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Current Opinion in Colloid and Interface Science 8 (2003) 5–14

**Current Opinion in
COLLOID and
INTERFACE SCIENCE**

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Novel surfactant mesostructural topologies: between lamellae and columnar (hexagonal) forms

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Abstract

Recent developments in theoretical and experimental studies of amphiphilic lyotropic ‘intermediate’ mesophase formation are summarized. For the purposes of the review, we consider intermediate mesophases to be self-assemblies with novel geometries and topologies, excluding lamellar, bicontinuous sponge, columnar (hexagonal) and micellar mesophases. Intermediates include novel branched bilayer topologies, enclosing multiple interwoven channel systems and inclined rod packings, and punctured bilayer morphologies, including mesh phases and bicontinuous monolayers.

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Keywords: Polycontinuous lyotropic mesophases; Bilayer aggregates; Membrane fusion; Mesh mesophases

1. Introduction

Surfactant and lipid self-assemblies still present a number of theoretical and experimental mysteries. This review attempts to expose some of the more fundamental unresolved issues surrounding lyotropic self-assembly in equilibrium systems in the light of recent experimental and theoretical works. We focus particularly on recent observations of ‘intermediate’ phases of complex micellar topologies, membrane fusion intermediates and developments in the theory of phase stability of novel mesh, sponge and polycontinuous structures. (We exclude, for example, geometrically deformed hexagonal phases, such as the centred rectangular ‘ribbon’ intermediates).

The now classic work of Fontell, Larsson and colleagues in Sweden and Luzzati and colleagues in France from the 1960s established the canonical forms of phase diagrams of lyotropic systems based on studies of soaps,

lipids and model surfactants [1^* , 2^* , 3^*]. Lamellar, micellar, columnar (usually hexagonal), mesh and bicontinuous mesophases can form equilibrium phases, in addition to the disordered mesophases, dominated by microemulsions (and, more recently, sponge mesophases). On the theoretical front, the real advance in understanding lyotropic self-assembly came about through the marriage of thermodynamics and molecular geometry proposed by Ninham and colleagues in Canberra in the late 1970s and 1980s, that owed some debt to earlier concepts, such as that of hydrophobic–lipophilic balance, and work of Tanford [5]. Modern theories rely crucially on the concept of membrane bending energy dominating the self-assembly process. It is noteworthy that this theoretical contribution derived from theoretical and experimental studies of bending of biological membranes, associated with Canham [6], and Helfrich [7].

Today, activity and interest in the field of molecular self-assembly has largely moved beyond the study of equilibrium aggregation and phase studies. Much effort is now devoted to dynamic features, such as rheology, phase transformation kinetics, and synthesis of novel mesostructured inorganic materials using lyotropic systems as templates. Nevertheless, reassessment of now less glamorous studies of self-assembly are worthwhile, as these are fundamental to understanding non-equilibrium and dynamic processes in amphiphilic systems and germane to other fields, particularly new materials and membrane fusion in biological systems.

Abbreviations: AFM, atomic force microscopy; DDAB, didodecyl dimethyl ammonium bromide; DMPG, dimyristoylphosphatidylglycerol; DOC, disordered open cylinders; DOPG, dioleoylphosphatidylglycerol; DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; LH, left-handed; RH, right-handed; SAXS, small-angle X-ray scattering; TEM, transmission electron microscopy; TPMS, triply periodic minimal surfaces.

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2. The canon of lyotropic self-assembly

The experimental and theoretical study of mesophase formation in lyotropic systems is now considered sufficiently mature to be explored in some detail in textbooks, with emphases on chemical, physical and materials science aspects [8,9]. The most commonly discussed mesophases are those deliberately excluded from this review. They are lamellar, discrete micellar (packed in various cubic and hexagonal close-packed arrays), columnar or ribbon phases (rod-shaped micelles stacked in a 2D hexagonal or rectangular array) and the cubic bicontinuous mesophases, based on the P, D and gyroid triply periodic minimal surfaces (TPMS, also abbreviated as IPMS). The usual experimental techniques to identify these mesophases are NMR lineshape analysis, diffusion measurements, small-angle scattering data (with neutrons and X-rays) as well as optical texture analyses. In addition, reconstruction of electron density profiles [10] and cryo-transmission electron microscopy (TEM) [11••] have been used to elucidate the details of these mesostructures.

The theoretical understanding of these orthodox assemblies is today dominated by the coupled notions of a preferred molecular shape and a preferred membrane curvature. In a nutshell, deviations from that preferred shape or curvature cost bending energy, and the resulting membrane shapes are the result of minimization of that curvature energy. Amphiphile interfaces can be curved towards the (hydrophobic) oil regions (Type 1) or the polar (hydrophilic) regions (Type 2). The former are favoured by amphiphiles with bulky head-groups (e.g. single-chained charged detergents); the latter by molecules with bulky chain regions (e.g. double chained surfactants). Most biological lipids are finely balanced in their hydrophobic–hydrophilic steric geometries, so that lamellar mesophases, flat and planar, dominate their phase diagrams at biologically relevant physical conditions. Nevertheless, the concept of a well-defined defect-free, uncurved lamellar state is an idealization, and these systems are poised between neighbouring membrane geometries and topologies.

Those neighbouring structures are the mesh, bicontinuous and columnar states. Older reviews of mesh [12] and bicontinuous [13] mesophases remain relevant. Analysis of their geometries and topologies, and relations to the simpler discrete micellar, hexagonal, ribbon and lamellar phases has been done. The standard picture has it that there is a gradual evolution of mean curvature in both Type 1 and Type 2 mesophases, dependent on solvent concentration and also temperature to a greater or lesser extent.

The geometrical viewpoint is particularly simple in Type 2 systems [14]. Straightforward analysis of the shape parameters for mesh, bicontinuous, rod and discrete (globular) micellar forms and global surface to

volume ratios, leads to the conclusion that a sequence lamellar—mesh—bicontinuous (sponge)—rod—globule is expected on water dilution, assuming the molecular shape (defined by the shape parameter) changes little on dilution. In addition, the sequence of lattice crystallographies encountered in bicontinuous and discrete micellar phases can be explained on surface-to-volume grounds alone. If the assembled amphiphiles are chemically monodisperse, so that a single value of the membrane curvature or (equivalently) molecular shape is preferred, only those structures with a strongly peaked distribution of curvatures are admitted. In practice, that typically means one encounters a lamellar phase, then one or two bicontinuous cubic mesophases (based on the D or Gyroid morphologies), then a hexagonal phase and (possibly) a discrete micellar phase on water dilution.

The situation for Type 1 systems is similar, with lamellar, bicontinuous cubic, hexagonal and (discrete) micellar mesophases usually encountered, corresponding to a continuous increase in the magnitude of hydrophilic–hydrophobic interfacial (mean) curvature, or, equivalently, a continuous decrease in the value of the shape parameter of the amphiphiles.

3. Current theoretical analyses of novel mesophases

This simple picture is muddled by reality. In practice we remain unable to predict *ab initio* a phase diagram from the molecular constitution of the amphiphile(s) alone. That goal remains elusive, due in part to the vagaries of undulation forces, membrane bending elastic moduli and specific ion effects governing the delicate balance of hydrophobic, electrostatic and electro-dynamic (dispersion) forces and entropic contributions that dictate the self-assembly process. A fundamental problem remains the theoretical impossibility of deducing a complete phase diagram from first principles, even for a simple model amphiphile. Recent theories have invoked Landau-type approaches, with some success [15]. The underlying physics, however, is reminiscent of earlier theories, with some modifications [16]. That problem derives from the impossibility of writing down a complete Boltzmann-type distribution of all *possible* membrane geometries and topologies, appropriately weighted by their energies and entropies. Some examples of the wealth of structures not yet included in the theoretical canon will be discussed later.

The formation of bicontinuous cubic mesophases is comprehensible within the standard canon, as these TPMS geometries are favoured for Types 1 or 2 systems with preferred curvatures just marginally different from that leading to lamellar phases. They offer quasi-homogeneous forms with almost constant shape parameters throughout the structure. Rigorous modeling of mesh mesophase geometries has only very recently been

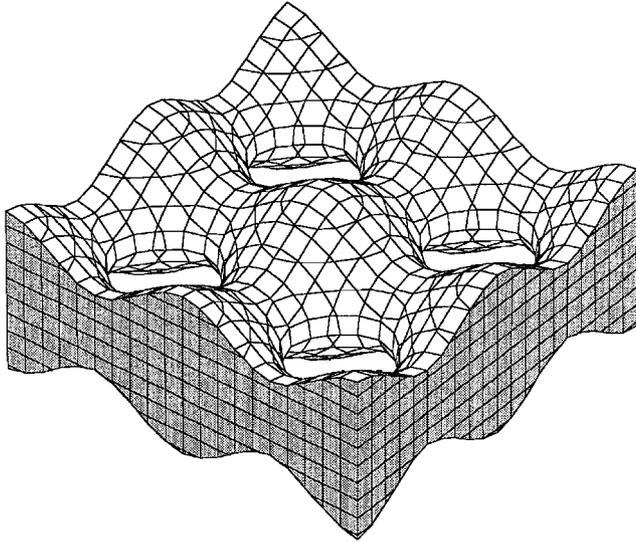


Fig. 1. Nodal surface description of a single layer of a tetragonal mesh structure. Successive layers that are typically stacked to form a body-centred tetragonal arrangement.

explored [17^{••}]. These authors have pointed out serious deficiencies in current theoretical studies of the relative stability of mesh and bicontinuous structures. At present, no model can compare their stabilities, as the relative energies of mesh phases—dependent on the curvature variations and global packing frustration constraints—remain difficult to ascertain. A reasonable *topological* model for mesh structures remains the original proposal of Luzzati for the structure of anhydrous soap phases: planar bilayers, interspersed with square (tetragonal phase) or—more commonly—hexagonal arrays of pores within the bilayers (leading to the body-centred tetragonal and rhombohedral structures with 3-dimensional space groups I422 and R3m, respectively) [18]. The details of the optimal mesh *geometry* remain uncertain. If the dominant contribution to the mesophase stability is the bending energy of the surfactant monolayers, the surfaces adopt a constant mean curvature retaining the topologies of Luzzati. The excellent freely available EVOLVER software developed by Brakke [19] can be used to generate mesh surfaces of fixed mean curvature to arbitrary accuracy. Reasonable geometric approximations to those geometries can be realised using a contour surface parametrisation involving simple trigonometric functions [20], equivalent to the nodal surface approximations for TPMS [21,22] exploited most recently by Schwarz and Gompper [15]. A nodal surface parametrisation of a single layer of the tetragonal mesh mesostructure is shown in Fig. 1.

Mesh surfaces of constant mean curvature resemble closely the nodal surfaces, though their curvature variations are very different to those of nodal surfaces or other mesh surfaces of equivalent topology [17^{••}].

Estimates of the bending energies of mesh surfaces thus depend crucially on the choice of surface geometry. That choice remains somewhat arbitrary, given our ignorance of the relative importance of other contributions to the monolayer stability, such as frustration terms, due to variations in surfactant chain lengths, electrostatic interactions, and so on. The relative importance of these contributions remain largely unexplored in recent years, in contrast to earlier studies [4[•],23] with the exception of some discussion of bending energies and chain stretching energies by Templer and colleagues [24], following earlier studies in reverse hexagonal (H₂) mesophases [25]. A number of alternative explorations of relative stability have been canvassed in the literature couple bending and stretching terms [26,27], though their value remains uncertain.

Theoretical investigation of stretching contributions to the relative energies of bicontinuous and mesh intermediates requires a robust algorithm for determining variations in monolayer thicknesses as a function of geometry and topology. A generic technique is emerging, involving the numerical estimation of ‘medial surfaces’, that define the centers of polar and apolar domains for a given monolayer geometry. To date, that algorithm has been applied only to the simpler TPMS found in bicontinuous mesophases (the P, D and Gyroid geometries, Fig. 2). The analysis suggests that the G structure has the most monodisperse distribution of domain thick-

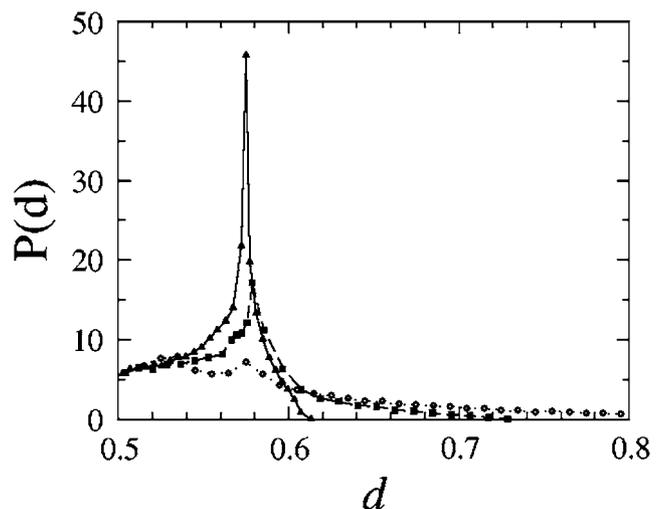


Fig. 2. Distribution of distances (d) from the P(rimitive), D(iamond) and Gyroid TPMS to the centers of their channels via a medial surface construction. The graphs describe the distribution of radii of ‘maximal spheres’ that just fit within the channels of the TPMS, grazing the surface at more than one point. The P distribution is marked by open circles, the D by filled squares and the G by filled triangles. The frequency of distances $P(d)$ are weighted by the percentage of area on the TPMS with that distance. The unit cells of the cubic TPMS are scaled to give equal Gaussian curvature distributions (so that they are isometric). Note the homogeneity of distances decreases in the order G–D–P.

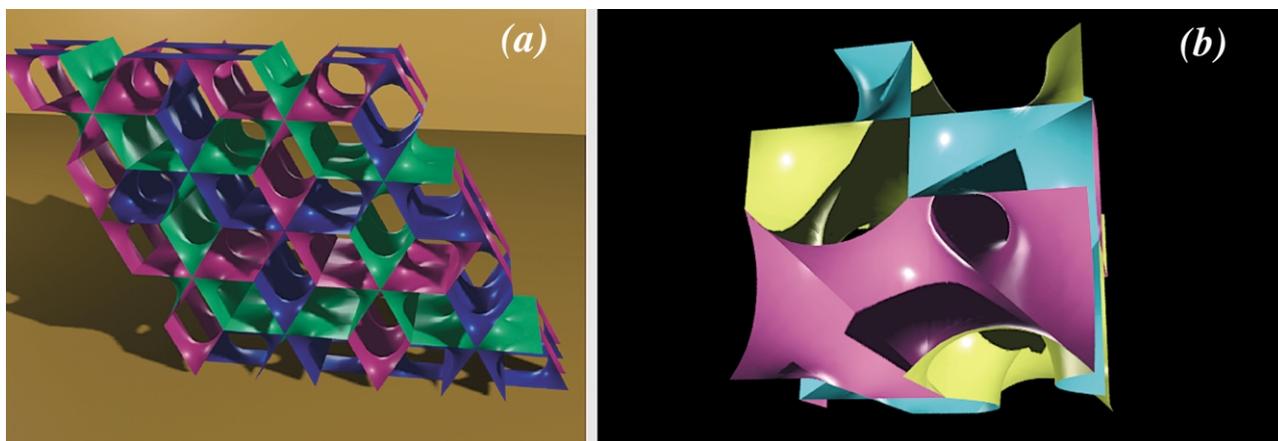


Fig. 3.

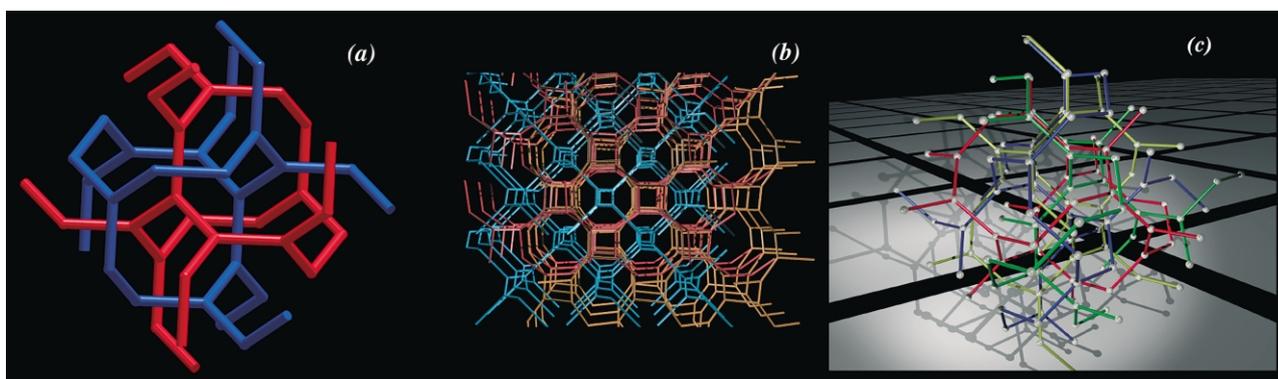


Fig. 4.

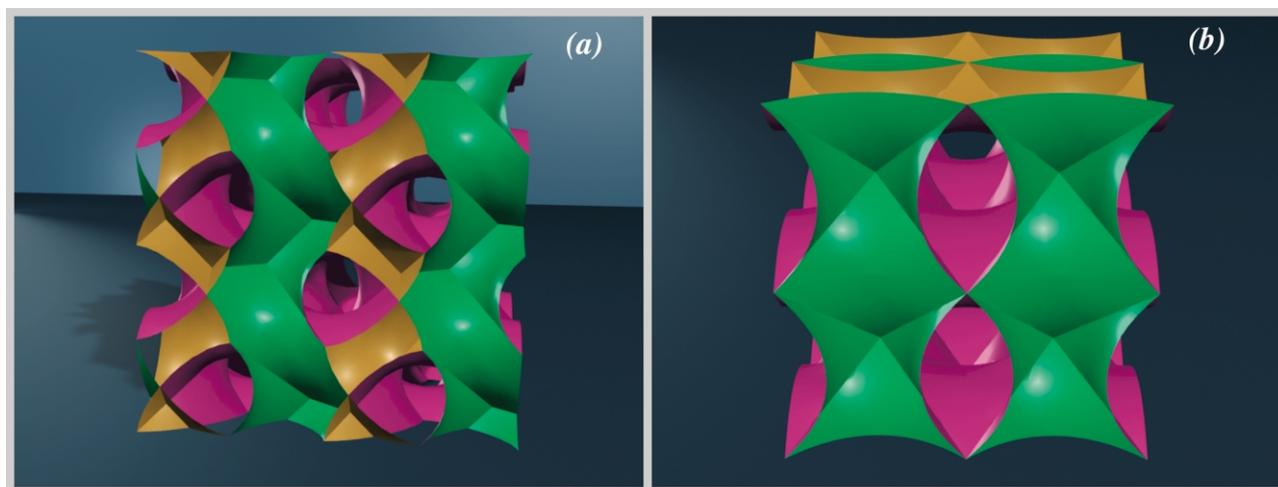


Fig. 5.

nesses, followed by the D structure (with the P far less uniform) supporting that supports the ubiquity of the G and D structures in bicontinuous mesophases. Extension of that analysis to various possible intermediate mesophase geometries is required, particularly to non-cubic deformations of the bicontinuous geometries and to mesh structures.

In addition to the need for better understanding of the relative importance of stretching and bending terms, as well as other interactions, in stabilizing mesophases of arbitrary topology, a better overview of *possible* shapes is needed. A thorny issue in comparing different mesophases is the virtual distinction between ‘monolayer’ and ‘bilayer’ mesophases. The distinction is one of convenience only; certainly any bilayer can be considered as a pair of monolayers. The converse holds, provided bilayer punctures and branches are admitted, discussed later in this review. The bilayer description is particularly succinct for those phases consisting of chemically identical monolayers, whose local mirror symmetry, with respect to a tangent plane inserted at the mid-plane of the (reversed for Type 1, or normal for Type 2) bilayers, imposes the constraint of zero mean curvature on that mid-plane, defined as the bilayer surface. (We note that the pair of monolayers making up the bilayer are generally not everywhere equidistant from the bilayer surface: the bilayer may be of varying thickness.) Bilayer surfaces are thus physically realisable as (stable or unstable) soap films, also of zero mean curvature, and a useful catalogue of possible structures with identical monolayers can be drawn from that field. Such films may contain branches, or punctures. For example, the reversed hexagonal mesophase (H_2), constructed of infinite, columnar monolayers, is a branched bilayer folded onto the faces of a hexagonal honeycomb, with infinite, parallel, threefold branch lines forming a 2D hexagonal ($p\bar{6}m$) lattice (and the Type 1 hexagonal phase, H_1 , consists of a reversed bilayer folded onto the same branched honeycomb). Similarly, discrete micellar mesophases, consist of cellular bilayer structures. Thus, the body-centred cubic ($Im\bar{3}m$) micellar phase, is a bilayer foam, with threefold bilayer branch lines along the edges of the truncated octahedral cells, and branch

points at vertices of those cells. Bicontinuous phases consist of sponge-like bilayer films lining TPMS, free of branch lines or branch points.

Recent studies of branched minimal surfaces suggest a number of intermediate structures, containing features of bicontinuous and columnar mesophases. Branched TPMS describe *polycontinuous* structures, containing more than two subspaces separated by the bilayer surface [28,29]. A $p\bar{6}m$ array of parallel infinite branch lines (identical to those in the H phase), spanning saddle-shaped films, is found in a hexagonal tricontinuous TPMS (Fig. 3a). A cubic array ($Ia\bar{3}d$ rod packing [30]) of (inclined) infinite branch lines is found in the ‘Archimedean screw’ minimal surface of Elser (Fig. 3b) [31]; this chiral surface too is tricontinuous (with symmetry $I4_132$): the three labyrinths are tetragonally distorted examples of a single labyrinth in the gyroid ($Ia\bar{3}d$) bicontinuous cubic mesophase (the Y^* net). Branch lines induce increased curvature in the constituent monolayers compared with the unbranched bicontinuous structures. These model polycontinuous examples are geometrically intermediate to the bicontinuous and hexagonal mesophases and offer attractive structural intermediates to sponge and columnar mesophases. Detailed calculations of their stability have not been done, though general arguments suggest they are particularly stable polycontinuous examples.

Still further curvature can be induced by imposing branch points in addition to branch lines. Quadra- and octa-continuous cubic minimal surfaces are known to date (both chiral). The quadracontinuous example, (space group symmetry $P4_232$) is particularly attractive, containing four chiral interwoven labyrinths, each identical to a Y^* net found in the gyroid phase (Fig. 4).

These polycontinuous examples are generalizations of bicontinuous patterns that consist of interwoven sponge-like labyrinths, each triply periodic. Mesh surfaces, with doubly-periodic labyrinths, can also be generalized to give novel spatial patterns. The known mesh mesophases (T and R) consist of smectic-like stacks of mesh surfaces, all oriented equivalently. Other mesh intermediates are geometrically and topologically feasible in

Fig. 3. Tricontinuous hexagonal branched minimal surface (space group $P6_3/mcm$), containing three identical interwoven hexagonal labyrinths. (Left) Tricontinuous cubic branched minimal surface (space group $I4_132$), a ‘cubic Archimedean screw’ of Elser [31], with three interwoven and identical channels (rods). Each channel is a distorted Y^* network, found in the cubic bicontinuous gyroid mesophase (see Fig. 4).

Fig. 4. Known and hypothetical channel structures in bi-, tri- and quadracontinuous cubic morphologies, all consisting of Y^* graphs: (a) The (RH and LH) Y^* labyrinths of the bicontinuous gyroid mesophase. (b) Trio of (RH or LH) distorted (tetragonal) Y^* labyrinths in Elser’s surface (Fig. 3). (c) Quartet of (RH) or (LH) Y^* labyrinths in the quadracontinuous chiral cubic (space group $P4_232$) branched minimal surface.

Fig. 5. (a) The chiral cubic ($I4_132$) β -Mn minimal surface, containing branch lines and points, that partitions space into the β -Mn cubic rod packing. (b) A β -W surface ($Pm\bar{3}n$), an alternative spatial partition into a cubic rod packing.

space, though they have not been identified in actual amphiphilic mesophases. For example, three differently oriented stacks of rhombohedral-like mesh monolayers, aligned along the edges of a Kagomé tiling [32] can be interwoven to form an infinite number of mesh cells [33]. The surface that partitions space into this labyrinth pattern has not yet been derived. Like the previous examples, it is a minimal surface (and is therefore a feasible bilayer surface for simple surfactant–water systems), containing parallel, infinite threefold branch lines. Branched minimal surfaces containing an infinite number of columnar cells can be found, in addition to the hexagonal honeycomb. These structures consist of rod packings [30,34] of (rod-shaped) micelles, more complex than the hexagonal array characteristic of H_1 and H_2 mesophases. Minimal surfaces whose channels define the cubic I432, β -W and β -Mn rod packings have been derived recently (Fig. 5).

Other examples of rod, mesh and sponge labyrinth systems can be found among the fascinating list of self-intersecting TPMS (that contain even-fold branch lines) of Koch and Fischer [35,36] though we expect those examples to be less stable bilayer morphologies in simple amphiphile-water systems than those mentioned above. Some discussion of the *possible* relevance of the latter examples to surfactant self-assemblies can be found elsewhere [28]. Given the wealth of possible intermediate structures, combining elements of bicontinuous (sponge), mesh and columnar (hexagonal) geometries, estimation of their relative stabilities is needed. Within each of the new structural classes introduced here—sponges with branch lines, sponges with branch lines and branch points, interwoven meshes with branch lines and columns with branch lines and points—we have described the likely most stable examples. Measures of relative stability, however, require estimates of the contributions of (bilayer) branch lines and points on stability.

One further ‘defect’ of the bilayer surfaces, additional to the insertion of branches, needs to be considered in any discussion of possible intermediate phase geometries: bilayer punctures. The insertion of punctures in the bilayer surface, resulting from the removal of discs from the surface, decreases the curvature of the bilayer, opposite to the effect of branches. The shape parameter of a Type 2 structure is *reduced* by puncturing of the bilayer surface, that of a Type mesostructure is *increased* [37]. Indeed, mesh mesophases are punctured planar bilayers, whose detailed mesostructures depend on the ordering of the punctures with and between lamellae. In addition to those examples, 3d mesostructures result from puncturing more complex bilayer surfaces. For example, a punctured bicontinuous bilayer is smoothly deformable into a bicontinuous monolayer. Indeed, a continuous bilayer–monolayer–reversed bilayer transition can be effected by inserting, growing and then

shrinking punctures within a sponge [37]. Bilayers can be turned inside–out by this process, transforming from Type 1 to 2 without passing through the flat lamellar mesophase geometry! That possibility is best described graphically. The pathway is illustrated in part in Fig. 6.

If the puncture radii are comparable with the bilayer thickness, the structure—a punctured bilayer—is geometrically indistinguishable from a bicontinuous monolayer. The route relies on the possibility of forming structures whose mean and Gaussian curvatures remain nonzero, but balance, to allow a shape parameter of unity. That situation is identical to a bicontinuous monolayer morphology, equivalent to the disordered open cylinders (DOC) cylinder model invoked to describe some bicontinuous microemulsions in cationic surfactant systems [38]. Analysis of punctured bilayers is particularly important to the understanding of membrane fusion, discussed below.

4. Current experimental understanding of novel mesophases

The prime mystery remains that of the relative stabilities of bicontinuous vs. ‘intermediate’ mesophases. The theoretical challenge is clear following the extensive surveys of phase diagrams of surfactant–water systems by Tiddy and colleagues (largely in non-ionic systems, from Ref. [12]). The analysis in the previous section suggests that intermediate mesophases of novel topologies and geometries fall into three topological classes, non-cubic bicontinuous sponges, and ‘defective’ bilayers, containing branched or punctured bilayers. (A fourth class, ribbon mesophases, consisting of geometrical distortions of the columnar micelles in hexagonal mesophases, is discussed in detail by Holmes [12].) Experimental studies of surfactant–water phase diagrams suggest that intermediate structures form in place of bicontinuous cubic mesophases once the surfactant chains exceed a certain length, or their rigidity is enhanced (e.g. by replacing hydrocarbon with fluorocarbon chains [39]).

To date, the only examples of non-cubic bicontinuous intermediate mesophases are those of Kékicheff et al., already noted in Holmes’ review [12]. In contrast, a number of mesh intermediate mesophases have been reported since then. Recent experimental work confirms earlier reports that fluorocarbon surfactants form intermediate mesh lyotropic mesophases in place of bicontinuous (cubic) phases found in their hydrogenated counterparts [40]. The phase behaviour of tetramethyl ammonium perfluorodecanoate in water is dominated by mesh phases, with distinct phase boundaries between a random mesh and conventional rhombohedral mesh phases [41]. That finding is of some interest, as charged perfluorinated surfactants have been reported previously to form tetragonal mesh phases. A mesh phase, with no

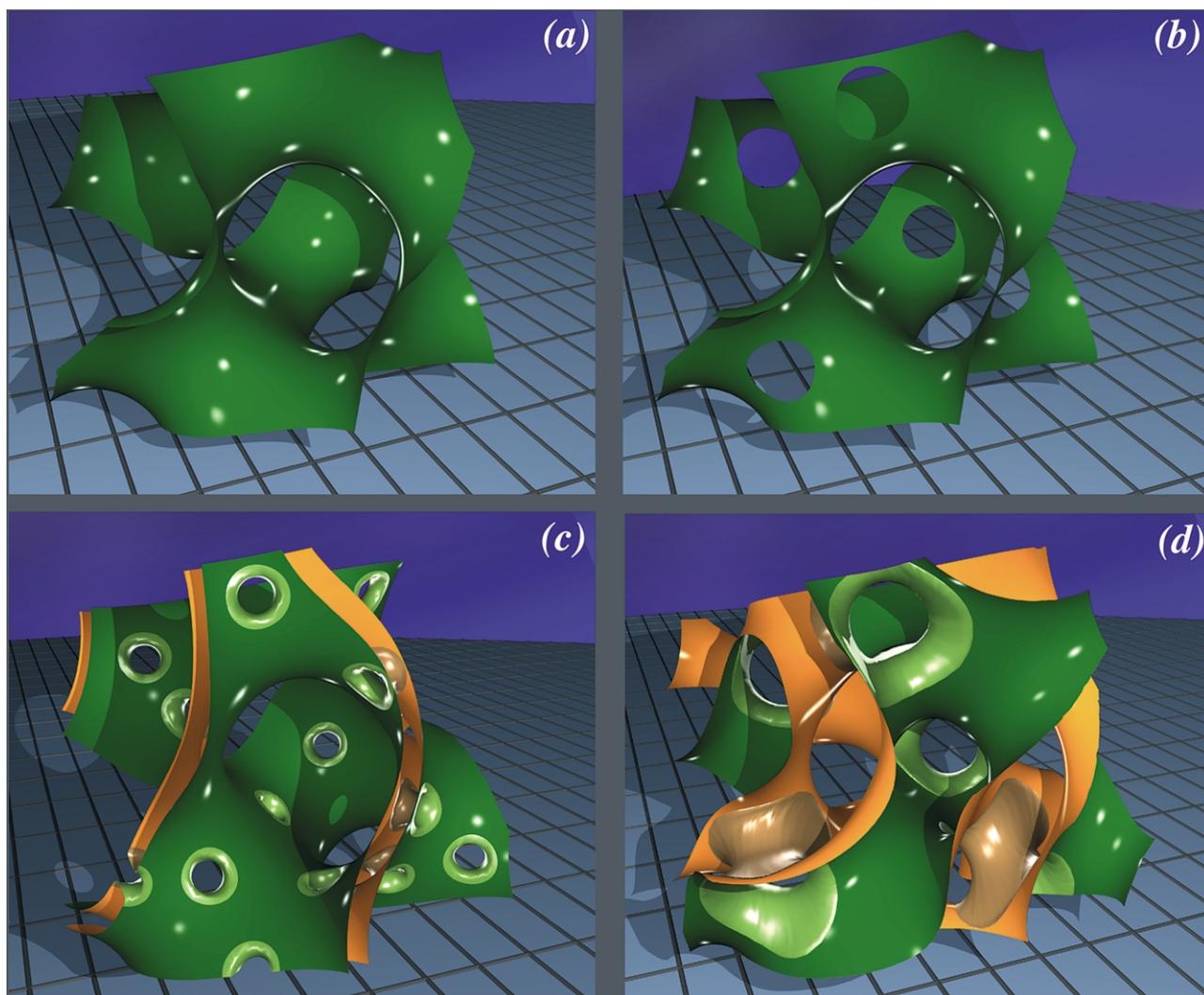


Fig. 6. (a) Fragment of a bicontinuous bilayer surface (a sponge, of zero mean curvature) (b) Punctured sponge bilayer surface. The bilayer surface runs through the mid-surface of the bilayer (between the chain ends for Type 2 mesophases; through the midplane of the polar layer for Type 1). (c, d) Monolayer interfaces, separating hydrophobic from hydrophilic domains. A number of catenoidal surface patches, corresponding to bilayer punctures, are shown connecting the parallel monolayer sheets. (c) Small punctures (shape parameter just larger than one for Type 2 systems, with chains in the thin film between the parallel sheets). (d) Larger punctures, with an average shape parameter close to one. The bilayer surface corresponding to (d) is shown in (b). The swollen punctured sponge in (d) is geometrically and topologically indistinguishable from a bicontinuous monolayer geometry.

correlations between the bilayer punctures, has also been reported in a more complex mixed surfactant–lipid system, though its structure is more difficult to ascertain [42].

The most detailed study of a hydrated intermediate mesophase to date is that of Leaver et al. The authors focus on the geometry of the intermediate $R\bar{3}m$ mesophase observed in binary systems containing water and the nonionic polyoxyethylene surfactants $C_{30}EO_9$ and $C_{16}EO_6$ [17••]. That work confirms Luzzati's model topology for the rhombohedral mesh structure, though it offers a timely reminder that the monolayer curvatures distributions (and resultant bending energies) are

extremely sensitive to the model, more sensitive than topology and even geometrical details of the interfacial shape in space.

The experimental focus has been largely on liquid crystalline mesophases. Recent work on microemulsions suggests the importance of novel intermediate mesostructures in disordered mesophases also and is consistent with the topological analysis introduced in the previous section. The ternary cationic system didodecyl dimethyl ammonium bromide (DDAB), water and tetradecane, exhibits a broad microemulsion region in the phase diagram, that is distinct from those of DDAB, water, alkane systems with shorter chained oils. The latter form

bicontinuous monolayers (the DOC cylinder morphology). Earlier investigations suggested that the tetradecane microemulsions consists of a disordered bilayer structure [43,44]. New studies, combining small-angle X-ray scattering (SAXS) and NMR relaxation measurements reveal a more complex situation. The mesostructure evolves continuously from a disordered bicontinuous bilayer (similar to a sponge L_3 phase, and adjacent to a bicontinuous cubic mesophase in the ternary phase diagram) to a disordered bicontinuous monolayer on addition of tetradecane [45]. The mesostructural evolution on oil dilution is that shown in Fig. 6: tetradecane opens punctures in the sponge-like bilayer, that grow to form a DOC monolayer configuration.

5. Biological implications

The relevance of mesh and other intermediate mesostructures extends beyond the finer details of phase studies. The overwhelming focus on proteins and their influence on biological function has surely overshadowed the equally important area of control of membrane topology through lipid biochemistry. Despite that, some significant experimental reports have appeared recently, that shed light on membrane fusion and dissolution and suggest the importance of lipids in determining membrane form and function.

A number of direct observations of lipid membranes have revealed punctures in bilayers of physiological relevance. For example, atomic force microscopy (AFM) images of A–B bilayers on silicon wafers, formed by sequential Langmuir–Blodgett monolayer deposition (with A deposited first onto the silicon substrate, then B), show bilayer punctures of circular (or coalesced circular) aspect, occupying between 10 and 20% of the membrane area, in bilayers of composition DMPE–dipalmitoylphosphatidylglycerol (DPPG), DMPE–dimyristoylphosphatidylglycerol (DMPG), DMPE–dioleoylphosphatidylglycerol (DOPG). Punctures and polygonal linear puncture networks were seen in dipalmitoylphosphatidylcholine (DPPC)–DPPG, DPPC–DMPG, DPPC–DOPG bilayers [46]. The relevance of these ‘defects’ to biological membranes is therefore certain.

Almgren has published a comprehensive review of his work on dissolution of bilayers by addition of (charged and uncharged) detergents and bile salts to lipid water mixtures [11••]. A number of cryo-TEM images are contained in that report, that suggest the importance of novel ‘intermediate’ membrane topologies in biological processes and the possibility of topological and geometric control of membranes by biochemical variations of the bilayer composition. The experiments explore changes in membrane morphology on addition of surface active additives with small shape parameters (less than unity) to standard bilayer-forming lipids

(whose shape parameters are close to, and probably slightly exceed, unity). From the discussion in the previous section, the bilayer is expected to shift from a lamellar or sponge state to a punctured bilayer state, i.e. mesh or bicontinuous monolayer topologies. This expectation is confirmed in many cases, with the formation of mesh bilayer fragments (‘lace-like’ bilayers), bicontinuous monolayers (interwoven and likely interconnected thread-like micellar assemblies) and punctured vesicles in both charged and uncharged systems. In many cases, the punctures grow to form extended lines rather than loops, resulting in thread-like columnar micelles. Novel twisted bilayer ribbons are reported in some cases. Connected networks of twisted ribbons are a novel geometry that emerges by growth of punctures within sponges [37]; the twist relaxes the hyperbolic bilayer shape. Almgren’s report of this form thus provides some support for the notion of punctured bilayer intermediates. In addition, isolated bilayer discs form, that may relax to standard Type 1 closed micelles. The formation of finite bilayer fragments is due to addition of detergents to vesicles as starting structures, of finite size. The final configuration of the mixed systems is set in part by the effective shape parameter of the mix; as its value decreases (towards its minimum possible value, $1/3$), the final state is expected to be, respectively, mesh, bicontinuous monolayer, columnar and globular micelles. Specific interactions dependent on charged species etc. must also be at play [47], as some intermediates are dependent on the presence of salt.

The real space reconstruction of the electron density distribution in a rhombohedral mesh phase have been achieved for the first time very recently by Yang and Huang [48••]. The result is particularly noteworthy as the mesh structure is formed by a bilayer-forming lipid, diphytanoyl phosphatidylcholine. In their study, lamellar, mesh and hexagonal (H_2) structures are induced by varying the relative humidity of the lipid stack. In order to induce a preferred orientation, Yang and Huang confine the sample to a flat silicon nitride substrate, thus it is misleading to refer to their structures as true mesophases. The resulting mesh intermediate is topologically identical to the original proposal of Luzzati, and close to model mesh geometries described above, although some rippling of the mesh layers is detected.

Some uncertainties are likely in the structural solution, dependent on accurate phasing of reflections, and noise-free data. (In addition, their assignation of the $R\bar{3}$ space group is presumably in error: the description suggests the usual $R\bar{3}m$ space group). The technique used is a potentially useful one, allowing in principle accurate structural reconstruction from diffraction data. (Similar techniques have been employed by Luzzati and coworkers to ‘solve’ structures of bicontinuous cubic mesophases [10]).

Both Almgren's and Yang and Huang's work demonstrates the continuing relevance of studies of self-assembly. The importance of their work lies in its biological implications: for the first time, it is clear that membrane-forming lipids found in biological systems can form intermediate (mesh) structures under biologically reasonable constraints (assuming the influence of the inorganic substrate is only to align the structure!). The data suggest a conclusion of profound biological importance: punctures can form spontaneously, and reversibly, in lipid bilayer membranes through control of water content alone. The long-held suspicion that biological activity may be controlled by lipids in addition to the conventional protein-governed mechanisms is beginning to be confirmed. That progress imposes a new motivation for continuation of careful experimental and theoretical study of possible mesostructures in surface-active systems. The full wealth of possible mesostructures and mesophases remains uncertain, and further work is required to restore the imbalance induced by decades of focus on protein function as a principle control for biological systems. The older picture of lipids as a passive matrix, hosting the real bio-machinery, proteins, needs to be redrawn, with a better appreciation of the active role of lipids and other membrane-bound agents [3].

Acknowledgments

We thank Jevon Longdell and Stuart Ramsden for their advice and assistance.

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