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Molecular Organization in Lipids and Emulsions

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I. INTRODUCTION

Important knowledge on the lipid structure in emulsions is based on studies of simple systems containing only a few components, such as a ternary system consisting of a polar lipid, a triglyceride oil, and water. Structures and phase equilibria in such model system provide information on emulsion-stabilizing mechanisms which can be translated into the behavior of complex food emulsions. The structures in the different states of order of lipid and lipid–water phases will be summarized with special regard to emulsions. Additional information on the chemistry and physics of emulsions are found in Ref. (1).

Lipids are unique in their ability to form a wide variety of structures, ranging from micellar solutions to liquid-crystalline phases and different crystalline forms (polymorphism). An important factor behind the molecular organizations they exhibit is the amphiphilic character of the molecule—the possibility to orient hydrophilic groups toward polar regions, such as water, and hydrophobic groups separated into nonpolar regions.

II. THE SOLID STATE

In crystals, it is possible to obtain exact and detailed information on the molecular conformation as well as the lateral molecular packing.

Knowledge about lipid crystal structures is therefore of fundamental importance for discussions of the structure in disordered states, such as liquid-crystalline lipid–water phases. Furthermore, there is a close resemblance between crystal structures and the structures of lipid monolayers at interfaces (e.g., on a water surface). A remarkable property of lipid crystalline phases that should be mentioned in this connection is that complex mixtures like natural fats can form solid solution with wide variations in molecular size.

A. Experimental Methods

When single crystals are available, it is possible to perform a complete structure determination by the x-ray diffraction technique. Even if single crystals are not available, a considerable amount of information on solid-state packing can be obtained by powder diffraction. It is thus possible to obtain a so-called long spacing from which the main features of the arrangement of the molecules in layers can be obtained, such as the number of molecular layers in the repetitive unit layer and the angle of tilt of the molecules within each layer. In the wide-angle region of the diffraction pattern, it is possible to identify dominating so-called short-spacing lines, which, in many cases, are sufficient for identification of the hydrocarbon chain packing.

X-ray diffraction is by far the most powerful method used to determine the structures in the solid state, even if important complementary information has been obtained by other methods (e.g., by infrared spectroscopy) (1).

B. Molecular Orientation in Lipid Crystals

The characteristic feature of lipid crystal structures is the arrangement of molecules in layers with the polar groups at the surface of the layer, as shown in Fig. 1. The molecular layers usually form unit layers of double molecular length, lipid bilayers, in order to permit polar interaction between the molecular layers (Fig. 1a). This means that there are only relatively weak van der Waals attractive forces between these bilayers separated by the methyl end-group gap. This explains the crystal morphology; the crystals form very thin plates parallel to these planes, with a pronounced tendency for cleavage at the methyl gap interface.

In cases with very weak attractive forces between the polar head groups or when the polar groups are not end groups, a head-to-tail arrangement as shown in Fig. 1b can be adopted. An example is ethyl stearate (2), in which the polar group is too far from the end of the linear molecule to give dimerization. Methyl stearate (3), on the other hand, forms the usual head-to-head structure.

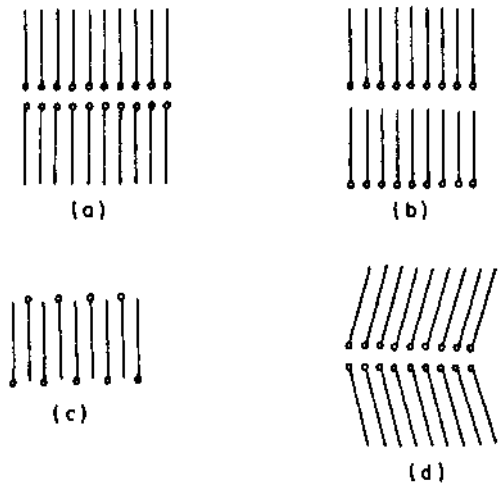


Figure 1 Schematic of the major types of molecular orientation in layers: (a) head-to-head arrangement, all molecules directed in the same way within each layer; (b) head-to-tail arrangement, all molecules directed in the same way within each layer; (c) head-to-tail arrangement within each layer; (d) tilted molecules within monomolecular layers (alternating directions of the tilt, as shown here, or nonalternating).

A head-to-tail arrangement within each layer, as shown in Fig. 1c, has been observed in cases with very bulky polar head groups. By mean of such a structure, the polar groups obtain a cross-sectional area twice as large as the hydrocarbon chains.

In cases where the molecules are tilted, they are usually all parallel. The tilt results in an increased space for the polar head group (see Section II. F). In some structures, however, the angle of tilt alternates between opposite directions in successive layer, as indicated in Fig. 1d. Such an arrangement possesses particular advantages in cases of solid-state phase transitions, as translations along the methyl end-group planes can be avoided. Finally, it should be mentioned that structures with two chain directions within each molecular layer have been observed in cases where the forces between the polar head groups are of dominating importance (e.g., in amides) (4).

C. Hydrocarbon Chain Packing

The hydrocarbon chains are always in the extended planar zigzag conformation in lipid crystal forms. There are a few alternative close-packing arrangements of such chains, which can be best described by the corresponding

subcell (the smallest repetition unit within the layer). All chain packing alternatives that have been observed are summarized in Fig. 2. The triclinic packing, *T parallel*, and the orthorhombic one, *O perpendicular*, represent the most efficient packing from an energetic point of view, and they are

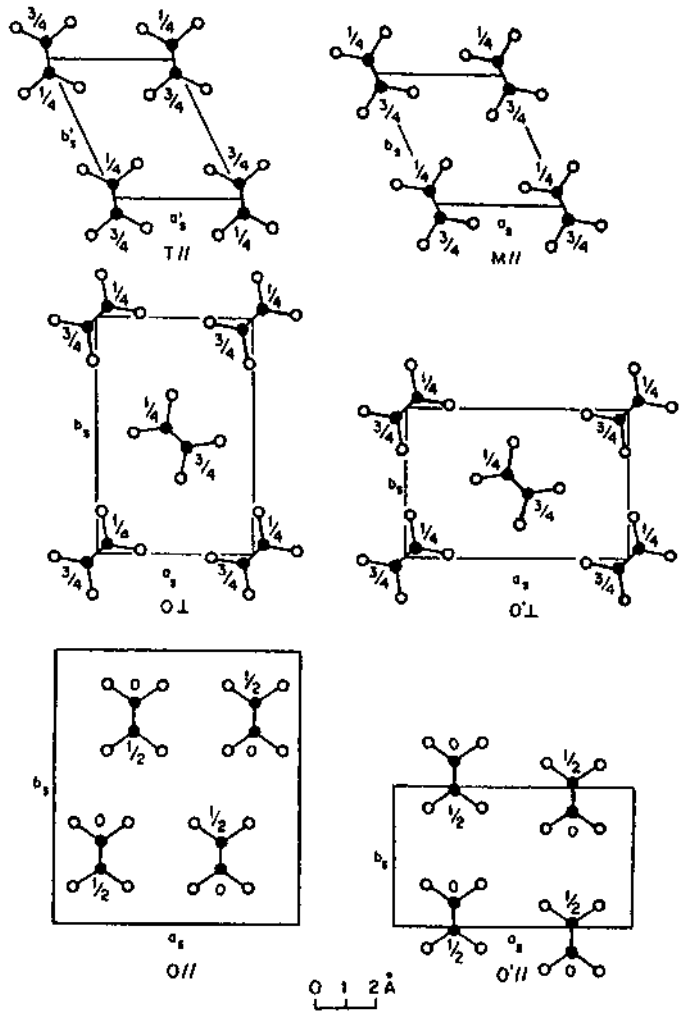


Figure 2 Hydrocarbon chain packing alternatives as defined by the corresponding subcell. One zigzag period is seen in the direction of the hydrocarbon chains with the atomic positions in the chain direction given by their fractional coordinates. Open circles represent hydrogen atoms and filled circles represent carbon atoms.

observed in *n*-paraffins, for example. All chain planes are parallel in the triclinic packing, whereas every second chain plane is perpendicular to the rest in the orthorhombic packing. The monoclinic packing, *M parallel*, has been observed in racemic 1-monoglycerides (5). An orthorhombic subcell, *O′ perpendicular*, closely related to the common one, *O perpendicular*, was found in a branched fatty acid (6). In other structures with disturbances in the hydrocarbon chain region, two more orthorhombic chain packing types have been observed: *O parallel* and *O′ parallel*, both with all chain planes parallel (7,8).

An interesting disordered crystal form, usually termed the alpha form (9), is observed near the melting point of many lipids. Its x-ray single-crystal data are in agreement with a hexagonal lateral symmetry of the hydrocarbon chains with rotational freedom (10). Due to the rotation of the molecules and its physical properties, this form is in fact a plastic crystal. In some lipids, however, the chains are anchored at the polar groups (e.g., in triglyceride), and it is obvious that only oscillation movements of the chains are possible. Finally, it should be mentioned that this hydrocarbon chain arrangement occurs in lipid–water phases and even in emulsions.

D. Polymorphism

The multiple melting phenomena exhibited by triglycerides was first explained by Malkin (11) as due to the occurrence of an alternative crystal form: polymorphism. One possibility for polymorphism is the arrangement of the different hydrocarbon chain close-packing types described earlier. The most common reason for polymorphism, however, is the possibility for variations in the angle of tilt of the hydrocarbon chains. The chains are successively displaced one or more whole zigzag periods in relation to adjacent chains so that the angle of tilt toward the end group plane is increased or decreased and the hydrocarbon chain region will not be changed.

Most lipids exhibit polymorphism. This can be illustrated by fatty acids. An even *n*-fatty acid in the chain-length range C_{12} to C_{18} can be obtained in three different crystal forms: the A, B and C forms. The crystal forms B and C have the same packing (*O perpendicular*) with different angles of tilt, whereas the A form shows another chain packing (*T parallel*).

E. Fatty Acids

The structure shown in Fig. 1a is characteristic for fatty acids with normal and saturated hydrocarbon chains. Branches can be accommodated into the

hydrocarbon chain region by relative displacements of adjacent chains or by changes in the tilt direction at the branch position (12).

When there are double bonds in the hydrocarbon chain, they will affect the chain packing differently according to the bond configuration. In oleic acid, where there is a cis double bond, there is a change in the chain direction at the double bond, whereas the corresponding trans isomer, elaidic acid, shows only one chain direction (8). The crystal structure of oleic acid is shown in Fig. 3.

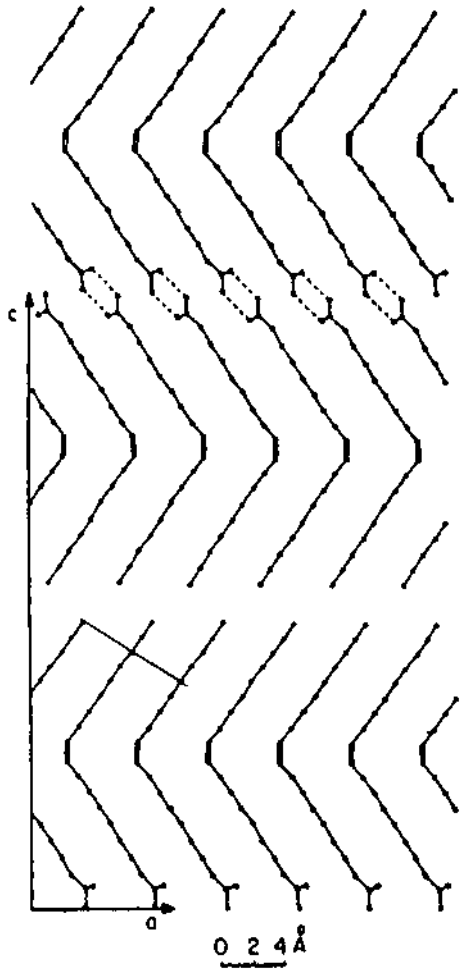


Figure 3 Crystal structure of oleic acid viewed along the *b* axis. (From Ref. 8.)

F. Monoglycerides and Emulsions Based on Crystalline Monoglycerides

Monoglycerides are most commonly used emulsifier in foods (see Chapter 2). An emulsification process, involving cooling through the crystallization of the monoglyceride solved in the oil, will result in the formation of a crystalline phase at the oil–water interface, as shown both from surface tension studies and phase identification (13). Organized lipid phases at the oil–water interface is a common emulsification-stabilizing mechanism in food emulsions; we will come back to this in connection with liquid crystals.

The crystal structure of a saturated 1-monoglyceride is shown in Fig. 4. There is a complex hydrogen-bond system linking the molecules together in two dimensions. When saturated monoglycerides are used as emulsifiers, a final emulsion product can be obtained stabilized by a crystalline monoglyceride film at the interface, with a methyl end group surface toward the oil phase and a monoglycerol head group surface toward water.

G. Diglycerides and Triglycerides

Crystal structure work has shown that there are two types of molecular conformation in diglyceride crystals. In one alternative, the two hydrocarbon

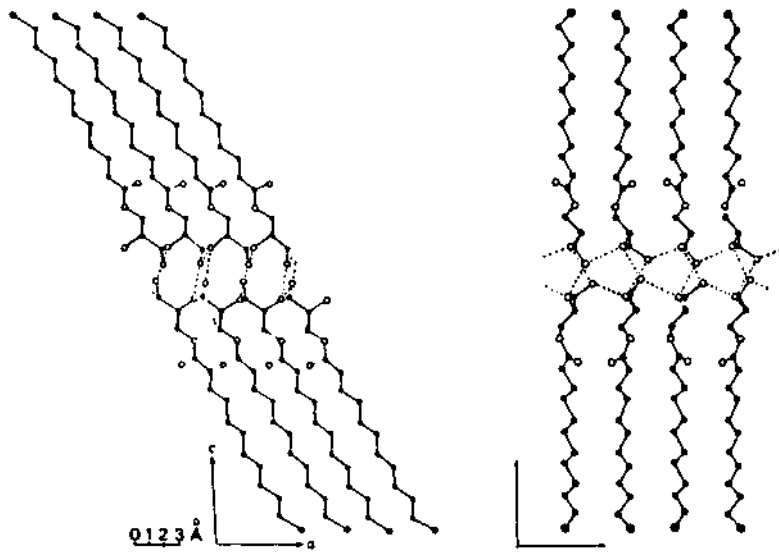


Figure 4 Crystal structure of a 1-monoglyceride viewed along two perpendicular directions. Also, 2-monoglyceride form similar to complex hydrogen bond networks linking the molecules in two dimensions. (From Ref. 5.)

chains have the same direction in relation to the head group, and then bilayers are formed just like the situation in monoglycerides. The other alternative is somewhat similar to triglyceride structures, with both chains pointing in opposite directions. Such a monolayer is then a unit layer in the crystal.

Simple saturated triglycerides crystallize in layers where extended molecules are arranged in the pairs as shown in Fig. 5. The acyl chains in the 1- and 3-position are extended, forming one chain, whereas the chain in the 2-position forms a branch, This is the characteristic molecular conformation of all triglycerides in the solid state. The presence of cis double bonds will result in a chain-sorting tendency so that unsaturated chains are localized in the same layer. This, in turn, will mean that sometimes the branching chain can be formed by the chain in the 1- or 3-position. Furthermore, the double-chain unit layer as shown in Fig. 5 cannot allow ideal chain sorting, and a triple-chain layer is instead the unit layer.

Simple triglycerides (all chains equal) show three polymorphic forms, and even complex triglycerides have a similar behavior although more forms may occur. The nomenclature now generally accepted to identify the polymorphic forms is based in x-ray diffraction with the following criteria:

Alpha form: One dominating diffraction line at 4.2 Å.
Beta prime form: Two diffraction lines at 4.2 and 3.8 Å dominate.
Beta form: Name for all other forms.

The crystal structure of the beta prime from and mechanisms involved in the polymorphic transitions have recently been determined by the pioneering work by Sato and co-workers (cf. Ref. 14).

The different polymorphic forms have different morphologies and this may influence emulsion stability. The beta prime form, for example, forms thin needles and they can sometimes extend through the oil–water interface and result in aggregation phenomena.

III. LIQUID-CRYSTALLINE PHASES

This term was introduced in order to describe structures with partial disorders like in liquids. Lipid–water phases form a wide variety of liquid-crystalline structures with technical significance, such as in food emulsions.

A requirement for the formation of liquid-crystalline phases is that the hydrocarbon chains are disordered, like in the liquid state of paraffin.

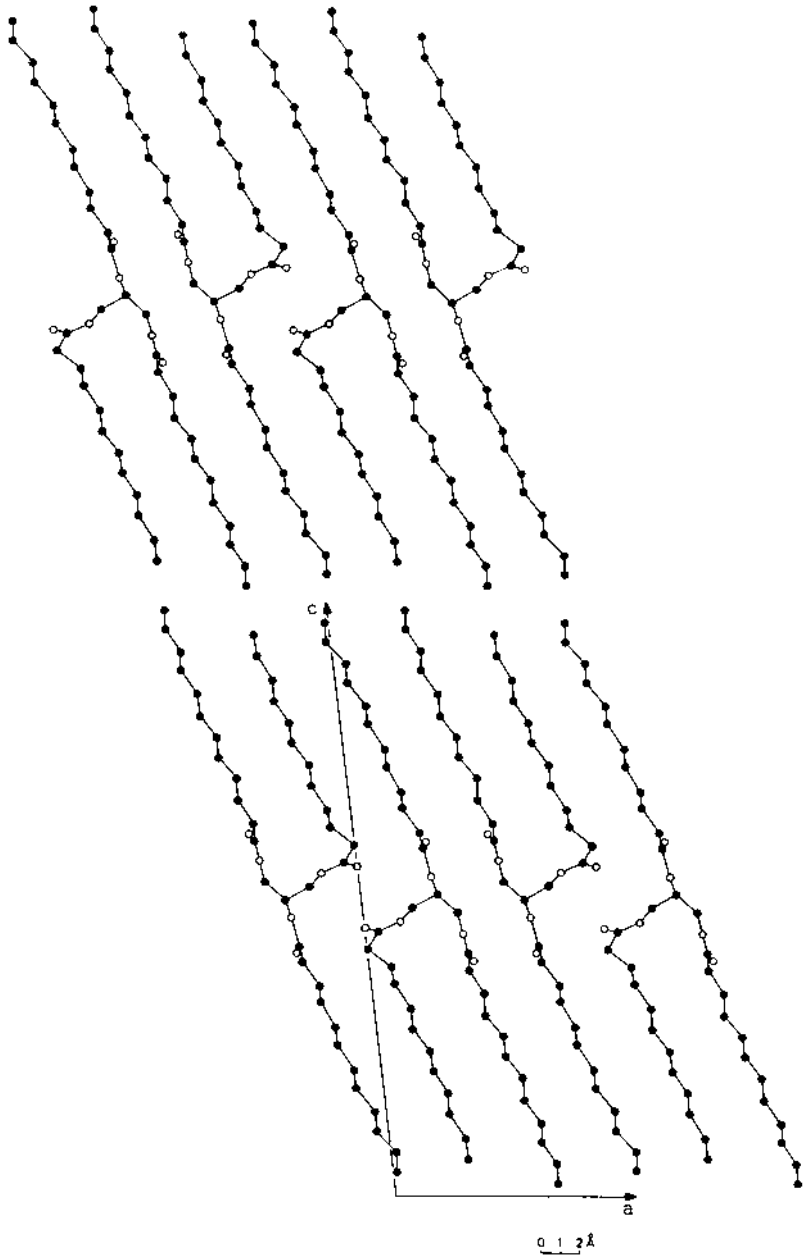


Figure 5 Molecular arrangement of trilaurin in the beta crystal form viewed along the shortest unit-cell axis. (From Ref. 5.)

The fundamental work in this field was reported more than four decades ago by Luzzati and co-workers (cf. Ref. 15). The different structures will be described and complementary information is found in Chapter 4.

A. Methods

The most informative method is x-ray diffraction (or x-ray scattering). Due to the long unit periods in these phases, it is necessary to use small-angle diffraction/scattering equipment. The identification of liquid-crystalline phase is straightforward and based on the existence of sharp diffraction lines corresponding to long spacings in the structure combined with no diffraction lines in the small-angle region, only a diffuse line at 4.5 Å. This line is due to the disordered chains, and the same line is obtained from a liquid triglyceride oil.

Spectroscopic methods, nuclear magnetic resonance (NMR) in particular, can also be used for phase identification and, in addition, provide important complementary information on dynamic properties.

Phase diagrams are useful tools in order to describe lipid–water system, and examples relevant to foods are shown in Chapter 9. Samples with different water contents are equilibrated and single phases are analyzed by x-ray diffraction. First, inspections should be performed in the polarizing microscope, and the birefringence is an important feature of the liquid-crystalline phases. Only the cubic phases lack birefringence, but they are easy to detect because of their high viscosity.

B. Lamellar Liquid Crystals

This phase is characterized by a series of diffraction spacings in the ratio $1:2:3:4\ldots$ corresponding to a one-dimensional repetition with the first-order spacing d equivalent to the thickness of the water layer and the bimolecular lipid layer. By simple geometry, the lipid bilayer thickness and the surface area per polar group can be calculated. The structure is shown in Fig. 6.

A special structure related to the lamellar liquid-crystalline phase is the so-called gel phase. It also consists of water layers alternating with lipid bilayers, but the hydrocarbon chains are crystalline. This phase is often obtained as a meta-stable state when the lamellar liquid-crystalline phase is cooled below the transition point to crystalline chains. The chains in this phase are usually arranged according to the hexagonal symmetry, indicating rotational or oscillation movements around the chains axes.

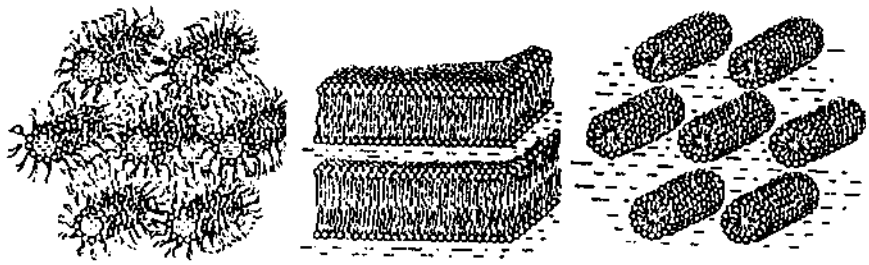


Figure 6 A fragment of the lamellar liquid-crystalline phase (in the middle), the hexagonal (to the right) and the reverse hexagonal (to the left) liquid-crystalline phases.

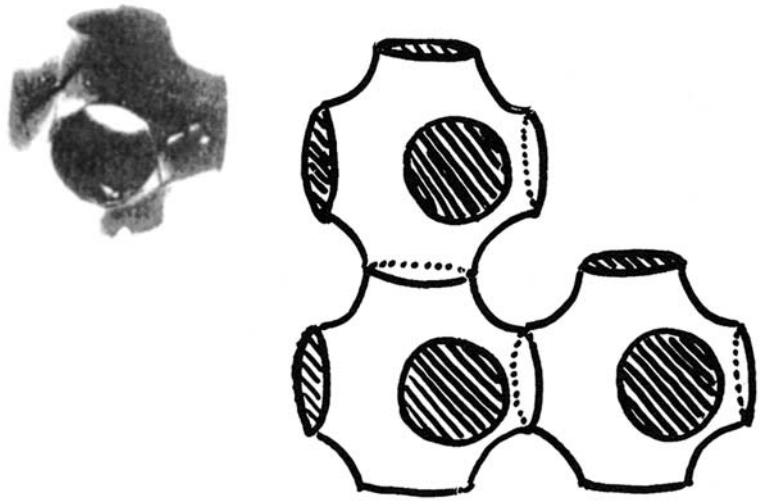
C. Hexagonal Liquid Crystals

A common aqueous lipid phase is often formed in lipids with a small hydrocarbon chain region at about equal amounts of water and lipid. It was called the middle phase in the earlier literature on soaps. This phase shows diffraction patterns with the square roots of the spacings in the ratios $1:3:4:7:12\ldots$, the condition of a two-dimensional hexagonal lattice. The swelling in water corresponds to an ideal two-dimensional swelling. The structure is shown in Fig. 6. Very polar lipids, such as lyso-phospholipids, form infinite cylinders with a liquid hydrocarbon chain core arranged in a hexagonal array and the polar heads facing the outside water. The space between the cylinders is taken up by water.

An alternative hexagonal structure of reversed type (i.e., water cylinders surrounded by lipid molecules) has been observed in many lipid systems with a relatively large hydrocarbon chain proportion. This phase, also shown in Fig. 6, is identified in the same way from x-ray diffraction pattern. By simple geometry, the diameter of the cylinders can be calculated from that the molecular length and the surface area per polar group. There is usually no problem in determining which is the true alternative of these two hexagonal structures, once the molecular dimensions have been calculated.

D. Cubic Liquid-Crystalline Phases

Cubic phases are the most complex of lipid–water phases and their bicontinuous structures have recently been revealed. Such a phase must show diffraction patterns where the square roots of the spacings form the ratios $1:2:3:4:5:6:8:9:10:11:12\ldots$. Absent lines are related to the actual symmetry (space group). The structure of one type of a cubic lipid–water



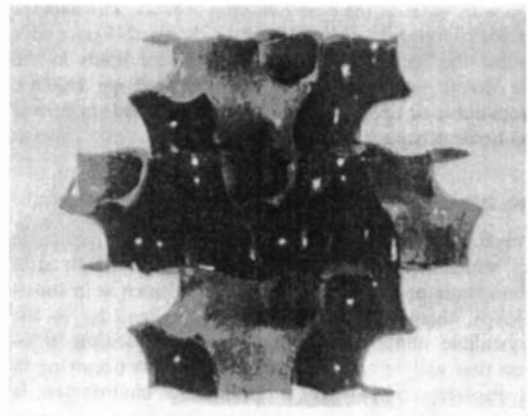


Figure 7 Structure of the simplest bicontinuous cubic lipid–water phase (space group *Im3m*) illustrated in (a) from the structure unit (plastic model) and the linkages of these bilayer units. Another phase (space group *Ia3d*) is shown in (b).

phase is shown in Fig. 7a. It has been shown that the lipid bilayer in such a phase follows an infinite, periodic, minimal surface (16). A minimal surface has zero average curvature in all points; that is, it is as convex as it is concave. The structure unit is illustrated in Fig. 7a, as well as the repetition of these units into an infinite three-dimensional surface. The lipid bilayer is centered on the surface, and on both sides, there is a water channel system.

Six water channels meet in the structure unit. The lipid bilayer is free from intersections and there is no connection between the two sides of the bilayer.

There are three different types of fundamental cubic minimal surface. They have different space groups, and x-ray data corresponding to all of them have been observed (16). All three have, in fact, been seen in the food-grade emulsifier monoolein. A second type of structure is shown in Fig. 7b, with three water channels joined into a structure unit. Finally, there is a structure with four channels joined in tetrahedral geometry.

E. Liquid-Crystalline Disperson of the Lamellar Phase and Emulsions

The lamellar liquid-crystalline neat phase can swell only up to a particular water-layer thickness, which depends on the nature of the lipid. The situation that will be considered here is the one occurring in most lipids, when a disperson of particles in an aqueous environment is formed when water is added above the swelling limit of the lamellar phase. The lamellar phase in neutral lipids swells up to a water-layer thickness of about 20 Å. The particles always have a closed structure with concentric bimolecular layers alternating with water layers. Such particles (termed liposomes) can encapsulate oil.

Friberg and co-workers more than three decades ago were the first to realize the significance of the lamellar liquid-crystalline phase in emulsion stabilization as demonstrated in the first edition of *Food Emulsions*. This stability criterium can easily be defined from phase equilibria; the oil phase and the water phase are in equilibrium with a lamellar liquid-crystalline phase.

A phase transition mechanism might also be utilized for destabilization purposes. If heating of the lamellar phase, for example, results in the formation of a reverse phase, this transition at the surface of the emulsion droplets will result in flocculation and coalescence.

Colloidal dispersions of cubic phases, cubosomes, can be prepared from food-grade components and may have applications in foods (cf. Ref. 17). In this connection, it should be mentioned that food additives that induce formation of a cubic phase with water has applications in order to obtain aggregation of fat globules (e.g., in artificial cream products).

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