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Liquid crystalline behavior of concentrated aqueous solutions of biosurfactants

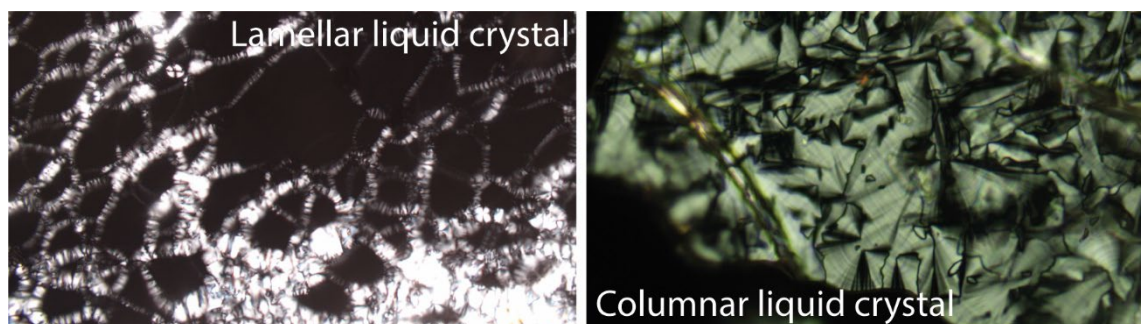
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Abstract

In the current global context of the search for renewable resources, bioamphiphiles appear as a promising alternative to conventional oil-based surfactants. However, to commercialize these compounds, all their structural and physical properties should be known. Although their self-assembly and interfacial properties at low concentrations are currently an active research topic, their self-organization into liquid crystalline (LC) phases at high concentrations has yet hardly been addressed. This article reviews the few studies devoted to the identification of LC properties of bioamphiphiles. It highlights the fact that only two bioamphiphile families (mannosylerythritol lipids, sophorolipids) have been investigated in some detail and that much more structural and thermodynamic knowledge is still needed to reach the level of understanding achieved with conventional surfactants.



ToC Graphic

1. Introduction

Bio-based amphiphilic molecules produced by micro-organisms (bacteria, yeasts, etc.), hereafter referred to as bioamphiphiles (BA), may in the distant future replace conventional oil-based surfactants in our daily lives.[1,2] However, for this to happen, their physical properties need to be thoroughly explored from a fundamental point of view. Among these, their self-assembly behavior in aqueous solution is of great importance, as it affects both product performance and industrial processing. Studies of BA self-assembly have recently been undertaken worldwide, but have too often been restricted to the case of dilute solutions, typically at weight fractions lower than 10 wt%.[3] In contrast, the self-assembly of oil-based surfactants is known in great detail, particularly with regard to their liquid crystalline (LC) character at high concentrations, typically at weight fractions higher than 50 wt%. Therefore, since the basic concepts and investigation techniques relevant to liquid crystal science may be somewhat unfamiliar to scientists interested in BA self-assembly, we will first recall them below.

Liquid crystals are materials that exhibit states of matter which are intermediate between the crystalline and liquid ones. By definition, an LC phase is anisotropic, like crystals, yet fluid, like ordinary (isotropic) liquids.[4] These different phases can be observed when either the temperature or the concentration in a solvent is varied. In the former case, the liquid crystals are said to be “thermotropic” and a typical example is the small rod-like organic molecules that are used in LC displays. In the latter case, the liquid crystals are called “lyotropic” and may appear when amphiphilic molecules in solution (above the critical micellar concentration) self-assemble into molecular aggregates such as membranes or rod-like (or spherical) micelles, in concentrated regions of the phase diagrams.

Being both anisotropic and fluid, LC phases are birefringent (except for cubic phases which are optically isotropic) but, unlike crystals, they flow, with some viscosity. Moreover, they can be classified in different categories according to the dimensionality of the positional long-range order of their constituents (molecules or molecular aggregates). LC phases that have long-range orientational order but only short-range positional order, are called “nematic” (see Figure 1). They are arguably the most common for thermotropic liquid crystals but they are less frequently observed with amphiphilic molecules. LC phases that, in addition to long-range orientational order, have long-range positional order in one dimension, are called “smectic” or “lamellar”. LC phases that have long-range positional

order in two dimensions are called “columnar”. The most frequent is the hexagonal columnar phase which is made of cylinders of circular cross section, but other columnar phases of lower symmetry (i.e. rectangular or oblique) can be found when the cylinders have an elliptical cross section, such as in surfactant mixtures. Finally, LC phases that have long-range positional order in the three dimensions of space, but with a unit cell much larger than the molecular dimensions are usually (but not always) of cubic symmetry. Their structure can be described either in terms of intertwined membrane networks (“bicontinuous” cubic phases) or in terms of “micellar” cubic packings. In contrast with ordinary crystals, molecules can easily diffuse within these structures. In addition, the so-called “sponge phase” is observed when the membrane networks are completely disordered. This phase is structurally isotropic and therefore should not be considered as liquid crystalline. Note that all these structures can be described in terms of different modes of organization of surfaces. The principal radii of curvature, R_1 and R_2 , at each point, are important geometric quantities that characterize each surface. They are used to define the mean curvature, $1/R_1 + 1/R_2$, and the Gaussian curvature, $(R_1 \cdot R_2)^{-1}$ of the surface. The L_α phase has no mean or Gaussian curvature, whereas the columnar phase has no Gaussian curvature but non-zero mean curvature. The micellar cubic phases have non-zero mean curvature and positive Gaussian curvature whereas the bicontinuous phases usually have zero mean curvature but negative Gaussian curvature (and can be described mathematically by “minimal” surfaces). The sponge phase is also thought to have negative Gaussian curvature.

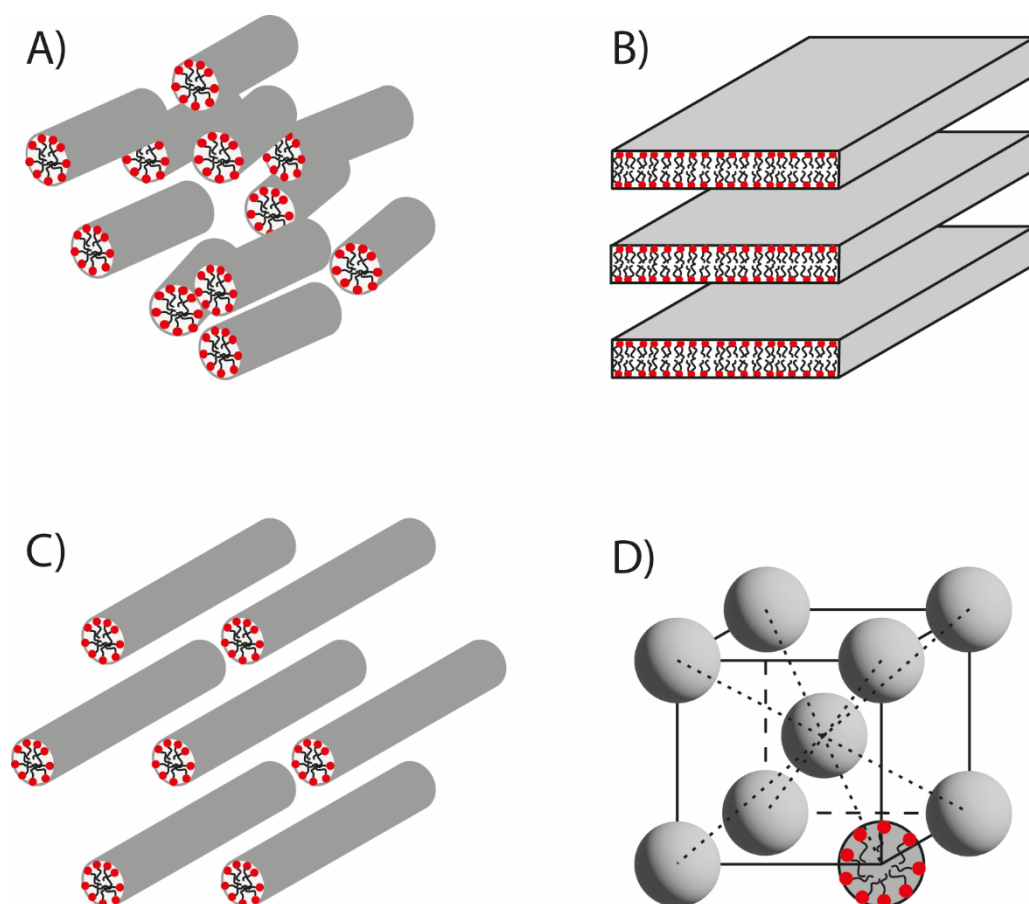


Figure 1: Schematics of the structures of the main liquid crystalline phases formed by aggregates of amphiphilic molecules. A) Nematic phase of small elongated micelles; B) lamellar (L_{α}) phase of infinite membranes; C) direct columnar hexagonal (Col_H) phase of infinite cylindrical micelles; D) direct body-centered cubic phase (as an example of cubic phase; one of the micelles is cut to show its internal structure).

From an experimental point of view, the structures of LC phases are mostly investigated by examining their textures with polarized-light microscopy (PLM) and by recording their X-ray diffraction (XRD) patterns. Optical textures provide valuable structural information because the topological defects that they reveal are characteristic of the order of the phases. The textures therefore directly reflect the basic symmetries of each phase: the constant periodicity of the lamellar phase leads to “focal conic” domains whereas the condition of constant lattice vector moduli of columnar phases leads to “developable” domains. (Both focal conic domains and developable domains refer to particular geometric objects that verify these conditions.) Historically, this is how the structures of the major thermotropic LC phases were first understood. However, the correct identification of LC phases based on PLM texture observation alone requires much expertise and, above all,

clear textures. For lyotropic LCs, this already difficult process is further complicated by solvent evaporation when samples are held between glass slide and coverslip. Instead, samples of well-defined concentrations should be prepared and introduced into flat glass optical capillaries that are flame-sealed after filling. If properly prepared, such samples can be stored indefinitely for convenient microscopic observation. Although this technique is perfectly adequate, it has the disadvantage of requiring point by point exploration of the phase diagrams, resulting in the preparation of numerous samples. To avoid this, the so-called “contact preparation” technique has been imported from the field of thermotropic LCs. The idea there is to bring a small amount of crystalline powder of the compound of interest into contact with pure water and examine the textures of the phases that appear along the concentration gradient between the powder and pure water. This provides a continuous scan of the concentration, using very small amounts of material, rather than discrete data points in the phase diagram. In the field of bioamphiphiles, this technique is often referred to as “water penetration scan” or “flooding penetration method”. However, apart from the evaporation problem already mentioned, the exact concentration at which a lyotropic LC phase forms along the gradient is not known. In addition, the sample may not reach full thermodynamic equilibrium and the texture may not coarsen sufficiently before the solvent evaporates. In fact, ideally, the two methods should be combined, as each has its own merits. Then, short of being a specialist in LC textures and topological defects, the PLM observations should be compared with the photographs shown in specialized books.[5,6] (An introduction to the detailed mathematical description of the specific topological defects that form the textures can be found in references [7,8].) However, identification errors are still commonly found in the literature of lyotropic LCs, such as confusing multilamellar vesicles suspended in excess water with focal-conic defects embedded in a homeotropically-aligned lamellar phase (i.e. with surfactant membranes parallel to the glass plates).

In general, the phase assignment based on PLM observations should therefore be systematically confirmed by XRD experiments, which can be performed either with in-house setups or at the beamlines of X-ray synchrotron facilities. The nature of each LC phase (in “powder” form, i.e., unoriented) can be identified by the detection of sharp diffraction lines indicative of long-range positional order. There are no sharp lines in the scattering patterns of the nematic phase; a series of equidistant sharp lines are observed in the patterns of the lamellar phase, due to its layered structure; sharp lines arising from a 2-dimensional (resp. 3-

dimensional) lattice correspond to a columnar (resp. cubic) LC phase. The reader unfamiliar with these concepts is referred here to X-ray scattering textbooks.[9,10] Here, samples of mixtures of well-defined concentrations should be filled into cylindrical X-ray glass capillaries which are flame-sealed after filling.

This review will only focus on low molecular weight BA produced by micro-organisms (see Figure 2): glycolipids such as mannosylerythritol lipids, rhamnolipids, sophorolipids, trehalolipids, and lipopeptides, such as surfactin. (For a more general, albeit older, review of the liquid crystalline behavior of biologically relevant carbohydrate-based liquid crystals, see reference [11].) Compared to conventional oil-based surfactants, these amphiphilic compounds tend to be more subtle because they can adopt more molecular conformations, even in their hydrophilic head group. For this reason, these molecules will be referred to as “bioamphiphiles” rather than “biosurfactants in this review”. They also sometimes exist in different forms, such as the lactonic or acidic forms of sophorolipids that are predominant in different pH ranges. They can also be sensitive to the temperature cycles often used to homogenize and anneal samples. In addition, they are generally produced by micro-organisms as mixtures of different molecules (congeners) that differ in the length of their lipid chain and the degree of acetylation of their sugar head group, a feature that should hinder the crystallization of the mixtures. Some of them are bolaamphiphiles, meaning that they have hydrophilic groups at both ends of their lipid chains, which adds additional complexity to the structures of their self-assemblies. Together with the small amounts of BA materials usually available, all these reasons may explain why so few studies have to date been devoted to determining the LC properties of BA compounds. (In fact, to the best of our knowledge, we are not aware of any other review article dealing with the LC behavior of bioamphiphiles in general.) In the following sections, we will review these studies according to the chemical nature of the bioamphiphiles.

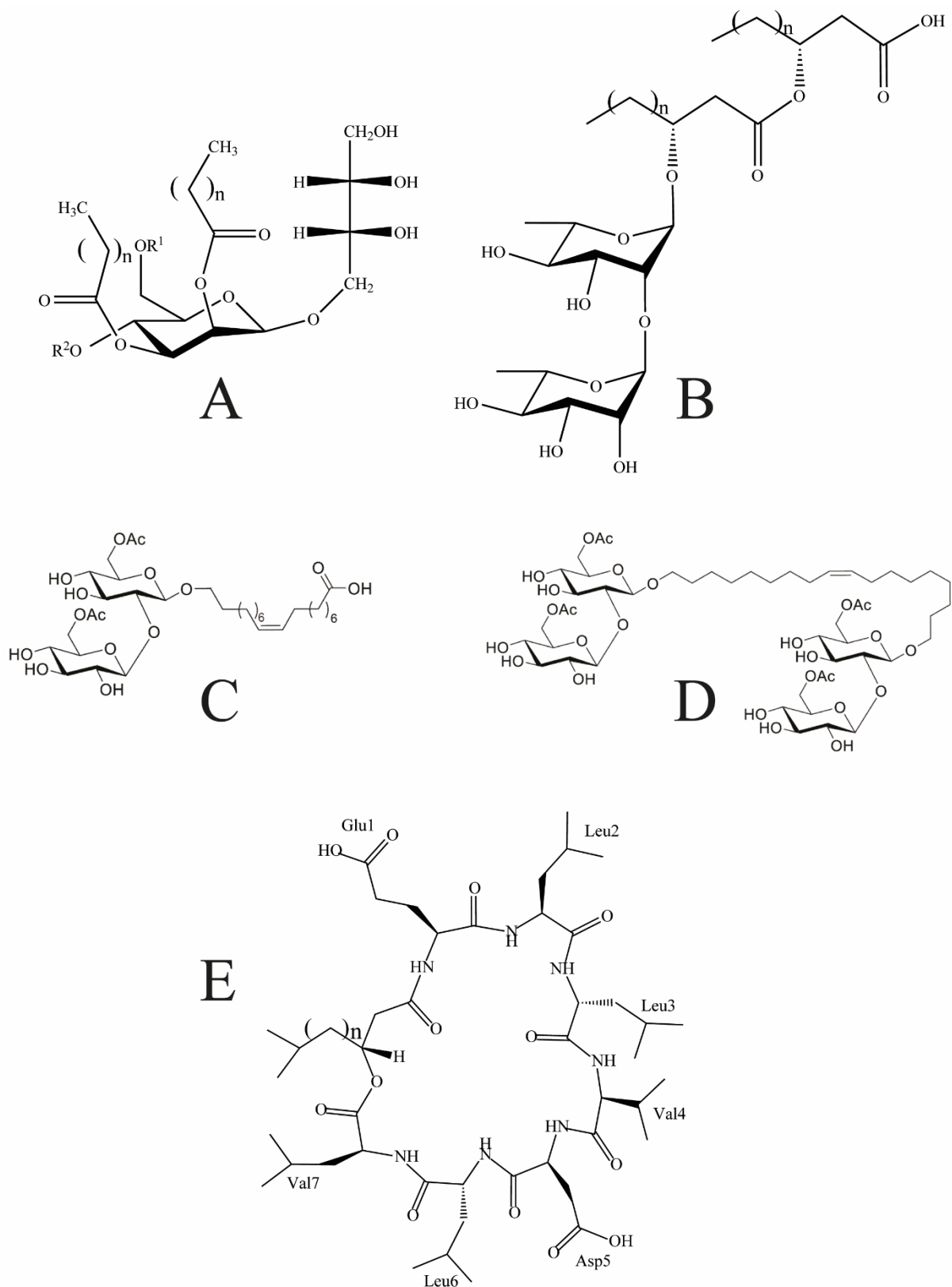


Figure 2: Chemical formulas of the families of bioamphiphiles discussed in this review. A) Mannosylerythritol lipids: MEL-A: $R_1 = R_2 = \text{Ac}$; MEL-B: $R_1 = \text{Ac}$, $R_2 = \text{H}$; MEL-C: $R_1 = \text{H}$, $R_2 = \text{Ac}$; MEL-D: $R_1 = R_2 = \text{H}$. B) Di-rhamnolipids. C) Mono-sophorolipid. D) Di-sophorolipid. E) Surfactin in lactonic conformation.

2. Mannosylerythritol lipids (and mannosylmannitol lipids)

This family of glycolipids has been the most extensively investigated to date, particularly by the pioneering group of D.Kitamoto and co-workers. The general chemical formula of mannosylerythritol lipids (hereafter referred to as MELs) is shown in Figure 2A. These BA are produced in high yields by yeasts of the genus *Pseudozyma* from renewable sources and they are already commercially available for applications in cosmetics and bioremediation. MELs appear as four main modifications, depending on the (de)acetylation at two different sites of the sugar polar head. In general, the hydrophobicity of MELs increases with their degree of acetylation, with MEL-A considered to be the most hydrophobic. Moreover, the samples are usually mixtures of several congeners that differ in the length of their fatty acid chains. In most cases, *Pseudozyma* yeasts secrete mainly MEL-A, with MEL-B and MEL-C being minor components.[12]

2.1. MEL A

a) “Standard” MEL-A

The liquid crystalline behavior of aqueous solutions of the di-acetylated MEL-A glycolipid (actually mixtures of different congeners differing in their C₆, C₈, C₁₀, chain length) has been explored as a function of both concentration and temperature [13] (it is called “standard” MEL-A here to contrast it with the tri-acetylated MEL-A described below in section 2.1.b). Its phase diagram is dominated by the presence, at weight fractions below 56 wt%, of a “sponge” phase (often called the L₃ phase), which is an isotropic and disordered phase made of interconnected membranes. Strictly speaking, this phase is not liquid crystalline because it is (structurally) isotropic but it bears similarities with the lamellar phase made of stacked membranes.[14] Moreover, the presence of a sponge phase which has negative Gaussian curvature often suggests the existence of a bicontinuous cubic phase.

Indeed, at slightly larger weight fractions (between 56 and 65 wt%), another (optically) isotropic and highly viscous phase was observed by polarized-light microscopy as it appears completely dark between crossed polarizers. Its X-ray scattering pattern displays several diffraction peaks that could be indexed with the Ia3d space group which corresponds to a bicontinuous type of cubic phase made of two equivalent infinite intertwined networks. Although this phase assignment

seems quite likely, at this stage, further structural studies are required to rule out other possible cubic space groups and to obtain more detailed information on the structure of this phase. The lattice parameter of the cubic phase is 11.4 nm, a large value suggesting the presence of many molecules per cubic unit cell, as is usually observed with cubic phases of conventional surfactants.[15]

Between 65 and 87 wt%, a lyotropic L_α lamellar phase was identified by its X-ray diffraction pattern, which shows a series of equidistant diffraction peaks. The lamellar period is 3.6 nm at 75 wt%, values from which the thicknesses of the BA membrane and its hydrophobic part were estimated to be 2.7 nm and ≈ 1.7 nm, respectively. The latter value is well below twice the length of the lipid tail, indicating that the structure of the BA membrane is interdigitated rather than fully bilayered. This is probably due to the large size of the hydrophilic head (≈ 0.7 nm², compared to the typical cross-section, ≈ 0.4 nm², of two lipid chains [16], and this feature is often observed with lamellar phases of bioamphiphiles. Finally, above 87 wt%, the system is in an isotropic fluid (molten) state, with no particular properties.

The data described above were obtained at room temperature but the effect of temperature on the LC phases was also investigated and the L_3 phase was found to remain stable up to 65°C. This wide range of stability led the authors to suggest that MEL-A has an underlying tendency for negative Gaussian curvature of the membranes, which probably drives the formation of both the sponge and the bicontinuous cubic phases. The melting enthalpies of the LC phases were measured by DSC. The values obtained ranged from 0.2 to 0.45 kJ/mol and are typical for lyotropic LC phases. The phase boundaries are mostly vertical (i.e. little sensitive to temperature), which is in contrast to the case of common non-ionic surfactants, such as the C_nEO_m family. The low sensitivity of the lyotropic LC phases to an increase in temperature was interpreted as being due to the presence of a hydrogen-bond network between the sugar moieties, which is another feature often mentioned for bioamphiphiles.

b) MEL-A variant

A new variant of MEL-A, called MEL-A2, was produced by a new strain of *Pseudozyma* yeast.[17] This variant differs from the usual MEL-A in that its hydrophilic head is tri-acetylated so that it carries only one fatty acid chain (albeit in C_{16} , which is longer than the chains of standard MEL-A). The lyotropic liquid

crystalline properties of this new compound were tackled by using the contact preparation method (“water penetration scan”), which revealed the presence of a lamellar L_α phase. More detailed investigations of samples at well-defined concentrations should be carried out to determine the range of stability of the phase, both in concentration and temperature, and X-ray diffraction studies could also help to determine the influence of the reduced number of aliphatic chains on the phase diagram and the structural parameters of the LC phases.

2.2. MEL B

a) Standard MEL-B

MEL-B differs from MEL-A in that it is deacetylated at the C_4 position of mannose and has an OH group there instead. It is often found as a minority component in MEL mixtures. Observation by polarized light microscopy of its concentrated aqueous solutions revealed beautiful lamellar textures called “myelin figures” (see Figure 3A) below for a beautiful example of these textures).[18] These patterns are typical of the way the L_α phase of phospholipids grows when the crystals are brought into contact with water. The observation of these quite distinctive textures is clear evidence for the presence of the L_α phase.[19]

It is therefore not surprising that the X-ray diffraction of these samples showed a series of equidistant diffraction peaks, thus confirming the assignment of the L_α phase. From the lamellar spacing and the composition, the thickness $\delta = 3.2$ nm of the bioamphiphile membrane could be deduced, a value slightly larger than that mentioned above for standard MEL-A, which might be due to a difference in lipid chain length.

Overall, compared to MEL-A, the wide range of stability of the L_α phase suggests that MEL-B strongly favors the formation of molecular assemblies without spontaneous curvature, such as flat membranes and large vesicles. The research team of D.Kitamoto argues that the main reason why MELs are such effective components of cosmetic formulations is that they retain moisture and therefore maintain the skin extracellular matrix, precisely because they form L_α phases. Note that similar conclusions have been reached by other teams using different glycolipids.[20,21]

b) MEL B variant

It was discovered that another species of *Pseudozyma* yeast produces a diastereoisomer, hereafter called MEL-B', of the usual MEL-B, which differs from it by the configuration of the erythritol moiety (and also by a somewhat wider distribution of fatty acid chain lengths).[22] This new bioamphiphile displays a L_α phase at concentrations above 60 wt% at 25°C. The phase remained stable up to fairly high temperatures, with a melting temperature located above 95 °C between 60 and 85 wt% and linearly decreasing to 35°C between 85 wt% and 100 wt%. This dramatic decrease in the melting temperature was interpreted by the authors as a sign that the hydrogen-bond network may play an important role in the formation and stability of the L_α phase.

Although the optical textures were not as clear as the myelin figures discussed above, the XRD data showed diffraction peaks located at q-vector moduli in a 1:2:3 ratio, allowing the identification of the L_α phase. Interestingly, the diffraction peaks showed an intensity inversion that may provide valuable structural information, as in similar cases [23,24], but this feature was not exploited.

The dependence of the lamellar period on the bioamphiphile concentration was also measured. This type of measurement is often referred to as the “swelling law”. In general, a simple geometric argument shows that, within the range of stability of the L_α phase, as the volume fraction of surfactant, ϕ , decreases, the lamellar period increases as $d = \delta/\phi$, where δ is the thickness of the surfactant membranes.[25] As ϕ is further decreased, deviation from this linear law to a constant lamellar period is most often a sign that the system has entered a biphasic region and expels excess solvent. In the present case, the measurement of the swelling law provided the thickness, $\delta = 3.1$ nm, of the bioamphiphile membrane, which is not significantly different from the value for the standard MEL-B.

c) (MEL-B, H₂O, n-decane) system

The mixtures of the ternary phase diagram of (yet another stereoisomer of) MEL-B, H₂O, and decane (oil) were investigated by PLM and XRD.[26] Surprisingly, it was found that the L_α phase exhibited by MEL-B can be swollen to incorporate up to 40 wt% oil, so that the lamellar spacing increases from about 4 to 8.5 nm. This swelling behavior is quite reminiscent of some lamellar phases of common

surfactants [27], although to a lesser extent. A similar observation was made by another team using mixtures of alkylpolyglucosides and fatty acids. [28]

2.3. MEL C

a) Standard MEL-C.

Like MEL-B, MEL-C is deacetylated once but at a different site (Figure 2). However, MEL-C is considered to be more hydrophilic than MEL-B and therefore MEL-A. [29] Common MEL-C samples are actually mixtures that differ in the length of their hydrophobic chains, which are usually in C_6 , C_8 , and C_{14} . The lyotropic liquid crystalline properties of concentrated aqueous solutions of MEL-C have been quickly assessed using the contact preparation method.[30] On this basis, the presence of a lamellar L_α phase has been reported but no XRD investigation has been carried out to confirm this assignment. Furthermore, the phase diagram of MEL-C still remains unknown. However, like MEL-B, MEL-C spontaneously organizes into flat assemblies, such as the L_α phase and vesicles, and is therefore thought to hinder any spontaneous curvature.

b) MEL-C variant

A variant of MEL-C, hereafter called MEL-C', with one short (C_2 or C_4) chain and one long (C_{16}) chain, therefore very different from the standard MEL-C which has two chains of medium length (C_{10}), has been isolated from the cultures of another strain of *Pseudozyma* yeast.[31] Based on texture observation using the contact preparation method, two phases have been reported in concentrated solution, namely the L_α phase and a hexagonal phase, as is often the case with synthetic surfactants. The report of the latter phase with MELs is rather unusual but has not yet been confirmed by any XRD study. A more detailed structural study of the L_α phase might also shed light on the influence of the hydrophobic chain length on the organization of the mesophase.

c) MEL mixture

Finally, the lyotropic liquid crystalline properties of a MEL mixture with 22 wt% MEL-A, 13 wt% MEL-B, and 65 wt% MEL-C have been examined by PLM.[29]

The observation of myelin figures proved the presence of a L_{α} phase. The MEL-C component of the mixture was also purified and, quite interestingly, no significant difference was observed between its behavior and that of the crude MEL mixture. This means that the molecular disorder inherent to the L_{α} liquid crystalline phase washes out the molecular differences between these glycolipids and would imply that MEL purification could be dispensed with for practical applications. Further detailed studies are now required to characterize this conclusion more quantitatively.

2.4. MEL D: a twice-deacetylated MEL

a) MEL-D produced by enzymatic hydrolysis

Since fully deacetylated MELs are not commonly produced by yeast strains, a novel MEL homologue, labeled MEL-D, without acetyl groups at neither C_4 , nor C_6 , in the mannose moiety was nevertheless obtained by the selective enzymatic hydrolysis of acetyl groups from MEL-B.[32] A priori, MEL-D is expected to be the most hydrophilic of all MELs due to the hydrolysis of the acetyl groups. Investigation of aqueous solutions of MEL-D by PLM and XRD revealed the existence of a lamellar L_{α} phase that is stable over a fairly wide range of weight fractions, from 50 to 100 wt%. Upon dilution, the lamellar period increases (swelling law) from ≈ 2.5 nm to ≈ 5 nm at 50 wt%, from which the thickness $\delta \approx 2.5$ nm of the MEL-D membranes can be inferred.

b) MEL-D produced by genetic engineering

MEL-D could also be obtained thanks to an acetyltransferase disruption mutant of a *Pseudomonas* yeast.[33] Although this MEL-D differs slightly from the one discussed above in the distribution of the fatty acid chain length, its concentrated aqueous solutions also form a L_{α} phase. More detailed structural studies of this compound would be required to determine if there are any differences between these two BA.

2.5. MML: Mannosylmannitol lipid

In a very elegant study, *Pseudozyma* yeasts were fed on mannitol (a C₆ alcohol) instead of erythritol (a C₄ alcohol), which resulted in the production of a type of glycolipid, labeled MMA, differing from MELs by the size of the linear alcohol moiety.[34] Like MEL-A, MML is twice acetylated but it should be more hydrophilic owing to the longer linear alcohol in its hydrophilic head. The study by PLM of concentrated aqueous solutions of this BA, using the contact preparation method, revealed the formation of myelin figures (Figure 3A) and thus the presence of a L_α phase. In contrast to MEL-A, neither the sponge phase nor any cubic phase was reported, which should be confirmed by more detailed studies. This would suggest that the MML bioamphiphile favors molecular assemblies without spontaneous curvature.

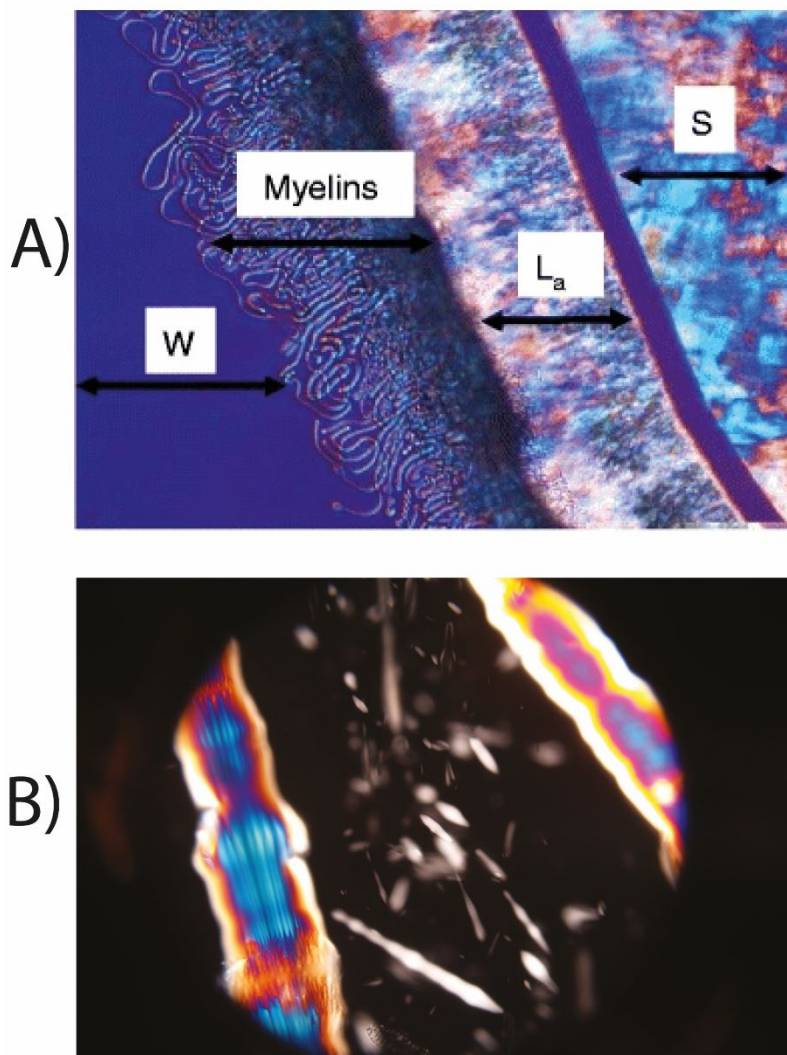


Figure 3: Two ways of formation of the L_{α} phase. A) Differential interference contrast microscopy image of a contact preparation of mannosylmannitol lipid in solid form (S, on the right) with pure water (W, on the left). “ L_a ” is the bulk L_{α} phase and “myelins” are the structures formed by the L_{α} phase as it grows within water. Adapted from T.Morita et al, Applied Microbiology and Biotechnology (2009) **83** 1017; DOI: 10.1007/s00253-009-1945-4. B) Polarized-light microscopy image of an aqueous mixture (70 wt %) of a mono-sophorose lipid (see Figure 2C) showing bâtonnets de Friedel-Grandjean growing from the isotropic liquid phase. (Adapted with permission from F.Saci et al, Langmuir (2022) 38, 8564; DOI: 10.1021/acs.langmuir.2c00807. Copyright 2022 American Chemical Society.)

3. Rhamnolipids

Rhamnolipids (RLs) are glycolipid-type bioamphiphiles with a hydrophilic head based on the rhamnose sugar moiety (Figure 2B). They are commonly produced by strains of *Pseudomonas aeruginosa*, which are ubiquitous and highly pathogenic bacteria. At least, six different types of RLs are known, which differ in the number of hydrocarbon chains and rhamnose sugars in their hydrophilic heads.

The liquid crystalline properties of solutions of two RLs, a mono-rhamnolipid and a di-rhamnolipid, both with two fatty acid chains, have been investigated by Ozdemir et al.[35] The concentration of these BA was not well controlled as drops of 10 wt% aqueous solution were simply left to dry on glass plates. Nevertheless, the authors reported the observation of Maltese crosses by PLM, which they interpreted as a sign of the formation of a L_{α} phase. A subsequent study by another group, using XRD, of solutions of a di-rhamno-di-lipid (with a longer fatty acid chain) also reported the presence of the L_{α} phase and moreover suggested the existence of a cubic phase.[36] However, in the absence of PLM data, it is difficult to confirm the nature of these lyotropic LC phases and, in particular, whether or not the samples are monophasic at the high water contents (75 and 90 %) used here. These interesting preliminary studies call for additional work to fully determine the phase diagram of these compounds.

The lyotropic LC phase diagrams of two complex systems based on a mono-rhamno-di-lipid and a di-rhamno-di-lipid were mapped out based on PLM

data.[37] These two systems are quaternary mixtures of the respective RL, water, n-heptane, and n-alcohol where the linear alcohol was varied from n-propanol to n-octanol. The alcohol/bioamphiphile mass ratio was kept constant at 2/1. Some of the phase diagrams show wide regions of LC phases identified as lamellar, cubic, and hexagonal. (Note that a somewhat similar situation occurred with the mixtures of alkylpolyglucosides and fatty acids mentioned earlier. [28] However, only lamellar textures were clearly documented. A complementary XRD study of these interesting systems is therefore necessary to confirm these claims. Furthermore, the influence of the alcohol/bioamphiphile mass ratio on the phase diagram should also be explored.

4. Trehalolipids

Trehalolipids (TLs) are a class of bioamphiphiles produced by a specific strain of *Rhodococcus* bacteria. These BA are based on a hydrophilic trehalose (a disaccharide) head esterified by fatty acid chains. We are aware of only one study that mentions the LC behavior of these bioamphiphiles. The lyotropic liquid crystal properties of a mixture of several TLs of different chain lengths, obtained from a specific strain of *Rhodococcus* bacteria, have been examined by polarized-light microscopy.[38] Observations of the textures of aqueous solutions, made with the contact preparation method at different temperatures, suggested the existence of a L_{α} phase at room temperature and a hexagonal phase at 70°C. Since the reported textures do not really allow for unambiguous identification, and in order to better identify the domain of stability of each liquid crystalline phase, this research topic is certainly worth revisiting not only by texture observations but also by XRD.

5. Sophorolipids

Sophorolipids (SLs) are common bioamphiphiles produced by yeasts such as *Candida bombicola*. Depending on the pH, they can adopt an acidic (AS) or a lactonic (LS) form and, due to the thermodynamic equilibrium between the two forms, it is difficult to obtain one form completely free of the other. Although all SLs have a hydrophilic head based on sophorose sugar, there are many kinds of SLs that differ by the number (one or two) of sophorose head groups and by some modifications of their fatty acid chain (based on oleic acid). Moreover, the

sophorose moieties may be more or less acetylated at specific sites. This molecular variability results in many different SL compounds (congeners), which can be difficult or even impossible to purify. SLs are often classified as “bolaamphiphiles” because they have polar heads at each end of their fatty acid chain. Because SLs are usually only weakly charged, their self-assembly properties are more reminiscent of those of non-ionic surfactants with large polar heads than of those of ionic ones [39] and their hydrophilicity decreases with increasing degree of acetylation. To date, we know of only one work that deals with the liquid crystalline behavior of concentrated solutions of sophorolipids.[40]

A family of six sophorolipid BA were investigated by PLM and DRX at weight fractions ranging from 20 to 100 wt%. Some of them, hereafter referred to as mono-sophorose BA, had only one hydrophilic sophorose head with a carboxylic acid at the other end of the fatty acid chain whereas the others, hereafter referred to as di-sophorose BA, had one sophorose head group at each end (Figure 2C,D). They also differed by a methyl substitution at the sophorose end of the fatty acid chain.

A detailed study by PLM showed that the mono-sophorose BA displayed typical optical textures of the lamellar L_{α} phase, i.e. the focal conic texture, oily streaks (Figure 4) and lamellar spherulites, and Friedel-Grandjean bâtonnets in the isotropic / L_{α} phase coexistence region of concentration (Figure 3B).

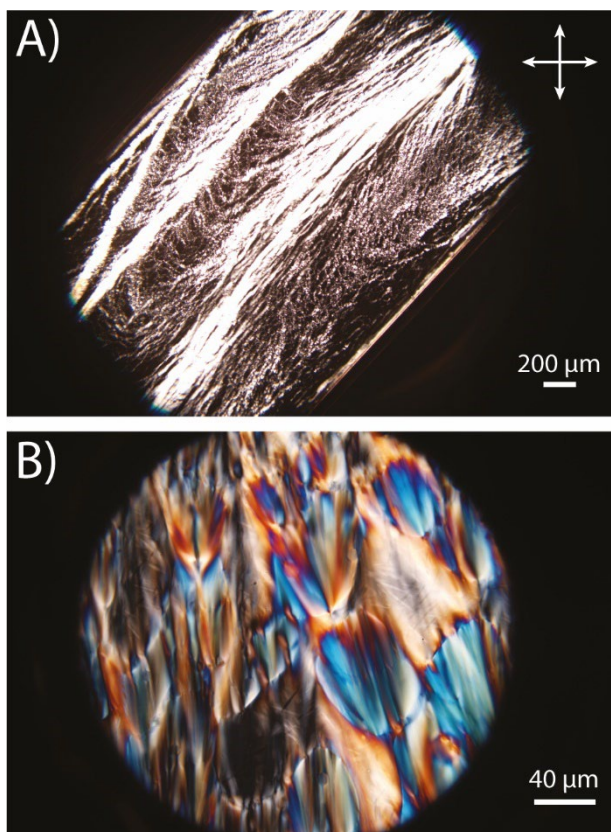


Figure 4: Polarized-light microscopy images of aqueous mixtures of a monosphorolipid BA (see Figure 2C) in flat glass capillaries. A) Oily streaks (80 wt%). B) Focal conics (80 wt%). (Adapted with permission from F.Saci et al, *Langmuir* (2022) 38, 8564; DOI: 10.1021/acs.langmuir.2c00807. Copyright 2022 American Chemical Society.)

This phase assignment, based on clear liquid crystalline lamellar textures, was further confirmed by XRD, with the observation at small angles of only two sharp diffraction lines at wave vector positions in a 1:2 ratio (Figure 5). At wide angles, a diffuse scattering ring is observed, which shows that the lipid chains are in a fluid state, as expected. The L_{α} phase was observed in concentrated aqueous solutions at weight fractions of 60 wt% and above. From the period of the lamellar phase and the BA concentration, the thickness, $\delta = 26 \pm 1 \text{ \AA}$ was deduced. Comparison of this value with the molecular length in an extended conformation led the authors to conclude that the membranes are made of fully interdigitated molecules. This conclusion is quite consistent with the interdigitated structure of the self-assembled ribbons previously observed at lower concentrations by other authors and interpreted as being due to strong disaccharide-disaccharide hydrogen bonding. [41] Indeed, like many other BA, SLs with their many OH groups and

ether oxygens on each hydrophilic sophorose head groups must be prone to hydrogen bonding. Moreover, the lamellar L_{α} phase could be easily aligned by flow or by applying a high-frequency alternating electric field, opening the way to a more detailed study of its physical properties. Finally, one of the mono-sophorose BA, in pure form (i.e. in the absence of water), also showed the L_{α} phase between 140 and 150 °C, which gives it an amphitropic (i.e. both thermotropic and lyotropic) character.

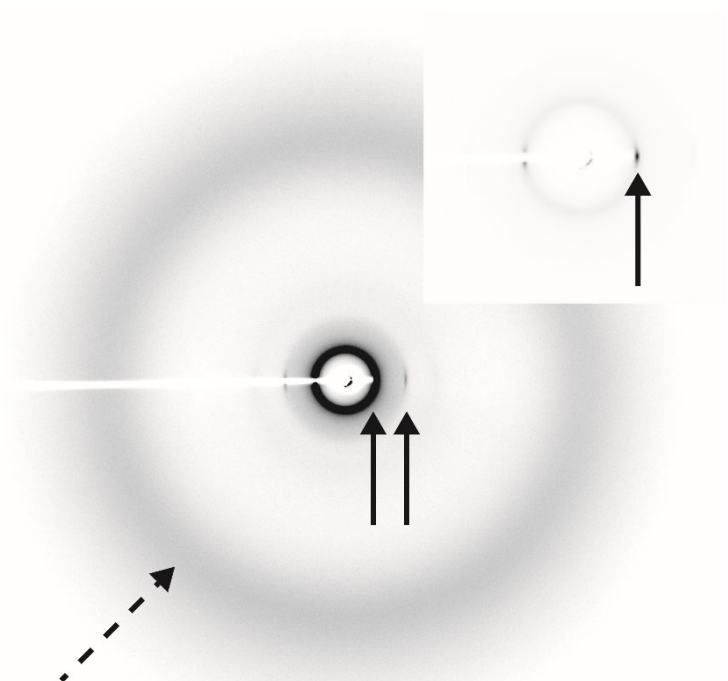


Figure 5: X-ray scattering pattern of a mono-sophorolipid BA (see Figure 2C) at 70 wt% in water. The inset at the top-right shows the underexposed central part of the pattern. The solid black arrows point to the first and second-order lamellar reflections, whereas the dashed black arrow points to the wide-angle diffuse ring. (Reproduced with permission from F.Saci et al, *Langmuir* (2022) 38, 8564; DOI: 10.1021/acs.langmuir.2c00807. Copyright 2022 American Chemical Society.)

The di-sophorose BA in concentrated aqueous solutions showed an entirely different liquid crystalline behavior, forming a columnar hexagonal phase instead of a lamellar L_{α} phase. The study by PLM revealed the presence of liquid crystalline textures that were much more difficult to interpret. However, the use

of the contact preparation method allowed for the observation of the so-called “developable domains” (Figure 6), which is the clear signature of the columnar phase. This phase assignment was further confirmed by an XRD study, which revealed the presence of the columnar phase at weight fractions of 70 wt% and above by the detection of two diffraction lines at wave vector positions in a $1:3^{1/2}$ ratio. The hexagonal lattice parameter of this SL compound is $34 \pm 1 \text{ \AA}$ at 70 wt%. Assuming a direct structure for the columnar hexagonal phase, a column diameter of about 30 \AA can be estimated, which seems reasonable for di-sophorose compounds with the same fatty acid chain length as the previously discussed mono-sophorose compounds. However, the direct character of the structure, although likely, could not be rigorously demonstrated in this study and a more detailed structural investigation therefore remains to be performed. Yet, we note that a synthetic bolaamphiphile, with a similar structure but with only a single sugar moiety in each head group, showed a lyotropic lamellar phase over the whole concentration range investigated. [42] This suggests that the size of the head group plays a major role in determining the phase behavior.

In addition, the columnar phase could not be aligned either by flow or by application of an electric field, which probably only reflects the fact that it has a higher viscoelasticity than the L_α phase, as is usually observed with amphiphiles. Furthermore, these di-sophorose BA, in pure form, did not show any thermotropic liquid crystalline properties.

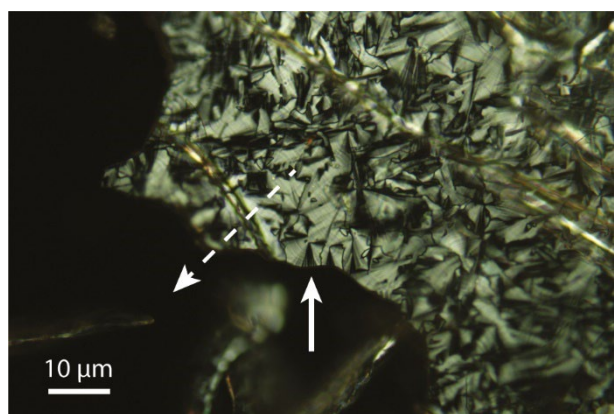


Figure 6: Polarized-light microscopy image of a contact preparation with water of a di-sophorose lipid (see Figure 2D), showing developable domains (e.g. white arrow). (Adapted with permission from F.Saci et al, *Langmuir* (2022) **38**, 8564; DOI: 10.1021/acs.langmuir.2c00807. Copyright 2022 American Chemical Society.)

6. Surfactin

Surfactin differs from the previously discussed bioamphiphiles in that it belongs to the lipopeptide family (Figure 2E). It consists of a fatty acid chain linked to a hydrophilic peptide moiety that can have either a linear conformation or a cyclic, lactonic, conformation, depending on the physico-chemical conditions, such as the pH. This BA is produced by various strains of *Bacillus subtilis* and is considered to be a very good bioamphiphile. Moreover, it has a strong antibiotic effect as it incorporates into the cell membrane and makes it permeable.

D.Kitamoto's team also studied the liquid crystalline behavior of concentrated aqueous solutions of surfactin.[43] This compound was actually a mixture of three congeners that differed in the fatty acid chain length (C₁₃: 17%; C₁₄: 52%; C₁₅: 31%). The linear form (LS) was obtained from the cyclic form (CS) by alkaline hydrolysis and it was found that LS is more hydrophilic than CS.

The lyotropic LC properties of both systems were investigated by PLM and XRD. These techniques showed that CS forms a lamellar L_α phase at weight fractions of 50 wt% and beyond (Figure 7A). Moreover, the lamellar spacing decreases from 4 to 3 nm as the weight fraction increases from 50 to 100 wt%. This swelling law provided the thickness of the BA membrane, $\delta = 3$ nm, which strongly suggests that CS forms an interdigitated bilayer, like all other BA described previously.

In contrast, in its LS form, surfactin, at high concentration, did not present a L_α phase but instead displayed both a bicontinuous cubic phase and an inverted hexagonal phase. Indeed, an optically isotropic phase was observed by PLM, but this phase showed many diffraction lines by XRD (Figure 7B). The case for a hexagonal phase is more doubtful since it should have presented a specific birefringent texture and a specific XRD diffractogram, which were not described in this work. Therefore, more detailed investigations are required to clarify this point.

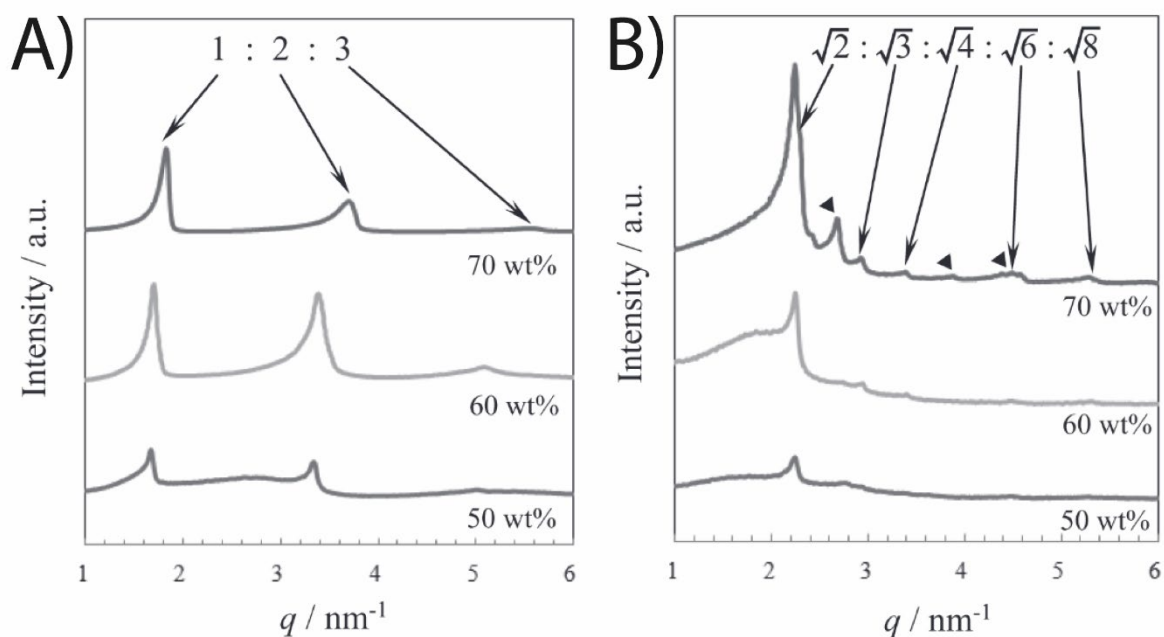


Figure 7: X-ray diffractograms of aqueous mixtures of surfactin. A) Cyclic form in the L_α phase. B) Linear form in the cubic phase. Reproduced from T.Imura et al (2013) Journal of Oleo Science, **62**, 499; DOI: 10.5650/jos.62.499.

7. Conclusion

Compared to their conventional oil-based counterparts, the study of the liquid crystalline properties of bioamphiphiles, as shown in this review, is still at an early stage. Only a few families of bioamphiphiles have been investigated to date and even fewer in detail. Moreover, these investigations are limited to simple phase identification and to the measurement of a few structural parameters. Furthermore, the number of studies is still far too small to rationalize the relationship between molecular structure and liquid crystalline organization. In addition, almost nothing is known about the physical properties of these phases (elastic constants, viscosities, anchoring properties ...) that are crucial for processing and industrial applications. Consequently, this review tried to show that this area of research has been yet little explored and that engaging in such studies is timely.

The liquid crystalline phase behavior of common synthetic surfactants is governed mostly (but not only) by two parameters: the surfactant concentration and its packing parameter. The case of bioamphiphiles appears to be much more complicated because the packing parameter, as usually defined, does not seem to be as relevant. [3] This may be due to the complex nature of the BA head group which can adopt a variety of molecular conformations and often bears hydroxyl groups that interfere with the H-bond network of water. Therefore, as illustrated by the MEL series, the number, precise location, and stereochemistry of the hydroxyl groups may also affect the phase behavior to some extent, as has been suggested for other glycolipids.[44] This is in stark contrast to the case of synthetic surfactants, for which the most relevant degrees of freedom are related to the aliphatic chains, which can be molten or frozen, straight or tilted.[45] In addition, it would be interesting to change the grafting positions of the fatty acid chains on the head group to examine the consequences on the phase diagram, as has recently been done for synthetic glycolipids.[42]

From a practical point of view, a number of experimental difficulties specific to bioamphiphiles must still be overcome. Purified BA materials are available in lower amounts than for oil-based surfactants, which hampers the systematic exploration of phase diagrams. Bioamphiphiles are complex molecules which can exist in different forms, depending on the pH and the temperature. Therefore, in addition to the usual temperature and concentration parameters, the influence of pH also needs to be assessed. This also applies to the ionic strength if the hydrophilic head group bears an electrical charge, such as mono-sphorolipids at high pH, for example. The electrostatic repulsions between charged head groups may indeed drive a transition from flat to curved interfaces and these repulsions depend on the ionic strength. In addition, kinetic effects can prevent the systems of interest from reaching thermodynamic equilibrium, in terms of molecular conformations and at the structural and textural levels. Consequently, the storage conditions of BA materials probably matter even more than for synthetic surfactants.

Another specificity of bioamphiphiles is that they are chiral, a feature which gives rise to the twisted structures that are often observed in the dilute regime. Although both lamellar and columnar liquid crystal phases in principle expel twist, there are other liquid crystal phases, such as the chiral nematic phase (also called “cholesteric”) and other more complicated chiral phases, that express molecular chirality at the macroscopic level. To the best of our knowledge, this aspect has

never been considered to date in the literature relevant to concentrated BA systems.

A final interesting aspect of these materials that has been so far somewhat overlooked is the connection between the self-assembled structures observed at low concentrations and the lyotropic liquid crystalline phases that occur at high concentrations. One example of an open question is to understand how the self-assembled structures (e.g. rod-like micelles or twisted ribbons) coalesce to form LC lamellar, columnar, or cubic phases. Another is to follow the evolution of the shape of these structures with concentration, up to the pure compounds, together with the conformation of the molecules that they are made of. Such questions have often been explored in detail for oil-based surfactants but remain to date *terra incognita* for bioamphiphiles.

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Declaration of interest: none

References

- [1] Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. *Microbiol Mol Biol Rev MMBR* 1997;61:47–64. <https://doi.org/10.1128/.61.1.47-64.1997>.
- [2] Mukherjee S, Das P, Sen R. Towards commercial production of microbial surfactants. *Trends Biotechnol* 2006;24:509–15. <https://doi.org/10.1016/j.tibtech.2006.09.005>.
- **[3] Baccile N, Seyrig C, Poirier A, Alonso-de Castro S, Roelants SLKW, Abel S. Self-assembly, interfacial properties, interactions with macromolecules and molecular modelling and simulation of microbial bio-based amphiphiles (biosurfactants). A tutorial review. *Green Chem* 2021;23:3842–944. <https://doi.org/10.1039/D1GC00097G>.
- This recent article reviews in great detail the self-assembly properties of biosurfactants, mostly in dilute solutions and at interfaces, both from experimental and numerical perspectives.
- [4] De Gennes P-G. *The physics of liquid crystals*. Oxford [Eng.]: Clarendon Press; 1974.
- [5] Demus D, Richter L. *Textures of liquid crystals*. 1. ed. Weinheim: Verl. Chemie; 1978.
- [6] Dierking I. *Textures of liquid crystals*. Weinheim: Wiley-VCH; 2003.
- [7] Pieranski P, Oswald, P. *Nematic and Cholesteric Liquid Crystals: Concepts and Physical Properties Illustrated by Experiments*. Boca Raton: CRC Press; 2005. <https://doi.org/10.1201/9780203023013>.
- [8] Pieranski P, Oswald, P. *Smectic and Columnar Liquid Crystals: Concepts and Physical Properties Illustrated by Experiments*. Boca Raton: CRC Press; 2005. <https://doi.org/10.1201/9781420036343>.
- [9] De Jeu WH. *Basic x-ray scattering for soft matter*. Oxford: Oxford University Press; 2016.
- [10] Als-Nielsen J, McMorrow D. *Elements of Modern X-ray Physics*. 1st ed. Wiley; 2011. <https://doi.org/10.1002/9781119998365>.
- [11] Vill V, Hashim R. Carbohydrate liquid crystals: structure–property relationship of thermotropic and lyotropic glycolipids. *Curr Opin Colloid Interface Sci* 2002;7:395–409. [https://doi.org/10.1016/S1359-0294\(02\)00091-2](https://doi.org/10.1016/S1359-0294(02)00091-2).
- **[12] Kitamoto D, Fukuoka T, Saika A, Morita T. Glycolipid Biosurfactants, Mannosylerythritol Lipids: Distinctive Interfacial Properties and Applications in Cosmetic and Personal Care Products. *J Oleo Sci* 2022;71:1–13. <https://doi.org/10.5650/jos.ess21358>.

The authors review the production, surfactant properties, self-assembly, and applications of mannosylerythritol lipids, including their own work on the liquid-crystalline phases observed at high concentrations.

- [13] Imura T, Hikosaka Y, Worakitkanchanakul W, Sakai H, Abe M, Konishi M, et al. Aqueous-Phase Behavior of Natural Glycolipid Biosurfactant Mannosylerythritol Lipid A: Sponge, Cubic, and Lamellar Phases. *Langmuir* 2007;23:1659–63. <https://doi.org/10.1021/la0620814>.
- [14] Quilliet C, Blanc C, Kleman M. Evidence of a Phenomenon of Epitaxy at the Interface between a Lamellar L_{α} Phase and a L_3 Sponge. *Phys Rev Lett* 1996;77:522–5. <https://doi.org/10.1103/PhysRevLett.77.522>.
- [15] Impéror-Clerc M. Thermotropic cubic mesophases. *Curr Opin Colloid Interface Sci* 2005;9:370–6. <https://doi.org/10.1016/j.cocis.2004.12.004>.
- [16] Small DM. Lateral chain packing in lipids and membranes. *J Lipid Res* 1984;25:1490–500. [https://doi.org/10.1016/S0022-2275\(20\)34422-9](https://doi.org/10.1016/S0022-2275(20)34422-9).
- [17] Morita T, Ogura Y, Takashima M, Hirose N, Fukuoka T, Imura T, et al. Isolation of *Pseudozyma churashimaensis* sp. nov., a novel ustilaginomycetous yeast species as a producer of glycolipid biosurfactants, mannosylerythritol lipids. *J Biosci Bioeng* 2011;112:137–44. <https://doi.org/10.1016/j.jbiosc.2011.04.008>.
- [18] Imura T, Ohta N, Inoue K, Yagi N, Negishi H, Yanagishita H, et al. Naturally Engineered Glycolipid Biosurfactants Leading to Distinctive Self-Assembled Structures. *Chem - Eur J* 2006;12:2434–40. <https://doi.org/10.1002/chem.200501199>.
- *[19] Benkowska-Biernacka D, Smalyukh II, Matczyszyn K. Morphology of Lyotropic Myelin Figures Stained with a Fluorescent Dye. *J Phys Chem B* 2020;124:11974–9. <https://doi.org/10.1021/acs.jpcb.0c08907>.
- Using polarized-light and fluorescence confocal optical microscopies, the authors elucidate the molecular organization of the myelin figures which are observed in the textures of the L_{α} phase of phospholipid amphiphiles.
- [20] Terescenco D, Picard C, Clemenceau F, Grisel M, Savary G. Influence of the emollient structure on the properties of cosmetic emulsion containing lamellar liquid crystals. *Colloids Surf Physicochem Eng Asp* 2018;536:10–9. <https://doi.org/10.1016/j.colsurfa.2017.08.017>.
- [21] Savic S, Lukic M, Jaksic I, Reichl S, Tamburic S, Müller-Goymann C. An alkyl polyglucoside-mixed emulsifier as stabilizer of emulsion systems: The influence of colloidal structure on emulsions skin hydration potential. *J Colloid Interface Sci* 2011;358:182–91. <https://doi.org/10.1016/j.jcis.2011.02.049>.

- [22] Worakitkanchanakul W, Imura T, Fukuoka T, Morita T, Sakai H, Abe M, et al. Aqueous-phase behavior and vesicle formation of natural glycolipid biosurfactant, mannosylerythritol lipid-B. *Colloids Surf B Biointerfaces* 2008;65:106–12. <https://doi.org/10.1016/j.colsurfb.2008.03.009>.
- [23] Davidson P, Strzelecki L. Synthesis, Characterization and X-Ray-Diffraction Studies of a New Homologous Series of Cyano-Substituted Mesomorphic Side-Chain Polyacrylates. *Liq Cryst* 1988;3:1583–95.
- [24] Poulos AS, Constantin D, Davidson P, Imperor M, Pansu B, Panine P, et al. Photochromic hybrid organic-inorganic liquid-crystalline materials built from nonionic surfactants and polyoxometalates: Elaboration and structural study. *Langmuir* 2008;24:6285–91.
- [25] Warriner HE, Keller SL, Idziak SHJ, Slack NL, Davidson P, Zasadzinski JA, et al. The influence of polymer molecular weight in lamellar gels based on PEG-lipids. *Biophys J* 1998;75:272–93.
- [26] Worakitkanchanakul W, Imura T, Fukuoka T, Morita T, Sakai H, Abe M, et al. Phase behavior of ternary mannosylerythritol lipid/water/oil systems. *Colloids Surf B Biointerfaces* 2009;68:207–12. <https://doi.org/10.1016/j.colsurfb.2008.10.009>.
- [27] Safinya CR, Roux D, Smith GS, Sinha SK, Dimon P, Clark NA, et al. Steric interactions in a model multimembrane system - A synchrotron x-ray study. *Phys Rev Lett* 1986;57:2718–21.
- [28] Terescenco D, Savary G, Clemenceau F, Merat E, Duchemin B, Grisel M, et al. The alkyl polyglucoside/fatty alcohol ratio effect on the formation of liquid crystal phases in binary systems. *J Mol Liq* 2018;253:45–52. <https://doi.org/10.1016/j.molliq.2017.12.149>.
- [29] Konishi M, Morita T, Fukuoka T, Imura T, Kakugawa K, Kitamoto D. Efficient production of mannosylerythritol lipids with high hydrophilicity by *Pseudozyma hubeiensis* KM-59. *Appl Microbiol Biotechnol* 2008;78:37–46. <https://doi.org/10.1007/s00253-007-1292-2>.
- [30] Morita T, Konishi M, Fukuoka T, Imura T, Yamamoto S, Kitagawa M, et al. Identification of *Pseudozyma graminicola* CBS 10092 as a Producer of Glycolipid Biosurfactants, Mannosylerythritol Lipids. *J Oleo Sci* 2008;57:123–31. <https://doi.org/10.5650/jos.57.123>.
- [31] Morita T, Konishi M, Fukuoka T, Imura T, Kitamoto D. Production of glycolipid biosurfactants, mannosylerythritol lipids, by *Pseudozyma siamensis* CBS 9960 and their interfacial properties. *J Biosci Bioeng* 2008;105:493–502. <https://doi.org/10.1263/jbb.105.493>.
- [32] Fukuoka T, Yanagihara T, Imura T, Morita T, Sakai H, Abe M, et al. Enzymatic synthesis of a novel glycolipid biosurfactant, mannosylerythritol

lipid-D and its aqueous phase behavior. *Carbohydr Res* 2011;346:266–71. <https://doi.org/10.1016/j.carres.2010.11.025>.

- *[33] Konishi M, Makino M. Selective production of deacetylated mannosylerythritol lipid, MEL-D, by acetyltransferase disruption mutant of *Pseudozyma hubeiensis*. *J Biosci Bioeng* 2018;125:105–10. <https://doi.org/10.1016/j.jbiosc.2017.08.003>.

Using genetic engineering, the authors created a mutant of *Pseudomonas Hubeiensis* that produces a MEL-variant which is completely deacetylated and which forms a liquid-crystalline phase at high concentration in water.

- [34] Morita T, Fukuoka T, Konishi M, Imura T, Yamamoto S, Kitagawa M, et al. Production of a novel glycolipid biosurfactant, mannosylmannitol lipid, by *Pseudozyma parantarctica* and its interfacial properties. *Appl Microbiol Biotechnol* 2009;83:1017–25. <https://doi.org/10.1007/s00253-009-1945-4>.

- [35] Özdemir G, Peker S, Helvacı SS. Effect of pH on the surface and interfacial behavior of rhamnolipids R1 and R2. *Colloids Surf Physicochem Eng Asp* 2004;234:135–43. <https://doi.org/10.1016/j.colsurfa.2003.10.024>.

- [36] Andrä J, Rademann J, Howe J, Koch MHJ, Heine H, Zähringer U, et al. Endotoxin-like properties of a rhamnolipid exotoxin from *Burkholderia (Pseudomonas) plantarii*: immune cell stimulation and biophysical characterization. *Biol Chem* 2006;387. <https://doi.org/10.1515/BC.2006.040>.

- [37] Xie Y, Li Y, Ye R. Effect of Alcohols on the Phase Behavior of Microemulsions Formed by a Biosurfactant—Rhamnolipid. *J Dispers Sci Technol* 2005;26:455–61. <https://doi.org/10.1081/DIS-200054576>.

- [38] Marqués AM, Pinazo A, Farfan M, Aranda FJ, Teruel JA, Ortiz A, et al. The physicochemical properties and chemical composition of trehalose lipids produced by *Rhodococcus erythropolis* 51T7. *Chem Phys Lipids* 2009;158:110–7. <https://doi.org/10.1016/j.chemphyslip.2009.01.001>.

- [39] Penfold J, Chen M, Thomas RK, Dong C, Smyth TJP, Perfumo A, et al. Solution Self-Assembly of the Sophorolipid Biosurfactant and Its Mixture with Anionic Surfactant Sodium Dodecyl Benzene Sulfonate. *Langmuir* 2011;27:8867–77. <https://doi.org/10.1021/la201661y>.

- *[40] Saci F, Roelants SLKW, Soetaert W, Baccile N, Davidson P. Lyotropic Liquid-Crystalline Phases of Sophorolipid Biosurfactants. *Langmuir* 2022;38:8564–74. <https://doi.org/10.1021/acs.langmuir.2c00807>.

Using polarized-light microscopy and X-ray diffraction, the authors show the existence, structure, and range of stability of various liquid-crystalline phases in concentrated aqueous solutions of mono- and di-sophorolipid biosurfactants.

- [41] Zhou S, Xu C, Wang J, Gao W, Akhverdiyeva R, Shah V, et al. Supramolecular Assemblies of a Naturally Derived Sophorolipid. *Langmuir* 2004;20:7926–32. <https://doi.org/10.1021/la048590s>.
- [42] Goodby JW, Görtz V, Cowling SJ, Mackenzie G, Martin P, Plusquellec D, et al. Thermotropic liquid crystalline glycolipids. *Chem Soc Rev* 2007;36:1971. <https://doi.org/10.1039/b708458g>.
- [43] Imura T, Ikeda S, Aburai K, Taira T, Kitamoto D. Interdigitated Lamella and Bicontinuous Cubic Phases Formation from Natural Cyclic Surfactin and Its Linear Derivative. *J Oleo Sci* 2013;62:499–503. <https://doi.org/10.5650/jos.62.499>.
- [44] Corti M, Cantù L, Brocca P, Del Favero E. Self-assembly in glycolipids. *Curr Opin Colloid Interface Sci* 2007;12:148–54. <https://doi.org/10.1016/j.cocis.2007.05.002>.
- [45] Nagle JF, Tristram-Nagle S. Structure of lipid bilayers. *Biochim Biophys Acta BBA - Rev Biomembr* 2000;1469:159–95. [https://doi.org/10.1016/S0304-4157\(00\)00016-2](https://doi.org/10.1016/S0304-4157(00)00016-2).