molecules.

As stressed above, a significant difference between GMO and egg lecithin is the presence of a cubic phase in the former in excess water, whereas the lamellar phase is the signature of the membrane-building lecithins. Only a small change of the spontaneous curvature is required to turn the cubic phase of GMO into a lamellar phase, and only about 0.07% bile salt is required in this case [48]. In the monoolein system both the cubic and the lamellar phases are strongly swollen in water, extending in narrow tongues towards the water corner in the phase diagram. In 0.150 M NaCl the swelling is weaker, and an island of L_3 phase is present between the tip of the lamellar phase and the aqueous corner. In all cases there are clear two- or multi-phase areas between the lamellar phase and the micellar phase. In the lecithin system both a hexagonal phase and at lower water content a cubic phase have been identified between the L_α phase and L_1. Neither of these

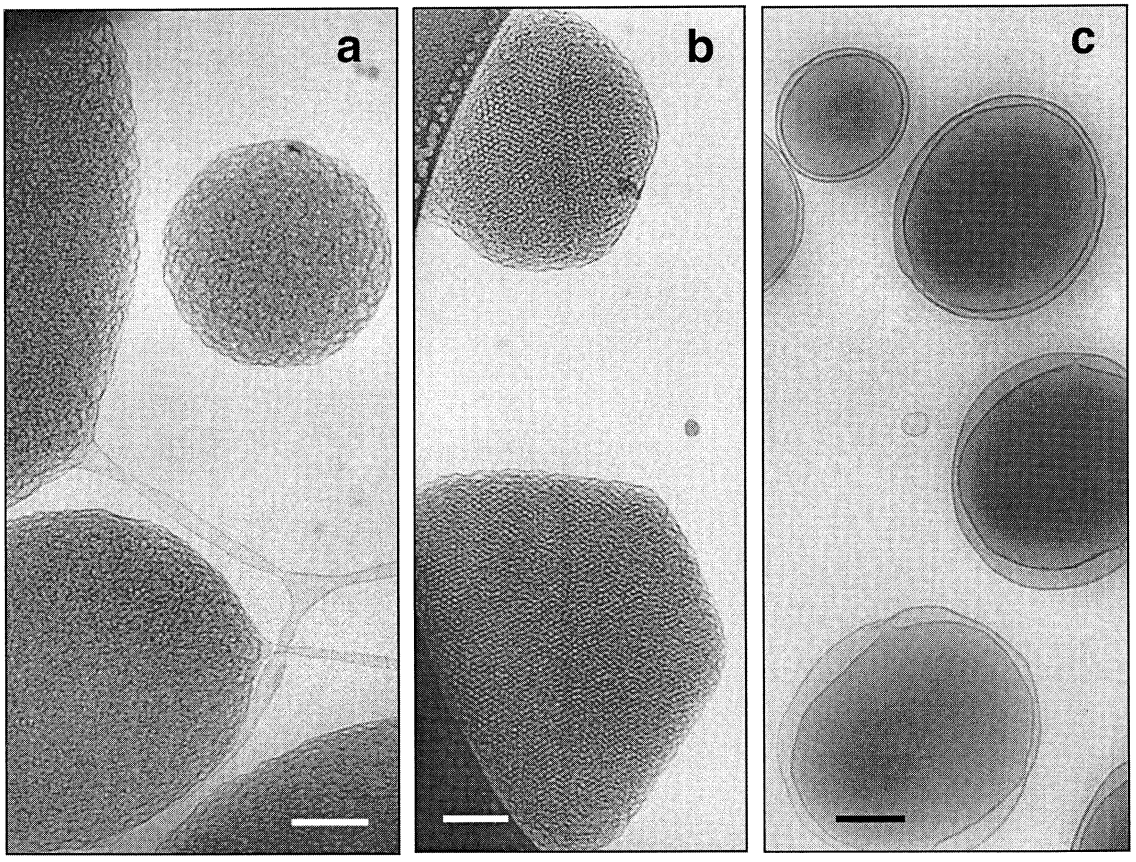

Fig. 13. (a, b) Particles observed in dispersions prepared by diluting the L_3 phase in excess brine (to 99% solvent). The particles in a have a non-periodic inner structure and may represent fragments of the L_3 phase, those in b have a periodic inner structure with hexagonal symmetry (and many defects) compatible with a cubic phase. (c) Passages and interconnections between the two membranes are found in this micrograph of double-walled liposomes formed on dilution of the lamellar phase in brine, sodium cholate/GMO = 0.15, 99% brine. Bar = 100 nm. Reproduced and adapted with permission from [48].

phases is in direct contact with the water corner, however. Instead a L_1, L_α, H_1 three-phase triangle blocks the contact. Addition of sodium cholate to a vesicle dispersion of the lamellar phase gives a rather typical three-stage course, where mixed rod-like micelles start to appear after saturation of the lamellar phase. The structures have been imaged by cTEM, and characterized in scattering studies, as discussed above. Interestingly, it was found that the solubilization was little dependent on the salt concentration in the solution [49]. Both R_{sol} and R_{sat} were practically the same in salt-free solutions and in 500 mM NaCl; it was observed, however, that growth of the vesicles prior to the formation of the mixed micelles occurred only with added salt, similar to other systems [31,34].

A dispersion of GMO in water or brine is not stable, but cubic phase particles quickly rejoin in domains of macroscopic dimensions. However, a little added charged surfactant increases the stability of the dispersion, at least so much that a cTEM investigation can be made, Fig. 13. Small dispersed particles of the cubic phase (cubosomes) have been found, and with salt present also particular dispersions of the L_3 phase. Dilution of the lamellar phase in excess brine, as in Fig. 13c, gives vesicles of interesting shape (without micelles). The vesicles are in general double-walled, with frequent interconnections between the two bilayers. This is understood as an influence from the nearby L_3 phase (which can be looked upon as a molten lattice of interpassages). When the lamellar phase is surrounded by excess aqueous solution, a substantial amount of the bile salt will escape from the membrane to the solution, and the composition of the bilayer will approach that of the L_3 phase. With more bile salt present, a broad coexistence region of bilayer structures, closed or open, and more or less globular micelles are found both in water and in brine. There are no thread-like micelles in this system. The mixed micelles seem to remain globular up to the L_1 saturation limit. This difference from the lecithin–bile system is in line with the absence of a hexagonal phase in the GMO system (Fig. 12). In the lecithin case, the phase diagram of Fig. 11 shows that the extrapolated L_1 border at lower water contents coincides with the hexagonal phase border, showing that this cholate/lecithin ratio favors formation of cylindrical aggregates.

3. Concluding summary and outlook

In this review I have discussed the solubilization of lipid bilayers by normal surfactants, and in particular the structures, intermediate or final, resulting from the process. As far as possible I have tried to relate the particularities to phase relations. It is obvious that the understanding of these processes would be much improved if the phase behavior of the pertinent systems were mapped out in greater detail.

The so-called three-stage model for liposome solubilization has been generally successful, in particular for the non-ionic systems. But often a closer examination reveals perturbing features, related to the interactions between the structures, and often accompanied by a macroscopic phase separation. Most noteworthy is the observation, first reported in [3], that on the solubilization of lecithin bilayers with OG, the saturated bilayers equilibrate in a three-phase region with two micelle phases, one dilute, the other more concentrated and viscous, containing rod micelles.

There seem to be two main types of solubilization behavior. In the case of non-ionic surfactants, the lipid bilayers seem to reach 'saturation' because mixed micelles start to form in the aqueous solution, and the chemical potential of the surfactant stays almost constant after that point. This implies that the composition of the bilayers also remains fixed during the dissolution. The phase behavior encountered with many charged surfactants is different, however. The lamellar phase seems to become destabilized at a certain content of surfactant in the membrane, and then disintegrates, forming mixed micelles, or a hexagonal phase, or an intermediate phase. In the phase diagram the stability limit is a straight line, indicating the bilayer composition where the spontaneous curvature of the monolayer becomes too large for the planar structure to persist.

In some systems, when salt is added, the two-phase area between L_1 and L_α becomes indistinct, and a direct transition from a defect lamellar phase to a micelle phase with unusual structure often seems to occur. It is possible that the unusual structures in reality are dispersed parts of the lamellar phase in a two-phase area; a decisive test, which can also be applied to vesicles proposed to be thermodynamically stable, is to investigate whether the structures

are equilibrium structures, in which case making and reversing a change of conditions should result in a reversible morphological change within the observation time after a change of conditions.

The defective intermediates, i.e. perforated vesicles and similar structures, are not entirely restricted to charged systems. There are suggestions, for example in [3], that a perforated lamellar phase is present in the EPC/OG/water system. Perforated vesicles, and a lamellar phase swelling in a narrow tongue to more than 97% of water, were also observed in a more unusual system, diglyceroldecanoate/glyceroldecanoate/water [50].

Bile salts are a special class of surfactants, and their behavior with polar lipids has been examined in great detail. The bile salts have a very good ability to solubilize polar lipids in mixed micelles, and seem to break down the bilayer already at comparatively low additions. Originally, disk-like mixed micelles were conjectured, with polar membrane lipids building the disk, and the bile salts covering the hydrophobic rim, but later work has shown that flexible cylinders are the dominant intermediates also in these systems, even if the disk-like structures have been re-established as transient intermediates in the transformation from mixed micelles to vesicles.

Disk-like intermediates – be they equilibrated micelles or bilayer fragments – have been established in many systems, with both non-ionic and ionic surfactants. Usually the disks are irregular in shape, but in some systems very planar and nicely circular disks have been found. An example was given in [51] where the effect of PEG lipids on DSPC and EPC liposomes was investigated. With about 10 mol% PEG lipid, circular and planar disks were obtained in both systems, instead of closed vesicles, when the lipid mixture of the bilayers had been fortified with 40 mol% of cholesterol, whereas irregular bilayer flakes were formed without cholesterol in the lipid mixture. The type of liposome membrane investigated in this example was devised for practical use (drug delivery), and is typically surrounded by hydrophilic polymer chains (such as PEG) for stabilization, anchored in the bilayer by covalent attachment to a polar lipid. Furthermore, they often have a high cholesterol content in the lipid mixtures, which improves the strength of the membrane and reduces leakage of entrapped hydrophilic substances, and also makes the bilayers more resistant to solubilization by surfactants [52,53]. The investigation of the stability of such more complex bilayer membranes has only begun. It can be anticipated that the rigid and mechanically tough membranes that are preferred for drug delivery applications will often give disk-like structures on disintegration by surfactants, but be harder to break down, since they withstand higher surfactant concentrations and solubilize slowly.

An important question is the effect on bilayer membranes of surfactants that are biologically relevant, such as lysolecithins and salts of fatty acids [54]. Except for systems with bile salts, such work has not been reviewed here, mainly because no systematic studies of the phase behavior seem to have been made as yet. Such investigations are needed. There are other topics that could have been given more attention in the review, such as studies of how variations of surfactant headgroup (charge [55], PEG size [56]), or the bilayer composition [57], influence the solubilization process. I have also chosen not to include studies of solubilization of 'synthetic' bilayer-forming surfactants, such as the extensive studies of the SDS–didodecyldimethylammonium bromide–water system [58,59] or the studies of dimer surfactants [60].

Theoretical treatments of the vesicle to mixed micelle transformation have not been covered here. Some simple models based on the differences in spontaneous curvature between surfactant and lipid have for example treated the transition from isolated bilayers to cylindrical micelles [61,62] and also discussed cylinder or disk issue [63].

Acknowledgements

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References

[1] J. Lasch, Interaction of detergents with lipid vesicles, Biochim. Biophys. Acta 1241 (1995) 269–292.

[2] U. Kragh-Hansen, M. le Maire, J.V. Møller, The mechanism of detergent solubilization of liposomes and protein-containing membranes, Biophys. J. 75 (1998) 2932–2946.
[3] S. Beugin-Deroo, Etude de la solubilization et de la formation de vésicules stabilisées non-ionique en présence d'octyl β-D-glucopyranoside, PhD thesis, Université Pierre et Marie Curie, Paris, 1997.
[4] A.S. Sadaghiani, A. Khan, B. Lindman, Liquid crystallinity of lecithin systems. Ternary phase diagram of lecithin-water with Triton X-100 and decanol, J. Colloid Interf. Sci. 132 (1989) 352–361.
[5] E.A. Dennis, Formation and characterization of mixed micelles of the nonionic surfactant Triton X-100 with egg, dipalmitoyl, and dimyristoyl phosphatidylcholines, Arch. Biochem. Biophys. 165 (1974) 764–773.
[6] A. Helenius, K. Simons, Solubilization of membranes by detergents, Biochim. Biophys. Acta 415 (1975) 29–79.
[7] D. Lichtenberg, R.J. Robson, E.A. Dennis, Solubilization of phospholipids by detergents, Biochim. Biophys. Acta 737 (1983) 285–304.
[8] M.-T. Paternostre, M. Roux, J.-L. Rigaud, Mechanisms of membrane protein insertion into liposomes during reconstitution using detergents. 1. Solubilization of large unilamellar liposomes (prepared by reversed phase evaporation) by Triton X-100, octyl glucoside and sodium cholate, Biochemistry 27 (1988) 2668–2703.
[9] K. Edwards, M. Almgren, J. Bellare, W. Brown, Effects of Triton X-100 on sonicated lecithin vesicles, Langmuir 5 (1989) 473–478.
[10] P.K. Vinson, Y. Talmon, A. Walter, Vesicle-micelle transition of phosphatidylcholine and octyl glucoside by cryotransmission electron microscopy, Biophys. J. 56 (1989) 669–681.
[11] K. Edwards, M. Almgren, Solubilization of lecithin vesicles by $C_{12}E_8$. Structural transitions and temperature effects, Colloid Interf. Sci. 147 (1991) 1–21.
[12] U. Kragh-Hansen, M. le Maire, J.-P. Noël, T. Gulik-Krzywicki, J.V. Møller, Transitional steps in the solubilization of protein-containing membranes and liposomes by nonionic detergent, Biochemistry 32 (1993) 1648–1656.
[13] E.A.G. Aniansson, S. Wall, M. Almgren, H. Hoffmann, J. Kielmann, W. Ulbricht, R. Zana, J. Lang, C. Tondre, Theory of the kinetics of micellar equilibria and quantitative interpretation of chemical relaxation studies of micellar solutions of ionic surfactants, J. Phys. Chem. 80 (1976) 905–922.
[14] M. Ollivon, O. Eidelman, R. Blumenthal, A. Walter, Micelle-vesicle transition of egg phosphatidylcholine and octylglucoside, Biochemistry 27 (1988) 1695–1703.
[15] B. Angelov, M. Ollivon, A. Angelova, X-ray diffraction study of the effect of the detergent octyl glucoside on the structure of the lamellar and nonlamellar lipid/water phases of use for membrane protein reconstitution, Langmuir 15 (1999) 8225–8234.
[16] J. Chopineau, S. Lesieur, M. Ollivon, Vesicle formation by enzymatic processes, J. Am. Chem. Soc. 116 (1994) 11582–11583.
[17] M. Ueno, Partition behavior of a nonionic detergent, octyl glucoside, between membrane and water phases and its effect on membrane permeability, Biochemistry 28 (1989) 5631–5634.
[18] J. Lasch, J. Hoffmann, W.G. Omelyanenko, A.A. Klibanov, V.P. Torchilin, H. Binder, K. Gawrisch, Interaction of Triton X-100 and octyl glucoside with liposomal membranes at sublytic and lytic concentrations – spectroscopic studies, Biochim. Biophys. Acta 1022 (1990) 171–180.
[19] A. De la Maza, J.L. Parra, Solubilizing effects caused by the nonionic surfactant dodecylmaltoside in phosphatidylcholine liposomes, Biophys. J. 72 (1997) 1668–1675.
[20] O. Lamberth, D. Levy, J.-L. Ranck, G. Leblanc, J.-L. Rigaud, A new 'gel-like' phase in dodecyl maltoside-lipid mixtures: implications in solubilization and reconstitution studies, Biophys. J. 74 (1998) 918–930.
[21] B. Carion-Taravella, J. Chopineau, M. Ollivon, S. Lesieur, Phase behavior of mixed aqueous dispersions of DPPC and dodecyl glycosides: aggregation states implicated in the micelle-to-vesicle transition, Langmuir 14 (1998) 3767–3777.
[22] B. Carion-Taravella, S. Lesieur, M. Ollivon, J. Chopineau, Enzyme-mediated formation of vesicles from DPPC-dodecyl maltoside mixed micelles, J. Am. Chem. Soc. 120 (1998) 10588–10595.
[23] I. Ribosa, J. Sanchez-Leal, F. Comelles, M.T. Garcia, Solubilization of large unilamellar liposomes by alkyl glycosides, J. Colloid Interf. Sci. 187 (1997) 443–446.
[24] K. Kameyama, T. Takagi, Micellar properties of octylglucoside in aqueous solution, J. Colloid Interf. Sci. 137 (1990) 1–10.
[25] L. Rydhag, T. Gabrán, Phase equilibria in the system dimyristoylphosphatidylcholine-N-hexadecyl-N,N,N-trimetylammonium bromide and water, Chem. Phys. Lipids 30 (1982) 309–324.
[26] L. Rydhag, P. Stenius, L. Ödberg, Phase equilibria and vesicle stability in dimyristoyl phosphatidylcholine-cetyltrimethylammonium bromide systems, J. Colloid Interf. Sci. 86 (1982) 274–276.
[27] J. Gustafsson, G. Orädd, G. Lindblom, U. Olsson, M. Almgren, A defective swelling lamellar phase, Langmuir 13 (1997) 852–860.
[28] B. Jönsson, P.K.T. Persson, Effects of charged amphiphiles on the stability of lamellar phases, J. Colloid Interf. Sci. 115 (1987) 507–512.
[29] H. Wennerström, The unbinding transition and lamellar phase-lamellar phase coexistence, Langmuir 6 (1990) 834–838.
[30] J. Gustafsson, G. Orädd, M. Almgren, Disintegration of the lecithin lamellar phase by cationic surfactants, Langmuir 13 (1997) 6956–6963.
[31] K. Edwards, M. Almgren, J. Gustafsson, G. Karlsson, Solubilization of lecithin vesicles by a cationic surfactant. Intermediate structures in the vesicle-micelle transition observed by cryo-TEM, J. Colloid Interf. Sci. 161 (1993) 299–309.
[32] W. Helfrich, Z. Naturforsch. 33a (1978) 305–311.
[33] J. Gustafsson, G. Orädd, M. Nyden, P. Hansson, M. Alm-

gren, Phase behavior of glycerol monooleate and cetyltrimethylammoniumbromide in aqueous solution: Defective lamellar phases and micellar polymorphism, Langmuir (1998) 4987–4996.
[34] M. Silvander, G. Karlsson, K. Edwards, Vesicle solubilization by alkyl sulfate surfactants: a cryo-TEM study of the vesicle to micelle transition, J. Colloid Interf. Sci. 179 (1996) 104–113.
[35] D.M. Small, M. Bourges, D.G. Dervichian, Biochim. Biophys. Acta 125 (1996) 563–580.
[36] D.M. Small, Physico-chemical studies of cholesterol gallstone formation, Gastroenterology 52 (1967) 607–610.
[37] D.M. Small, The physical chemistry of cholanic acids, in: P.P. Nair, D. Kritchasky (Eds.), The Bile Acids, Plenum Press, New York, 1971, Vol. 1, p. 340.
[38] N.A. Mazer, M.C. Carey, R.F. Kwasnick, G.B. Benedek, Quasielastic light scattering studies of aqueous biliary lipid systems. Size, shape, and thermodynamics of bile salt micelles, Biochemistry 18 (1979) 3064–3075.
[39] N.A. Mazer, G.B. Benedek, M.C. Carey, Quasielastic light scattering studies of aqueous biliary lipid systems. Mixed micelle formation in bile salt-Lecithin solutions, Biochemistry 19 (1980) 601–615.
[40] R.P. Hjelm, P. Thiyagaragan, D.S. Sivia, P. Lindner, H. Alkan, D. Schwahn, Small-angle neutron scattering from aqueous mixed colloids of lecithin and bile salt, Progr. Colloid Polymer Sci. 81 (1990) 225–231.
[41] R.P. Hjelm, P. Thiyagaragan, H. Alkan-Onyuksel, Organization of phosphatidylcholine and bile salt in rodlike mixed micelles, J. Phys. Chem. 96 (1992) 8653–8661.
[42] S.U. Egelhaaf, P. Schurtenberger, Shape transformation in the lecithin-bile salt system. From cylinders to vesicles, J. Phys. Chem. 98 (1994) 8560–8573.
[43] J.S. Pedersen, S.U. Egelhaaf, P. Schurtenberger, Formation of polymerlike mixed micelles and vesicles in lecithin-bile salt solutions: A small angle neutron scattering study, J. Phys. Chem. 99 (1995) 1299–1305.
[44] A. Walter, P.K. Vinson, A. Kaplun, Y. Talmon, Intermediate structures in the cholate-phosphatidylcholine vesicle-micelle transition, Biophys. J. 60 (1991) 1315–1325.
[45] J. Ulmius, G. Lindblom, H. Wennerström, L.B.-Å. Johansson, K. Fontell, O. Söderman, G. Arvidson, Molecular organisation in the liquid crystalline phases of lecithin-Sodium Cholate-water system studied by nuclear magnetic resonance, Biochemistry 21 (1982) 1553–1560.
[46] S.U. Egelhaaf, P. Schurtenberger, Micelle-to-vesicle transition: A time-resolved structural study, Phys. Rev. Lett. 82 (1999) 2804–2807.
[47] D.D. Lasic, The mechanism of vesicle formation, Biochem. J. 256 (1988) 1–11.
[48] J. Gustafsson, T. Nylander, M. Almgren, H. Ljusberg-Wahren, J. Colloid Interf. Sci. 211 (1999) 326–335.
[49] D. Meyhous, A. Bor, I. Pinchuk, A. Kaplun, Y. Talmon, M. Kozlov, D. Lichtenberg, J. Colloid Interf. Sci. 188 (1997) 351–362.
[50] H. Ljusberg-Wahren, J. Gustafsson, T. Gunnarsson, N. Krog, L. Wannerberger, M. Almgren, Micelles and liquid crystals in aqueous diglycerol monodecanoate systems, Progr. Colloid Interf. Sci. 108 (1998) 99–104.
[51] K. Edwards, M. Johnsson, G. Karlsson, M. Silvander, Effect of polyethyleneglycol-phospholipids on aggregate structure in preparations of small unilamellar liposomes, Biophys. J. 73 (1997) 258–266.
[52] S. Beugin-Deroo, M. Ollivon, S. Lesieur, Bilayer stability and impermeability of nonionic surfactant vesicles sterically stabilized by PEG-cholesterol conjugates, J. Colloid Interf. Sci. 202 (1998) 324–333.
[53] S. Beugin, K. Edwards, G. Karlsson, M. Ollivon, S. Lesieur, New sterically stabilized vesicles based on nonionic surfactant, cholesterol, and poly(ethylene glycol)-cholesterol conjugates, Biophys. J. 74 (1998) 3198–3210.
[54] K. Edwards, M. Silvander, G. Karlsson, Aggregate structure in dilute aqueous dispersions of oleic acid/sodium oleate and oleic acid/sodium oleate/egg phosphatidylcholine, Langmuir 11 (1995) 2429–2434.
[55] M.A. Urbaneja, A. Alonso, J.M. Gonzales-Manas, F.M. Goni, M.A. Partearroyo, M. Tribout, S. Paredes, Detergent solubilization of phospholipid-vesicles – effect of electric charge, Biochem. J. 270 (1990) 305–308.
[56] M.A. Partearroyo, A. Alonso, F.M. Goni, M. Tribout, S. Paredes, Solubilization of phospholipid bilayers by surfactants belonging to the Triton X series: Effect of polar group size, J. Colloid Interf. Sci. 178 (1996) 156–159.
[57] M.A. Urbaneja, J.L. Nieva, F.M. Goni, A. Alonso, The influence of membrane composition on the solubilizing effects of Triton X-100, Biochim. Biophys. Acta 904 (1987) 337–345.
[58] E.F. Marques, O. Regev, A. Khan, M.D. Miguel, B. Lindman, Vesicle formation and general phase behavior in the catanionic mixture SDS-DDAB-water. The anionic-rich side, J. Phys. Chem. B 102 (1998) 6746–6758.
[59] E.F. Marques, O. Regev, A. Khan, M.D. Miguel, B. Lindman, Vesicle formation and general phase behavior in the catanionic mixture SDS-DDAB-water. The cationic-rich side, J. Phys. Chem. B 103 (1999) 8353–8363.
[60] D. Danino, Y. Talmon, R. Zana, Vesicle-to-micelle transformation in systems containing dimeric surfactants, J. Colloid Interf. Sci. 185 (1997) 84–93.
[61] D. Andelman, M.M. Kozlov, W. Helfrich, Phase transitions between vesicles and micelles driven by competing curvatures, Europhys. Lett. 25 (1994) 231–236.
[62] D.R. Fattal, D. Andelman, A. Ben-Shaul, The vesicle-micelle transition in mixed lipid-surfactant systems: a molecular model, Langmuir 11 (1995) 1154–1161.
[63] M.M. Kozlov, D. Lichtenberg, D. Andelman, Shape of phospholipid/surfactant mixed micelles: cylinders or disks? Theoretical analysis, J. Phys. Chem. 101 (1997) 6600–6606.
[64] D.M. Small, M. Bourges, D.G. Dervichian, Nature 211 (1966) 816–818.
[65] D.D. Lasic, Biochim. Biophys. Acta 692 (1982) 501–502.