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### **Two sides of the coin. Part 1. Lipid and surfactant self-assembly revisited**

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# **Two Sides of the Coin.**

## **Part 1. Lipid and surfactant self-assembly revisited.**

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**Abstract.**

Hofmeister, specific ion effects, hydration and van der Waals forces at and between interfaces are factors that determine curvature and microstructure in self assembled aggregates of surfactants and lipids; and in microemulsions. Lipid and surfactant head group interactions and between aggregates vary enormously and are highly specific. They act on the hydrophilic side of a bilayer, micelle or other self assembled aggregate. It is only over the last three decades that the origin of Hofmeister effects has become generally understood. Knowledge of their systematics now provides much flexibility in designing nanostructured fluids.

The other side of the coin involves equally specific forces. These (opposing) forces work on the hydrophobic side of amphiphilic interfaces. They are due to the interaction of hydrocarbons and other “oils” with hydrophobic tails of surfactants and lipids. The specificity of oleophilic solutes in microemulsions and lipid membranes provides a counterpoint to Hofmeister effects and hydration. Together with global packing constraints these effects determine microstructure.

Another factor that has hardly been recognised is the role of dissolved gas. This introduces further, qualitative changes in forces that prescribe microstructure.

The systematics of these effects and their interplay are elucidated.

Awareness of these competing factors facilitates formulation of self assembled nanostructured fluids.

New and predictable geometries that emerge naturally provide insights into a variety of biological phenomena like anaesthetic and pheromone action and transmission of the nervous impulse (see Part 2).

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## PART 1 - THE PHYSICAL CHEMISTRY OF SELF ASSEMBLY

### 1 Introduction

In a world of automobiles and airplanes there is no doubt that the alkanes and their relatives would be the most important molecules. They would be kings, at least as far as energetics is concerned. But for biologists and biochemists they rate among the least in the hierarchy of interesting molecules.

The role of hydrocarbons in the self assembly of lipids that form membranes draws some interest from biophysicists. But even that interest usually assigns only a passive role to the alkyl chains of lipids. They just provide a backdrop for the activities of proteins. Most of the dynamic action in membrane structure and function, be it, *e.g.*, conduction of the nervous impulse or ion pumps, is ascribed to electrostatic forces due to salt and hydration forces between lipid and surfactant headgroups that operate on the aqueous side of a monolayer or bilayer. Over the last several decades the classical theories of physical chemistry and of colloid and surface science that informed our intuition, have been subject to re-evaluation [1,2]. It is that older intuition on which the early theories of self assembly were based. Ion specificity, Hofmeister effects are missing from classical theory. The same is true for classical theories of pH, buffers, interfacial tensions, activities and colloidal forces of interactions [3,4].

If we do not understand the specificity of forces completely, we know how to put them to work in microstructural design.

We hope this will become clear as our essay develops.

In the same time frame, say over the past three decades, there has been a developing awareness of the existence and central role for non Euclidean geometries, cubic,

random and other bicontinuous phases that are often the preferred geometries of Nature [5].

These advances have had a big impact. We now have more insights into factors behind complex nanostructures and their transformations than were previously imaginable [6,7].

Our intention here is to explore and demonstrate a parallel source of flexibility and complexity in self assembly: the proposition that hydrocarbons have their own systematic specificity. These forces, on the hydrophobic side of an interface, provide a counterpoint to action that determines curvature on the hydrophilic side of membranes. The alkanes and lipids are an integral part of the machinery of membrane structure and function.

The combination of the opposing tensions between “oil”, and ion specificity-hydration together with the availability of new geometries opens up access to a diversity of structures and functions.

It inevitably leads on to much more complex nano- and microstructures that we call supra-self assemblies that occur in biology.

In parallel with oil specificity we will show that other kinds of solute, in particular dissolved atmospheric or other gases can have equally dramatic effects in setting curvature and microstructure.

In Part 2 we will apply the new conceptual insights discussed in Part 1 to real biological systems and show how hydrocarbon specificity and gas effects can be and are exploited, *e.g.* in optimising drug action, anaesthetics, immunology, pheromones and more.

To draw out our theme we will avoid mathematical details as far as we can so as to focus on concepts.

## 2 Self Assembly without Specificity

### 2.1 First ideas

The first ideas to give a unified characterisation of self assembly were an extension of ideas of Tanford [8]. It purported to be a statistical mechanical theory of self assembly of surfactants and lipids. The theory claimed to span the gamut of micelles, cylindrical and vesicle aggregates, lamellar and reverse phases that had previously been lacking. It was limited to dilute solutions, *i.e.* interaggregate forces were ignored.

It relies on a continuum solvent model for water. It relies on a continuum model for the oil-like interior of a micelle or bilayer aggregate. When interaggregate forces occurred it relied on the classical physical chemistry of ionic solutions and colloidal interactions (DLVO theory). It appeals to hydration where it has to. It invokes a (measured) hydrophobic free energy of transfer of the hydrocarbon chain of a surfactant from water to bulk oil. But it ignored interactions between aggregates.

The required parameters were several. They were: the measured hydrophobic free energy of transfer of an alkane from water to the oil-like interior of a micelle (that is approximated to be as its bulk phase in standard conditions), and a surface free energy due to opposing forces at the (curved) oil-water interface. This reduced to the parametrisation of curvature energy in terms of surfactant parameter  $p$  shown in Figure 1 and defined as:

$$p = v/(al) \tag{1}$$

where  $v$ ,  $l$  and  $a_0$  are the volume and (roughly 80% of) the length of the alkyl chain in the fully stretched *all-trans* conformation, and the cross section area occupied by a single polar head group, respectively. Depending on the value of  $p$  we can predict the

shape of the self assembled aggregate: either spherical micelles, cylinders, planar bilayers, or a reverse curvature structures [8,9].

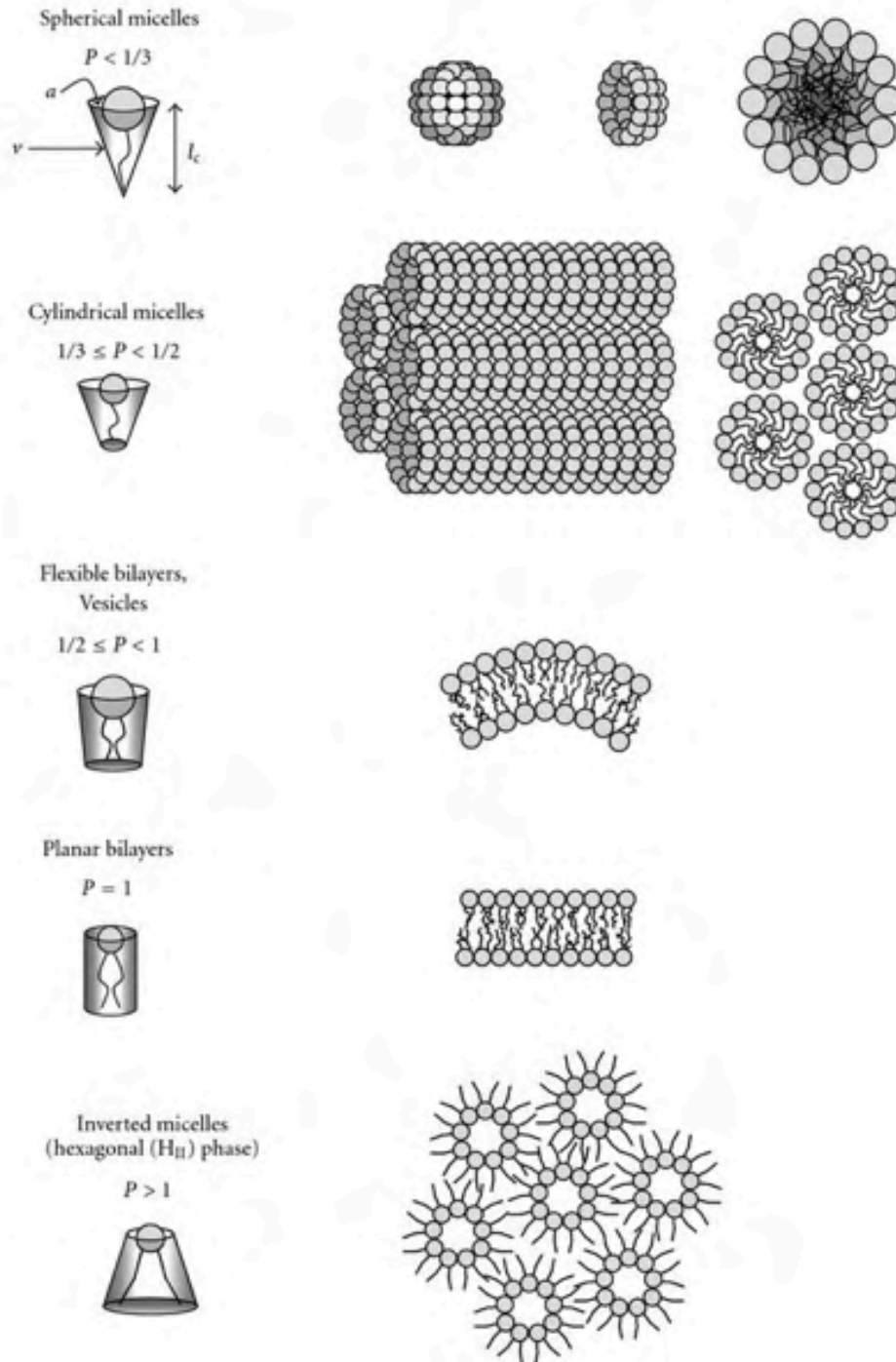


Fig. 1 Surfactant packing parameter  $p$  and corresponding geometries for the self-assembled aggregates. Reprinted from Ref. 10 with permission from Hindawi Publ. Co. under the Creative Commons Attribution License. Copyright 2011.



The theory captured a part of the story that had previously been lacking—in that it embraced in a simple framework a whole range of different structures. (However, even a proper description of micellisation of non ionic micelles is extremely complicated [11,12].)

It reduced ultimately to the statement embodied in Figure 1.

A still popular and useful characterisation, its virtues lay in its simplicity. However there are still several issues that need further reasoning. For example, the position of the interface is a hidden variable.

Nonetheless the fact that microstructure could be characterised at all, although imperfectly, was a surprise – the lifetime of typical micelle of sodium dodecyl sulfate is about  $10^{-5}$  s; that for the residence time of a surfactant in a micelle is about  $10^{-8}$  s; and the flip flop rate of a typical double chained surfactant from one side of a bilayer membrane to another is 3 months! Yet both are included in the one simple theory [13,14].

## 2.2 Asymmetry of vesicles and specificity of chain packing

Figure 1 is repeated and embellished in many papers. But the concept of an aggregate it conveys is too simplistic. Chains are fluid, not rigid; interactions between aggregates are ignored; the partition function of statistical mechanics averages over only euclidean geometries; asymmetry of chain packing is ignored; the assumptions necessary to even define a micelle are extreme. Taken over to light and neutron scattering experiments the preconception embodied in such cartoons is fraught and limiting. Some of the defects of the theory discussed above were removed in a later paper [11,12].

While maintaining generality, this paper gave both a proper description of oil-water–

surfactants ternary systems (microemulsions) [11]. It also resolved an important question – the existence and stability of vesicles. By vesicles we mean single walled bilayers. Here the inadequacy of a description of self assembly based on a bulk liquid, oil-like interior to describe hydrocarbon chains became evident. According to the simple Israelachvili-Mitchell-Ninham theory above, vesicles should form for  $\frac{1}{2} < p < 1$  (The surfactant parameter is that of the outer layer).

Careful rigorous optimisation of the forces in play at both sides of the closed membrane showed that matters were much more complicated, as illustrated by Figure 2. The inner and outside lipid hydrocarbon chains are in quite different configurations. With a mixture of lipids or surfactants of different chain lengths or headgroups the disposition of the mixtures will be even more asymmetric.

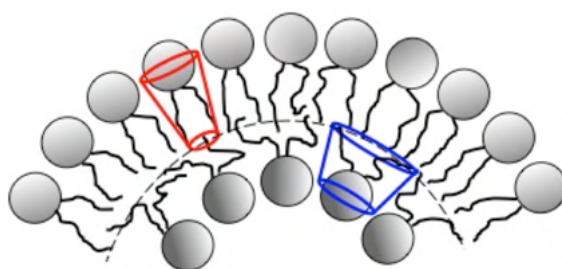


Fig. 2 Picture of a single walled vesicle showing chains stretched outside, compressed inside, with normal for outer and reverse curvature for inner lipids. The red truncated cone represents the curvature for outer lipids ( $p < 1$ ), the blue truncated cone represents the reverse curvature for inner lipids ( $p > 1$ ). The effects are enhanced for the sake of clarity. The radius of the dashed circle is not the average of the outer and inner layers' radii, showing that the inner layer is squashed.

If the lipid chains are flexible and can be compressed to about  $\frac{1}{4}$  of the fully extended chain length the prediction is that vesicles will form for  $p > \frac{1}{2}$ . As the surfactant parameter  $p$  increases further, *e.g.* with increasing chain length or reduced head group area, the vesicles grow and form multilamellar vesicle phases. If the chains are less flexible, the system will transit directly from hexagonal to lamellar phase for  $p > \frac{1}{2}$ . As the surfactant parameter increases further, single walled vesicles reappear and then

again lamellar phase for  $p \rightarrow 1$ . This complex behavior emerges from a quite general analysis and optimisation.

It became clear that depending on chain length and flexibility, some lipids could form stable vesicles and some had to be metastable.

The assumption of a *continuum* oil-like interior of an aggregate had to be forgone. A large literature that used a popular description of membrane curvature in terms of a “bending modulus” of a uniform membrane interior has to be revisited.

### 2.3 Origins of Supra Self Assembly

The asymmetry in packing for inner and outer lipids of a closed bilayer and multilamellar structures has another important consequence.

The inner chains are compressed. The outer chains are stretched. The curvature is significantly different for outside (normal average curvature) and inside (reverse curvature) lipids. Further the number of molecules inside and outside a bilayer is very different for typical vesicles say of 200 nm size [11].

For a multibilayer, or any topologically closed assembly, as the radius of the interior bilayers decreases, at a certain point the molecules on the inner side of the bilayer can no longer pack. Once this packing limitation is reached, the interior can remain empty. Or it can collapse into a (bicontinuous) cubic phase and/or micelles (see Figure 3).

For typical biological lipids the minimum size of vesicles that form is usually about 200 nm diameter. The lipids are typically double chained with about 14 to 20 methylene groups per chain.

Vesicles and multilamellar liposomes always have an aqueous core of at least that size. It can contain micelles and other structures (microphases) that can pack.

If the inside is just an aqueous core that core solution has a very different physico-chemical properties than the exterior.

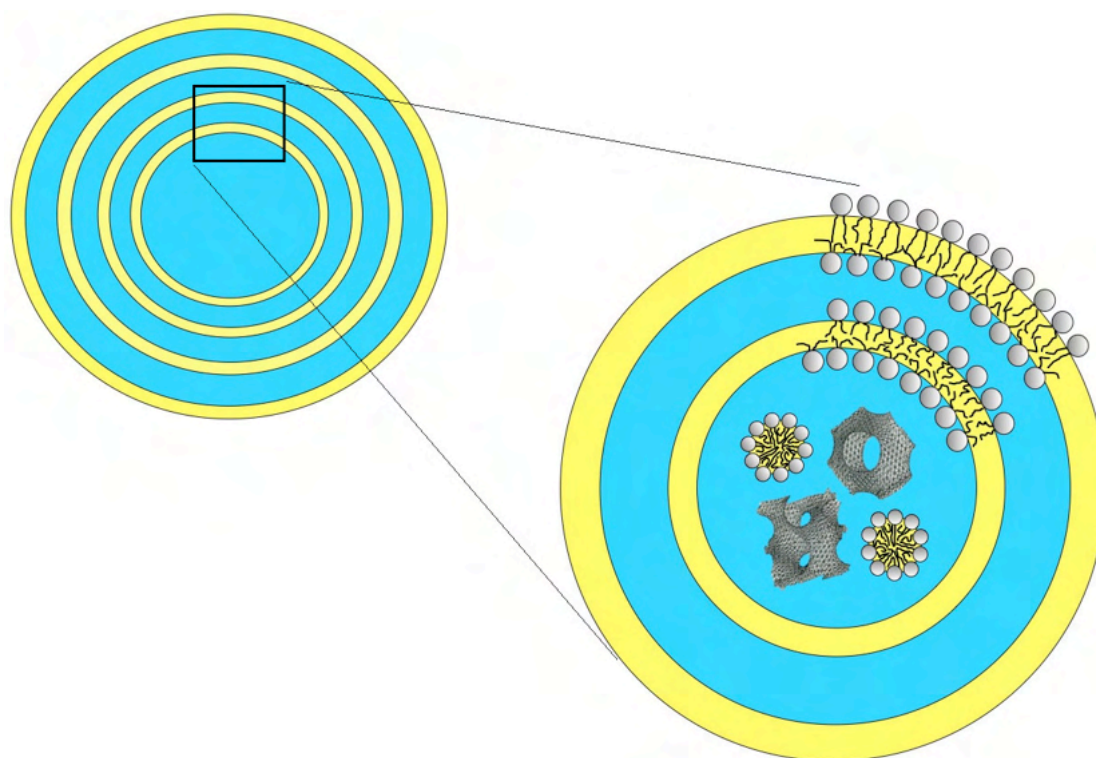


Fig. 3 Schematic representation of a multilamellar, onion-like curved structure. The enlargement shows that as the distance from the center decreases the amphiphiles in inner layers suffer from a severely squashed deformation until the bilayer assembly is no longer possible. The interior onion rings of surfactants can no longer pack and the interior is vacant. Either that or, because the interior can be all hydration water, the forbidden interior bilayers form micelles and different cubic phases in the central aqueous pool of the vesicle. The yellow and blue rings represent the hydrophobic chains and the aqueous interlayer compartments, respectively.

How different can be seen by doing the following *gedanken* experiment or calculation: Take a suspension of double chained ionic surfactant that forms vesicles at  $10^{-5}$  M total concentration. With a head group area of say  $50 \text{ \AA}^2$ , charge neutrality gives us an inside counterion concentration of ca. 1 M! The inside and outside water activities are very different. So the necessary asymmetry in chain packing has the further consequence that a good deal of care has to be taken in treating interior water as bulk water. Some consequences of this in biology are explored by Marc Henry [15-19].

In reverse microemulsions, *par excellence* the water must all be hydration water!

More curious phenomena can occur.

Sometimes, if the interior and outside media are very different, *e.g.* at the air-water interface, the vesicles can expand to form giant vesicles of the size of biological cells [20-22].

Since the work of Langmuir it has been assumed that only monolayers could exist at the air-water interface. In fact there are as many different phases at such an interface as there are in bulk. This obviously important class of phenomena can be illustrated quite dramatically as follows: Take a suspension of single walled vesicles made from *n*-dodecyl dimethylammonium hydroxide (or nitrate or acetate or other anions). Titration of HBr into the suspension immediately induces a transition to giant vesicles that do not fuse. They are stable at least for months. The pH inside remains at 12, that outside 7.

On shear the vesicles transit to giant “worms”, which on removal of shear go back to giant vesicles [23]. Another example of such memory effects in self assembled fluids occurs with didodecyl dimethylammonium bromide (DDAB), alkanes and water, and other microemulsions that are bicontinuous. A magnetic stirrer will wind the microemulsion up. It becomes so viscous that the stirrer stops. On reversal the system unwinds to its original state [24,25].

The role of hydrodynamics in self assembly, especially Gibbs Marangoni effects due to opposing diffusion gradients has hardly been noticed let alone explored.

## 2.4 Specificity in oil chain length

Alkanes show up specificity as a function of chain length in even the simplest situations.

At the air-oil interface the interfacial free energy *appears* to be well described. Knowing the bulk density of oil, through a pairwise summation of intermolecular dispersion interactions across the interface the interfacial free energy can be calculated — the Fowkes theory [26].

Not so. If the measured temperature dependent interfacial free energy is decomposed into enthalpy and entropy, it becomes clear that there is a profile of ordering of the alkane chains as one moves away from the interface. As chain length increases, the chains tend to line up more and more into cylindrical packing. For such a geometry the dependence goes like density to the power  $\frac{1}{2}$  instead of  $\frac{1}{3}$ .

A more explicit demonstration of specificity is the “even-odd” effect. Odd and even numbered alkanes are not happily miscible even as liquids. A quantitative explanation has been given in Ref. 27.

Specificity in biologically relevant organic molecules is mandatory. For example in molecular recognition as in enzyme-substrate interactions. We know that natural sugars belong to the D series, while most of the natural proteins comprise L-amino acids. Insect sex pheromones that are straight hydrocarbons usually contain an odd number of carbon atoms [28]. Furthermore organic chemists know very well that the physico-chemical properties of alkanes and their derivatives – such as the melting point - follow different trends for odd and even molecules, because the even-numbered alkanes pack closer together more easily, making it more difficult to break down the crystalline lattice. This is the reason why odd-numbered *n*-alkanes have lower melting points. In fact the even-numbered *n*-alkanes have optimal intermolecular interactions at both ends, while in odd-numbered the intermolecular distances are not optimal at both ends and this results in weaker intermolecular interactions [29]. In most marine organisms and bacteria an even or odd carbon-chain

preference is found in fatty acids and alcohols, while for the hydrocarbon odd chains predominate [30,31]. In organic chemistry this effect occurs most commonly in the solubility of solutes. Odd and even alkanes pack differently around solute giving rise to different dispersion free energies of solvation.

### 3 Hofmeister effects and quantification of ionic forces. A Necessary Diversion

#### 3.1 Specific ion effects and head group hydration vs. hydrocarbon chain length determine local curvature

The simple theory of micelles was quantified further for ionic micelles [32]. It assumed that electrostatic double layer forces at the curved interface of a micelle were opposed by forces of head group hydration and those due to chain repulsion in the hydrocarbon core of the constituent chains (see Figure 4).

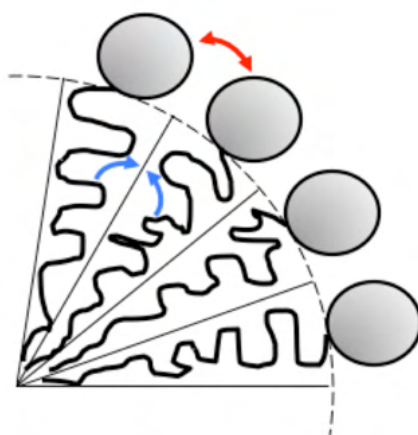


Fig. 4 Surfactants in a spherical micelle. The red arrow represents the repulsion between the hydrophilic heads, the blue arrows indicate the attraction between the lipophilic chains that occupy the micellar core.

Optimisation, analytically, of the opposing surface forces together with measured hydrophobic free energies of transfer led to quantitative prediction of critical micelle concentrations (cmc), aggregation numbers, and ion binding as a function of added salt and temperature.

These were all measured and up to temperatures of 130° C. They agreed with experiment [33,34].

As opposed to, and in apparent contradiction to this theory, the classical phenomenological characterisation used to extract ion binding to micelles, proteins and membrane surfaces used by NMR and other techniques, seems puzzlingly different. In brief for an anionic surfactant  $S^-$  we write down the equation 2:



$N$  monomers produce a micelle  $M$ , some  $(i)$  of the  $N$  counterions ( $I^+$ ) bind at the micellar interface, and  $(N-i)$  is the charge of the dressed micelle. Treating the micellization as a chemical equilibrium between the  $N$ -aggregate and the monomers with their counterions we have:

$$x_N = K(x_1)^N (x_2)^i \quad (3)$$

$$\Delta G_{mic}^0 = -RT \ln K \quad (4)$$

where  $N$  is the aggregation number,  $x_N$  the mole fraction of surfactant in micelles,  $x_1$  the mole fraction of surfactant monomers,  $\Delta G_{mic}^0$  the hydrophobic free energy of transfer of  $N$  monomer hydrocarbon tails to the hydrophobic interior of a micelle,  $x_2$  the mole fraction of counterions, and  $i$  the fraction of charged counterions “bound” to the micellar surface.

The standard phenomenological equation used in applications was usually correct. It turned out that the characterisation was valid, but *only* asymptotically, in the limit of strong binding [32]. Loosely speaking, the ion binding model holds for cosmotropic ions only (according to the classical Hofmeister terminology) [3]. Similar conclusions are derived when the counterion of cationic surfactants is replaced, as in the case of micelles and vesicles [35,36].

The same electrostatic vs. hydration forces theory worked to quantify the “Manning



theory” a phase transition of polyelectrolytes as a function of added salt [37].

### 3.2 Ion Binding and Bilayer Forces

For micelles of the surfactant dodecyl trimethylammonium bromide (DTAB) or force measurements between bilayers of the double chained didodecyl dimethylammonium bromide DDAB) above, the binding is about 80%, as is typical too for counterion binding of common surfactants like sodium dodecylsulfate [34].

But if we take the same surfactants with different counterions in the Hofmeister series, *e.g.*,  $\text{OH}^-$ ,  $\text{NO}_3^-$ , or  $\text{CH}_3\text{COO}^-$  the micelles have cmcs twice as large and aggregation numbers half those of the other class. There is here no binding measureable [38,39].

So theory “worked”, but only for particular head group-counterion pairs, the alkali halides and a few others, not to all univalent ions.

If it works for the single curved charged surface of a micelle, it ought to work also for direct (double layer forces) between bilayers. It does [40]. It does but only for the apparently anomalous ions. A nearly perfect fit for predicted double layer forces occurs for counterions  $\text{OH}^-$ ,  $\text{NO}_3^-$ , or  $\text{CH}_3\text{COO}^-$  for which the micellar theory fails.

It is for these that the theory, applied to a single (micellar) surface fails. On the other hand, for bromide and other halide anions the force measurements require for fitting the postulation of an extra, chemical, ion binding. The differences between the two kinds of measured forces are an order of magnitude [41].

Something is missing then that is responsible for this contradiction. In fact the large specific dispersion forces acting on ions that affect ionic adsorption and the inevitable specific ionic hydration induced by those forces need to be taken into account [42,43].

The complexity of the situation regarding specific ion, or Hofmeister, effects on forces remains. The consequence of this apparently baffling situation is worth

emphasis: The meaning of  $pK_a$ s, pH, buffers and the interpretation of ion binding and zeta potentials is problematic. Resolution waits on an emerging theory that includes hydration and dispersion forces missing from classical theory of electrolytes as well as electrostatic forces. Considerable progress is presently being made [2,4,44-49].

### 3.3 Changing Microstructure and Vesicles again

The complexity and unresolved nature of our understanding of forces between self assembled aggregates is a problem for theoreticians [1]. It is a gift for the experimentalist who now has a powerful tool with which to prescribe curvature via specific ion effects.

To illustrate: the insoluble double chained DDAB surfactant ( $p \sim 0.8$ ) forms lamellar phases in water. If we ion exchange the  $Br^-$  for  $OH^-$ ,  $CH_3COO^-$  or  $NO_3^-$  it forms monodisperse single walled vesicles of the usual 200 nm size seen with typical phospholipids [35,39,50].

These vesicles are stable. The interior and exterior have different pH. If we titrate back with the original bromide the clear vesicular system turns into a system of giant vesicles of micron size (biological cells) which slide about easily and do not fuse [51,52].

Exploitation of the differences in counterion and coion forces between aggregates can be used to precipitate and crystallise proteins. Similarly, differences in ion binding could be used to unravel entangled DNA to advantage in molecular biology.

The same kind of dramatic expression of specific ion effects occurs also for anionic double chained sulphosuccinate surfactants like AOT. With sodium they form lamellar phases, with lithium they form dispersed vesicles. Like the bromide vs. acetate story for DDAB, the interaggregate forces are an order of magnitude larger for

$\text{Li}^+$  counterion than for  $\text{Na}^+$ , even at 1 M [53].

## 4 Global Packing vs. Local Curvature Determines Microstructure and Phases

### 4.1 Equivalence of forces and geometric packing

So far the intuition that provides a backdrop to make sense of microstructure derives from, and is contained in, a surfactant packing parameter  $p$ . This characterises surfactant interfacial curvature.

We can change that parameter and therefore microstructure in several ways:

1. Change electrostatic head group forces (by adding salt)
2. Change electrostatic head group forces by changing counterion – Hofmeister effects
3. Change interaggregate interactions (*e.g.* by concentration, volume fractions)
4. Vary head group hydration (by changing the temperature or the background electrolyte or counterion)
5. Changing surfactant chain length
6. Admixing two surfactants of different  $p$  (requires compatibility of surfactant hydration)
7. Adding oils that change volume per head group (microemulsions, emulsions)
8. The biggest elephant in the living room is dissolved atmospheric gas. This we ignore at the moment as it takes us into a qualitatively different world.

Consider (1) and (2) in the phase diagram of the anionic surfactant SDS (sodium dodecylsulfate), sodium chloride and water [54].

The phase behavior of this system was explored in a classic paper of Håkan Wennerström, Bengt Jönsson and Per Linse [55]. They used a “cell model” to

describe electrostatic interaggregate interactions. Water was characterised as *continuum* with bulk dielectric constant.

Surface electrostatic curvature interactions were evaluated by a Poisson-Boltzmann description. An almost complete description of the phase diagram was obtained. Except for one region, where reverse micelles occurred. The theory breaks down at low water content. The *continuum* solvent assumption is inadmissible.

Nonetheless this statistical mechanical model calculation makes an important point. Local curvature and long range interactions between aggregates are intimately related and coupled. The long range electrostatic interactions between aggregates are described by a cell model, the radius of which is the inverse Debye length. Effectively this is a hard sphere interaction.

To make this explicit suppose that salt is added to a micellar solution. If the micelle with surrounding cloud of free ions is assigned a radius measured by the Debye length and treated as an effective hard sphere, then:

When the volume fraction of the effective spheres reaches close packing (between 50% and 74%) the system can no longer pack and changes to a hexagonal (cylindrical micelle) array. With more salt, electrostatic screening increases and reduces surfactant head group area. The cylinders can no longer pack (between 70% and 91% volume fraction). They change to lamellae, and so on.

The precise details are unimportant — we know these things are ion specific. The important point is that interfacial curvature set by local packing, and global packing are together the determinants of microstructure. These two variables are, if we like the key renormalised thermodynamic parameters that matter.

Note that the global packing is determined by volume fractions of aggregates, and interaggregate interactions acting together.

## 4.2 The Demonstrations of Lissant on Emulsion Structure

Some beautiful work of Lissant reinforced this point. He made some emulsions of a commercial surfactant, kerosene and water with a kitchen mixer. The emulsions could form with as little as 2% water external phase [56].

Discontinuous transitions in structure occurred at different close packing fractions of flattened spheres (see Figure 5).

This old, beautiful quantitative illustration of packing as the determinant of microstructure is dramatic.

The interface is soft with virtually no forces apart from global packing constraints.

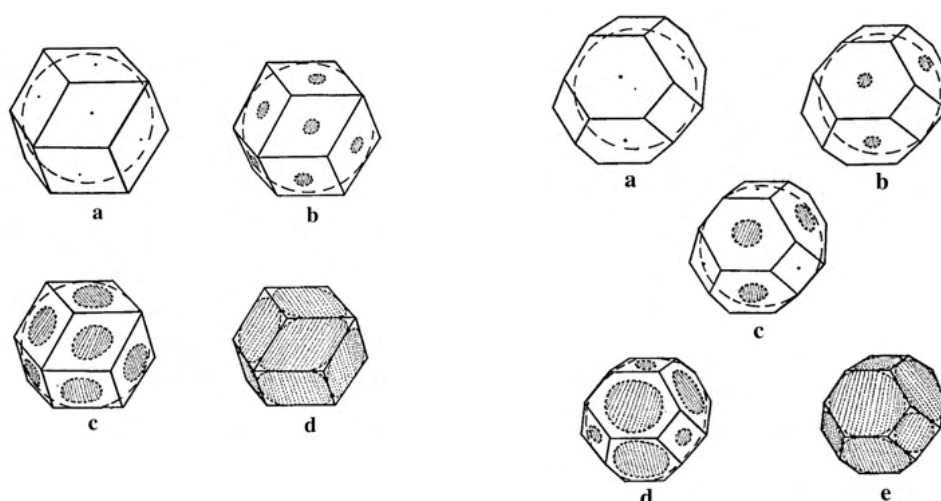


Fig. 5. Left: transition from a sphere to a rhomboidal dodecahedron (RDH) for a 74% to 94% internal phase packing. Above 94% internal phase the emulsion will follow a tetrakaidecahedron or truncated octahedron (TKDH) packing. Reprinted from Ref. 56, Copyright 1966, with permission from Elsevier.

The same is illustrated quantitatively by calculations of phase behavior of reverse micellar systems [17].

## 4.3 Remark on Ionic Surfactants and Cloud Points. Compatibility of Hydration

On a more technical level an important challenge to taking theoretical ideas seriously

occurs in the observation that zwitterionic surfactants (*e.g.* short chain phospholipids like dioctanoylphosphatidylcholine, diC<sub>8</sub>PC) exhibit an upper consolute (cloud) point in their temperature vs. concentration phase diagram [57-59]. On the other hand non ionic surfactants such as C<sub>i</sub>E<sub>j</sub> exhibit a lower consolute cloud point [60].

For ionic surfactants the cloud point does not occur at all. This appears to conflict with thermodynamics which would us to expect universality of phase diagrams. The reasons that zwitterionics and non ionics exhibit a cloud point phase separation with increasing or decreasing temperature at a molecular level are clear.

In the case of nonionics, with increasing temperature, the micelles grow at fixed concentration and this is possible only if  $p$  increases, which implies a reduction in the area per polar group  $a$ . This is known from NMR experiments that show the dehydration of the polar heads (two molecules of water per ethylene oxide unit of the surfactant head group) at the cloud point. Then the micelles grow to cylinders. At the same time the interactions between aggregates due to the changed hydration become attractive. However weak, an attractive force between very long cylinders will lead to phase separation. This is known from direct force measurements between monolayers [61].

With cationics, by comparison,  $p$  decreases with temperature increase.

The real variable is local curvature versus the global packing.

The apparent conflict can be reconciled if instead of plotting temperature as the ordinate of the phase diagram we use local curvature, *i.e.*  $p$ . This increases for nonionics like temperature, on the  $y$  axis. One achieves consistency for ionics by admixing increasing amounts of single chained ( $p \sim 1/3$ ) with double chained ionic

surfactants: So the effective packing parameter  $p_{eff} = \frac{x_d + (x_s/3)}{x_d + x_s}$  (where  $x_d$  and  $x_s$  are

the mole fraction of double chained and single chained surfactants, respectively) now increases on the ordinate. The cloud point now appears, and the phase diagrams then coincide in form.

LEMMA 1. The lesson is that global packing constraints, volume fractions that include the range of interaggregate forces are a hidden and ignored variable. *Global packing vs. local curvature are the renormalised thermodynamic variables that give us microstructure.*

LEMMA 2. This conclusion is correct. However the argument is too simplistic. It will not work for mixtures of cationics with anionic and nonionic surfactants. The reason is that head group hydration for the different surfactants in this case are incompatible. This is the reason that cationic surfactants act as detergents on cell membranes and are extremely effective above the cmc. The hydration of cationics with head groups like trimethylammonium or pyridinium is compatible with the choline moiety of the dominant cell membrane lipid phosphatidylcholine. For anionics or nonionics of the same chain length, although the hydrophobic free energies of the surfactant tails to the core of the membrane is the same, the incompatible hydration prevents surfactant uptake.

Further complications designed to upset our consistent world view will emerge with dissolved gas and long range hydrophobic interactions. They will come to our story later.

#### 4.4 Cubic Phases

Another consequence of chain flexibility is the existence of cubic phases — so called because of their cubic symmetry that is easily revealed by visual inspection through crossed polars. These are bicontinuous structures formed from lipids that occur

everywhere. They will play a central part in our story. If the chains have difficulty in both stretching on one size and compressing on the other, they can relieve this local average (normal) curvature frustration by adopting opposing curvatures as in a saddle, illustrated in Figure 6. The system takes up a nonzero Gaussian curvature [5].

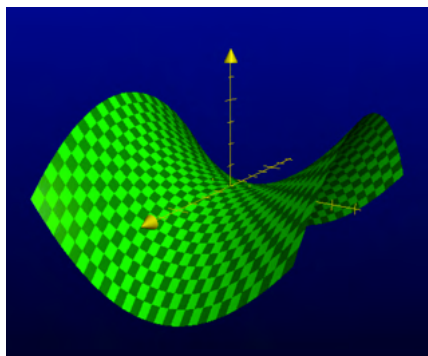


Fig. 6. The “saddle” or “potato chip” surface with two opposite curvatures.

There are a variety of such structures that can switch from one form to another with extravagant ease. These non euclidean geometry structures have increasing importance in solid state physics also. Figure 7 shows the G-, D- and P-surface arrangements respectively.

They can be expected to occur in biology also as supra aggregates, nanocubic phases surrounded and protected by layers of lamellar [5,6,62-70].

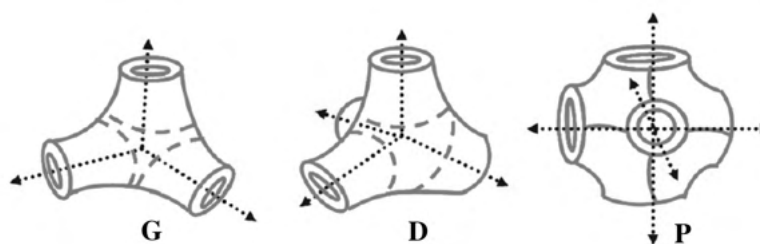


Fig. 7. G or Ia3d, D- or Pn3m, and P-surface or Im3m structures. Reproduced with permission from Ref. 71. Copyright 2011 American Chemical Society.

Two dimensional analogues, sometimes termed “mesh phases”, are also universal and occur whenever the same frustration appears. For the potato chip reduction of surface



area at constant interior volume causes the shape transition to occur. For the surface of drying of mud, or for the myriad patterns of bark of trees the same simple processes are involved.

## **5. Alkane Specificity**

### **5.1 Alkane Specificity in Microemulsions**

Ions change interfacial curvature at a surfactant-water interface through highly specific forces between headgroups. So too alkanes change curvature on the other (hydrophobic) side of the water-surfactant-oil interface. These changes due to oil are again highly specific [24,25].

We can exhibit this specificity by considering now three component systems of oil, water and (ionic) surfactant.

Microemulsions are spontaneous thermodynamically stable microstructured mixtures. The condition for forming ternary microemulsions requires a double chained surfactant with  $p \sim 1$  as for vesicles and lamellar phases [1,11].

When microemulsions are formed with single chained surfactants, invariably a cosurfactant like an alcohol, or cholesterol is necessary to give an effective  $p \sim 1$ .

While the condition is necessary, it is not sufficient.

That can be seen as follows. A mixture of two different single chained surfactants can achieve the necessary condition. *E.g.* an equimolar mixture of SDS and CTAB. A positively and a negatively charged surfactant ought to associate. However the differences in hydration mean that they do not and do not form a microemulsion. On the other hand a mixture of SDS and an alcohol does work in the much studied SDS-pentanol-toluene-water-salt mixture [43].

Another is catanionics, mixtures of SDS and dodecyl trimethylammonium bromide

(DTAB), single chained surfactants that individually form micelles [71]. And the effect of pentanol adsorption on the forces between bilayers of DDAB [42].

It is usually asserted and generally believed that the formation of microemulsions requires ultralow interfacial tension between oil or water and microemulsion. This is incorrect theoretically [11] and experimentally [72].

## 5.2 Microemulsions with Double Chained Cationic Surfactants as Lipid Mimetic Systems

We use for illustrative purposes the much studied double chained ionic surfactant DDAB.

It has the properties that it is very weakly soluble in both alkanes and in water. So it must reside substantially at the interface between oil and water. Further, its headgroup has two tightly bound water molecules of hydration. The headgroup mimics that of the common lipid phosphatidylcholine and, although charged, is therefore more likely to yield biological insights than other microemulsion forming surfactants. The bromide counterion is strongly bound and the head group remains at fixed area at the oil-water interface. The alkane oil is taken up penetrates into the hydrocarbon tails of the surfactant, depending on chain length. The uptake is most for hexane and follows the sequence: hexane > octane > decane > dodecane.

Since there are no other interactions between oil and tails than almost identical dispersion forces, the oil specificity effect is *mostly entropic*.

The effective surfactant parameter and effective interfacial curvature ( $p > 1$ ) increases as alkane length decreases.

With tetradecane there is no oil uptake and the oil is indifferent to the chains.

This oil specificity was studied first by D. Haydon with black lipid films and

quantified theoretically by Marčelja and Gruen [73-76].

### 5.3 Phase Diagrams in Ternary Microemulsions

The phase behavior is very rich in microstructure as can be seen for the DDAB-water-dodecane system plotted in Figure 8 [77].

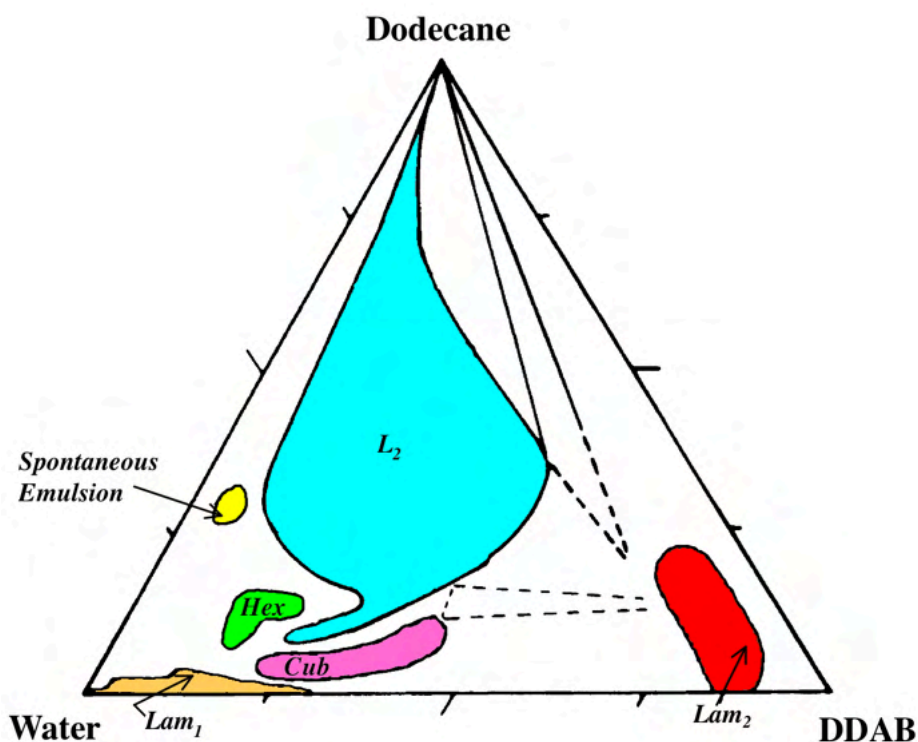


Fig. 8. Within the ternary phase diagram that demarks phase boundaries as a function of volume fractions of components, there are two lamellar phases ( $Lam_1$  and  $Lam_2$ ), three cubic phases (Cub), hexagonal phases of reverse curvature (Hex), a large single phase w/o microemulsion region ( $L_2$ ), a spontaneous emulsion region, and other regions containing one, two and three phase systems. Readapted from Ref. 77.

In the single phase regions, the viscosity varies along a water dilution line marked by 5 orders of magnitude. The conductivity varies by 10 orders of magnitude (see Figures 9 and 10) [78].

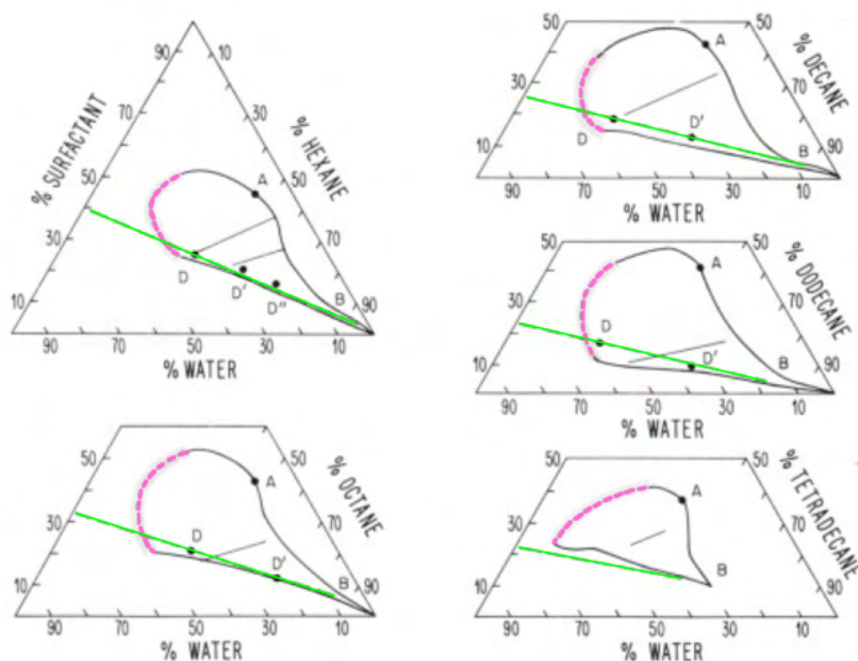


Fig. 9 Partial-phase diagrams showing the one-phase microemulsion region and the water dilution paths studied for hexane, octane, decane, dodecane, and tetradecane, respectively. The axes represent the weight percent for each component. The green line passing toward the oil corner is drawn from the oil corner through the end points of the conducting regions [79]. The pink region to the left of the single-phase region marks the gradual transition to a viscous gel-like system. Adapted with permission from Ref. 79. Copyright 1985 American Chemical Society.

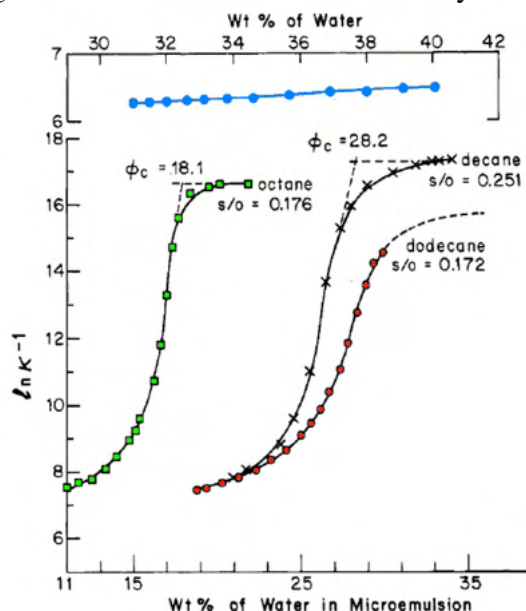


Fig. 10 Inverse of specific conductance of octane (green squares), decane (black crosses), dodecane (red circles) and tetradecane (blue circles) microemulsions at specific surfactant/oil ratios. The dotted lines indicate extrapolation to critical conductivity threshold  $\Phi_c$ . Tetradecane microemulsions are always conducting and comprise normal o/w spheres with a surfactant/oil ratio of 0.343. Adapted with permission from Ref. 24. Copyright 1984 American Chemical Society.

There is a percolation boundary across which the conductivity becomes zero. The system is mostly bicontinuous across the large one phase region, a random bicontinuous connected network as depicted in Figure 11 [80,81].

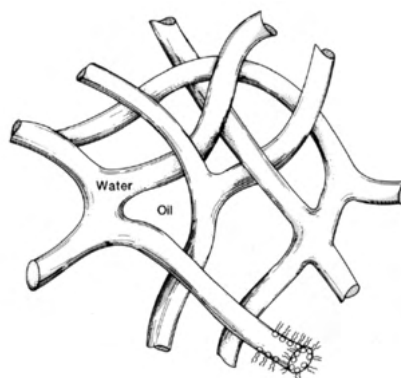


Fig. 11. Model structure for a conductive bicontinuous microemulsion. Reprinted with permission from Ref. 80. Copyright 1980 American Chemical Society.

Recently Talmon has confirmed through cryo-SEM the nanostructure evolution in microemulsions composed of DDAB, water, and iso-octane (see Figure 12) [82].

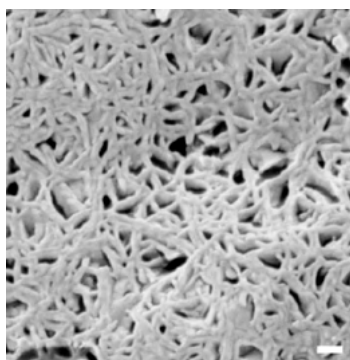


Fig. 12. Cryo-SEM micrograph of an iso-octane/DDAB/water (48:32:20) microemulsion. The bar corresponds to 100 nm. Reprint from Ref. 82, with permission. Copyright De Gruyter.

There is a white spontaneous emulsion (not a microemulsion) region which is completely stable and impossible to break by temperature or other methods. (We will return to that later. It is a stable supra-self assembled aggregate with complex microstructure which is a consequence of global packing constraints). There are three different connected cubic phases [83,84].

For further studies on supra aggregates see Ref. 85.

The important observation is knowing that head group area is fixed then the entire microstructure can be predicted by simple global packing constraints, in agreement with NMR, conductivity and neutron scattering [86-90].

#### 5.4 Microstructure with Alkanes

This shows up explicitly in the measured physical parameters for each alkane's phase diagram as shown in Figure 9 (Section 5.3).

This shows that the microemulsions form with smaller and smaller amounts of water as  $p$  increases.

The conductivity at these very low water contents  $\sim 2\text{-}4\%$  show transitions that correspond precisely to the packing fractions calculated by Lissant.

Beyond dodecane, tetradecane does not penetrate the hydrocarbon region of the surfactant at all. The phase diagram appears similar to those of the smaller alkanes but the microstructure is quite different [91].

#### 5.5 Alkenes a more subtle Example of Specificity

To further show up the specific role of oils in setting curvature, we can consider unsaturated hydrocarbons like 1-hexene, 2-*cis*- and 2-*trans*-octene, with more polarisable double bonds [25]. The phase behaviour here shows the oils penetrate more strongly into the headgroup region than their alkane homologues reflecting their higher polarisabilities. The alkene microemulsions exhibit and even more striking conductivity behavior than the alkanes. Comparing with Figure 10, the inverse conductivity at first decreases with complex microstructural changes between 2 and 4% water content. It then increases with further water addition as for Figure 10. This

apparently mystifying conductivity behavior is predicted by geometric packing, and follows exactly as predicted by Lissant [56]. The differences in *trans* and *cis* oils are reflected in the corresponding behaviour of phospholipid cell membranes [92], and in the different amount of *cis* and *trans* hydrocarbon chains in living organisms [93-95].

## 5.6 Spontaneous Emulsions and Supraaggregation

The spontaneous emulsion region of Figures 8 and 9 exist for all the microemulsion phase diagrams with alkanes and alkenes. No work is required for their formation, they are impossible to break and reversible to temperature cycles.

While little explored experimentally, calculation shows that they form at volume fractions as discussed above.

The interior of a topologically closed aggregate like multilamellar phase can no longer pack, the physicochemical conditions in the interior are different to those in the exterior.

The interior then collapses to a different microphase, *e.g.* hexagonal or cubic phase. These states of supraaggregation occur frequently, see for example the formation of reverse structures by replacing calcium with gadolinium in phospholipid's vesicles, the formation of complexes from chitosan and nonaoxyethylene oleyl ether carboxylate [96] and in biology *e.g.* in calcium containing vesicles across the synaptic junctions [97,98] or in the supra self-assembly of siliceous vesicles [99].

## 6. Several Sides of the Coin: Hofmeister and Hydration vs. Oil Specificity

Changing curvature from the aqueous side of interfaces with Hofmeister effects opposing those from the oil side allows flexibility in design of microstructures.

## 6.1 Specificity of Halide Counterions and Hydration

The oil specific sensitivity of phase behavior to alkanes and alkenes in setting interfacial curvature and therefore microstructure is mirrored by that of the counterion. Even a change from  $\text{Br}^-$  to  $\text{Cl}^-$  and  $\text{I}^-$  produces big changes that are reflected in corresponding force measurements between bilayers [100].

The most remarkable expression of this sensitivity are the microemulsions formed with sulphate as counterion instead of bromide. These are oil droplets in water systems, with normal, not reversed curvature. This is quite the opposite of classical expectation. One would have thought that the divalent sulphate ion would adsorb more strongly than the univalent bromide ion.

Evidently not. The microstructure and phase diagram is easily calculated from geometrical packing constraints again.

Even a small amount of added bromide induced a change back to the usual reversed curvature phase [101].

This example with microemulsions shows the irrelevance of electrostatics in this system (the concentration of counterions in the water channels of the microstructures fluid is typically 0.3 – 1 M, so the Debye length is irrelevant).

The effects of buffers and of addition of cations show up specific ion effects due to bulk and surface hydration and dispersion forces [102]. These effects are incomprehensible unless one understands that the classical intuition in physical chemistry on electrostatic forces is misleading. It fails here for at least two reasons:

One is that the the water channels are entirely hydration water. The second is that electrostatic forces are screened due to the very high density of counterions. So one has the apparently mysterious observation that cations of the same sign as headgroup adsorb strongly due to dispersion forces and compatible hydration. Similarly for



buffer anions which is very low concentration can adsorb also. Both effects change local curvature [1].

## 6.2 Further Apparent Anomalies

### 6.2.1 SDS/Alcohol/Toluene/Brine/Water System

A much studied microemulsion system involving several laboratories over many years in Europe and the USA. This was a mixture containing SDS, alcohol, toluene, brine and water [103]. Since the alcohol partitions between saline water, toluene and the surfactant interface, from the 5 dimensional phase diagram, the system's microstructure was impossible to unravel. It was modelled as spherical droplets to fit neutron scattering and data from other complex instruments. In fact it is bicontinuous. As we have seen the formation of a microemulsion requires  $p \sim 1$ . This is achieved by a mixture of single chained surfactant (SDS) and a cosurfactant (alcohol).

Alcohol first penetrates into micelles. This provides a favourable environment for toluene. The toluene expels the alcohol to the surface of the micelles, allowing swelling and uptake of the oil. We mention this to show that theory of self assembly is consistent.

### 6.2.2 AOT Methacrylate Water Polymerisation

Another example that appears to sit in opposition to the theory developed here is that of formation of methacrylate monodisperse polymer nanospheres from a reverse micelles of AOT in oil [104]. The droplets normally contain water and are spheres. If methacrylate monomer is added to the system with an initiator it forms spherical polymer spheres. How that happens is as follows: The methacrylate monomer is a cosurfactant that changes the AOT reverse micelle ( $p > 1$ ) to  $p \sim 1$ . The system is

bicontinuous with the same microstructure as the DDAB microemulsions—connected cylinders. When the reaction begins the initiator is in the pool of water where the cylinders intersect. As more and more monomer is drawn into the polymerisation center the interface reverts to AOT and breaks off to surround the new particle.

This classical example caused much confusion because it was thought that neutron scattering confirmed the reverse sphere microstructure before polymerisation. (Since the neutron scattering data was programmed to model the results as spheres only, this is not surprising. However to do so requires a huge attractive interaction between reverse micelles) In fact it is bicontinuous which can easily be established by use of a conductivity meter.

Not so obvious is some work of Larsson *et al* [105]. Here there is insufficient vitamin K<sub>1</sub> to change the surfactant parameter. In fact the vitamin K<sub>1</sub> associates within the aqueous phase to impose local curvature changes that induce the phase change. It was found that the oil soluble vitamin K<sub>1</sub> is solubilized in the lipid domains with similar mobility as the lipid.

This is reminiscent of the “rafts” that form in membranes to be discussed below.

A similar more explicit example of complex microstructure and phase behaviour induced by small amounts of additives is given in the paper above [102].

### 6.3 Hydration, Hydrophobicity and Other Known Unknowns

The separation of forces between molecules that underpins our attempts to organise observations into a predictable theory is always going to be fraught.

Terms like hydration, hydrophobic and hydrophilic are all difficult to be defined, being solute, solvent and temperature dependent.

1. The term “hydrogen bond” derives from a quantum mechanical calculation of

interactions between TWO water molecules. Hydrogen bonding energies quoted in different situations range from as low as 0.1 to as high as 100  $k_B T$ ! In the world of real water any bonding is a multibody multimolecular affair. Why ice floats on liquid water is a problem that goes back to Galileo [106,107].

2. The most extant and obvious classical known unknown in this connection is the very existence of jellyfish has underlined this problem [108]. Jellyfish comprise 98-99 % water and the last serious work on them was done by Gortner in 1930 [109].

Perhaps the water is held together in a gel by very long range non additive forces between permeating conducting polymers, but the matter is open and challenging [110].

3. The beginning of Physical Chemistry can probably be best dated to Napoleon's expedition to the Egypt in 1798. Berthelot, one of the accompanying scientists, observed that on the banks of the Nile river were rocks of soda lime, sodium carbonate. At usual temperatures one would have expected limestone, calcium carbonate to have been precipitated from the solution containing  $Ca^{2+}$ ,  $Na^+$ ,  $Cl^-$  and  $CO_3^{2-}$ . At high summer temperatures the reaction is reversed: calcium carbonate dissolves and  $Na_2CO_3$  precipitates [111-113]. This can happen if "ionic hydration" and "water structure" are different at the different temperatures.

The changes in "water structure" with temperature offer enormous opportunities for the synthesis of nanoparticles that have not even been recognised. Size, shape and structure can be varied more or less at will. More flexibility still is available if water structure is changed by exploiting Hofmeister effects and other solutes.

4. Hydrophobicity and hydrophobic interactions depend on dissolved atmospheric gases. Hydrophobic interactions are switched off on removal of gas as already remarked. Molecular simulations of water are therefore irrelevant to real water that

contains dissolved gas, in amounts depending on other solutes and temperature.

5. “Hydration” water in the microemulsions and vesicles, and reverse curvature structures we have considered is evidently the rule not the exception.

And so too in most biological situations. This has been very nicely argued by Henry [15,108] and through the extensive work of Philippa Wiggins [114-116].

These few examples provide a *caveat* to any attempts to draw out a coherent systematic picture of self assembly.

## **7 Molecular Forces: Changing Concepts and Complexity with Dissolved Gas, Bubbles, Salt and Heat**

D’Arcy Thompson in his famous book tells us that the early founders of the cell theory of biology and the early physiologists believed that progress in their sciences to depended critically on our knowledge of molecular forces [117]. The hubris of a new science that followed the application of X-rays and other techniques to the structure of proteins, the focus on DNA as the hidden source of life and kinetics of transport in neurophysiology is understandable. In that progress, lipids and the environment they provided to the perceived main players were poor cousins. And molecular forces were borrowed from physical and colloid chemistry.

Changes in the foundations of colloid science and physical chemistry took place over the last 70 years since the theory of Deryaguin, Landau, Verwey and Overbeek took center stage. Three connected advances occurred.

The first is in the quantification of Hofmeister (specific ion) effects. These, due to dispersion and related hydration forces, had been inaccessible to classical theories of electrolytes and molecular forces. This defect rendered theory impotent for prediction.

A second advance recognised a key role for hyperbolic (non Euclidean, bicontinuous) geometries in the self assembly of lipids, surfactants and proteins; and in nanostructure generally. That has been our concern here. And here we can be on solid ground because the theories depend on only local global packing of molecules, essentially mass conservation.

These advances have revolutionised the subject. Our whole intuition which relied principally on electrostatic forces and euclidean geometry, has been turned upside down.

The third has to do with the startling recognition that dissolved atmospheric gas, at a molecular level has qualitative effects that cannot be disregarded.

Also ion specific, they are ubiquitous and range over enzyme activities to protein structure and emulsion stability – “Hydrophobic interactions” apparently disappear when gas is removed. Further “non-Hofmeister” universal ion specificity occurs for bubble bubble interactions.

These developments are missing from intuition. We have explored these matters extensively elsewhere [1,3].

Despite D’Arcy Thompsons’ and the early biologists plea, the theories of forces that inform our intuition are about as informative as the Book of Genesis in explaining the early history of the earth, but perhaps less useful.

We have claimed in this article to have gained some insights into self assembly.

Progress seems real. But it is necessarily muddled when confusion exists due to misuse of molecular forces.

## **8 Conclusions**

In our search for organising principles behind lipid self assembly in membranes, several ideas emerged that seem to be right. The amphiphilic, yin-yang nature of lipid molecules with opposing head group and hydrocarbon chain solubilities is at the heart of it.

There are several matters that appear incontrovertible.

1. Local packing, that is conservation of volume in any curved bilayer, demands that a bilayer must itself be anisotropic, even for a single lipid.
2. The interior and exterior water of a vesicle or any closed membrane have to be different.
3. Partitioning of different ions like sodium and potassium and hydronium between interior and exterior domains is driven by their different self energies. This specificity is driven by dispersion energies ignored by present theories (Hofmeister effects and hydration) [44].
4. Packing constraints alone lead further to the necessary emergence of supraself assembly, which itself causes changes in water structure.
5. There is a necessary coupling between lipid membrane phase organisation and transmembrane structure and function.

The asymmetries occur naturally.

Asymmetries, in one form or another, as for example, charge distribution or liquid structure will impose electrical or diffusion potential gradients that drive transport or provide energy for enzymatic reactions.

These concepts are new.

We conclude by illustrating what consequences observations 1-5 might have.

On the idea of ion pumps the history is sad. It is told on the website of Gilbert Ling [118]. Ling's ideas tried to explain the difference between external and internal  $\text{Na}^+$

and  $K^+$  concentrations in cells in terms of the Donnan potential set up in a finite volume system. Based on classical theories of colloid science and electrochemistry, it could never have worked. This is because the extant theory did not take account of ion specificity due to non electrostatic forces. Two of Ling's associates abandoned the program and published a note in Science postulating an active pump. Where the considerable amount of energy comes from for the "pump" is still not obvious, especially for red cells which do not metabolise.

Revisiting the problem recently, two of us showed that in a finite volume system ion specific partitioning did occur [46,119].

Further, a potential is set up without an active pump. This makes sense in terms of modern theories of physical chemistry that includes Hofmeister effects.

How much this effect contributes to the biological "pump" has not been demonstrated. But contribute it must. The matter is open.

A related issue that illustrates our point is that of the disposition of phosphatidylserine in the plasma membrane of cells. This negatively charged lipid always sits on the inner side of the membrane.

If it is found on the outer side of the membrane it indicates that the cell is dead.

This asymmetric disposition is supposed to be maintained by two enzymes, flippase and scramblase [120]. How they operate and where their energy comes from is a mystery.

An alternative explanation is as follows:

1. The flip-flop rate of phospholipids across membranes has been measured by Mclaughlin. The energetic barrier to transfer from one side to another varies with chain length. And head group obviously. It varies from seconds to three months for

C<sub>18</sub> double chained lipids. With naturally occurring phosphatidylserine (PS) this is of the order of the lifetime of a cell.

2. But why would a cell membrane be formed with phosphatidylcholine on the outside and PS on the inside? One reason is incompatibility of head group hydration. Two observations reinforce and illustrate this.

The first comes from microemulsions: We know from Section 5.2 that to form a microemulsion the key condition is the presence of a double chained surfactant in the dispersion ( $p \sim 1$ ). Alternatively we can use a cosurfactant – to swell chain volume, or use a mixture of two single chained surfactants to achieve the same condition. A much studied example is the catanionic mixed surfactant formed from SDS and a carboxylate. But not all, indeed only few mixtures will work. This is because their hydration shells are not compatible.

The second observation comes from work on surfactants for sterilisation, *i.e.* destruction of cell membranes at the critical micellar concentration [1].

Cationic surfactants are much more effective than anionics or nonionics of the same chain length. The reason seems to be that the outer moities of the head group phosphatidylcholine head groups that predominate in the outer lipid layer are compatible with the trimethyl or similar groups of cationic surfactants in widespread use.

A third related observation is the effect of cationic surfactants in inducing immunosuppression in human and other mammalian T-cells discussed in Section 7.2. The mechanism and its chain length dependence is thoroughly explored and proven. The cationic surfactant, below the cmc, does not disrupt the membrane. It adsorbs following Langmuir isotherm with varying chain length, flips over to the inner side and neutralises the negative charge of the phosphatidylserine. This in turn lowers the



perimembrane calcium concentration, essential for the structure of the transmembrane major histocompatibility complex protein. Recognition of an antigen is switched off, and reversibly.

More observations can be adduced to reinforce the point, for example the mild anaesthetic effects of cationic surfactants, and the similar highly effective behavior of lanthanum in cell membrane destruction, more so than other trivalent ions with different hydration.

Our point is that effects that make no sense in biology within the framework of classical physical chemistry do not necessarily require the postulation of enzymes or active pumps as a source of energy.

And if the recognition of the serious flaws in the framework of physical chemistry allows the biological effects to be explained otherwise one has a conundrum.

And on that note we end our first part.

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