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REVIEW ARTICLE

Physical aspects of non-ideal mixing of amphiphilic molecules in solution: the interesting case of gangliosides

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Abstract. We review the interesting physical effects arising when bifunctional molecules, consisting of two moieties, one polar and the other apolar, are dissolved in a simple solvent. The peculiarity of such amphiphilic molecules is that they generate by themselves confined structures in which they are embedded and of which they dictate the topology according to their aggregative properties. Individual molecules are still free to redistribute within or across the aggregates. When two or more types of amphiphilic molecule are mixed together, the strong coupling between local interactions and the aggregate topology gives experimental access to a variety of non-ideal mixing effects. The couplings between the lateral distribution and the geometrical features of amphiphile assemblies are discussed separately for monolayers, bilayers, multilayers, and micelles. Concentration and temperature effects are taken into account. The case of non-ideal mixing of chemically identical molecules with mutually interchanging conformations is also discussed. It is found that bistability and thermal hysteresis phenomena can be important. Theoretical arguments are supported by a variety of experimental observations made of a class of amphiphilic molecules, the gangliosides, which are particularly suitable subjects for investigation as regards such non-ideality effects. Gangliosides, with a double-tail hydrophobic part, like phospholipids, and a bulky saccharidic headgroup, show relevant like–like and like–unlike interactions, and easily form large aggregates whose topology is quite sensitive to monomer redistribution.

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1. Introduction

Mixing properties of multicomponent fluids is a thoroughly investigated field in physics, chemistry, and biology. Systems like binary mixtures of small molecules which attract or repel each other with relatively simple potentials have been thoroughly investigated at various levels of approximation. Their mixing properties arise from the competing effect of mixing entropy, which favours the isotropic phase, and the like–like and like–unlike interactions, which may lead to phase-separated structures such as those depicted in figure 1(a) [1].

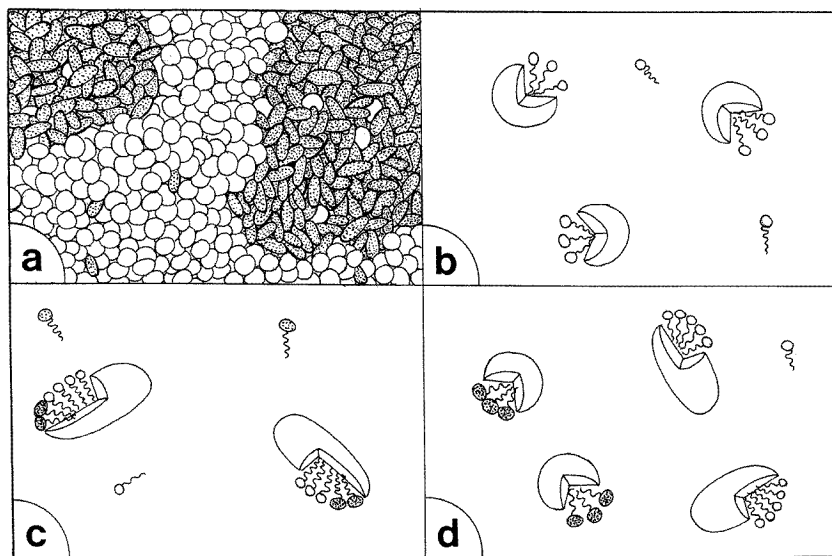


Figure 1. Supra-molecular assemblies of increasing degree of complexity: non-ideal mixing of simple molecules (a); micellar assemblies of bifunctional X–Y molecules of the same kind (b); and micellar assemblies of two different types of bifunctional molecule (the two types of molecule have been assumed immiscible) in the same aggregate (c) or in different ones (d). For the sake of simplicity, in all of the drawings the solvent molecules have not been indicated.

Dramatic reduction of the entropic forces can be achieved by considering molecules with unequal relative size [2] or, even better, by introducing molecular connectivity among the molecules of the same kind. The resulting object, a polymer chain dissolved in a solvent,

has a far lower mixing entropy, S_{MIX} , than the unconnected molecules (in a mean-field picture, S_{MIX} roughly behaves as $(\Phi/N) \log(\Phi/N) + (1 - \Phi) \log(1 - \Phi)$, where Φ is the polymer volume fraction and N is the number of monomers within the polymer chain), and the resulting phase diagrams exhibit a wider and asymmetric immiscibility region as compared with the monomeric case for which $N = 1$ [3]. Hence, for dilute solutions of polymer chains, the like–unlike interaction balance plays an even more important role in determining the mixing properties of multicomponent fluids.

Novel and interesting effects arise when one considers mixtures of bifunctional X–Y molecules dissolved in a simple solvent. Amphiphiles are bifunctional molecules consisting of two moieties, one polar, the other apolar. When they are dissolved in a polar solvent such as water, two main regimes can be identified. The first one relates to very low concentrations for which entropic forces prevail and monomers are dispersed isotropically in the solvent. The second regime holds when concentrations are higher than the so-called critical micelle concentration (CMC), where the entropic term is overridden by the quite complicated landscape of the like–like and like–unlike interactions. Complications arise from the fact that, while for the headgroups the favourable interaction takes place with the solvent molecules, the hydrophobic tails interact much more favourably with themselves rather than with the solvent. The result of such like–unlike interaction balance is the self-association of amphiphilic molecules, as sketched in figure 1(b), where the head X is represented by a circle and the tail Y is represented by a wavy line. Then, although the phase separation in an amphiphile-rich and a solvent-rich phase still depends on the balance between entropic cost and intermolecular interaction gain, the geometrical structures of these phases exhibit a stunning variety of different shapes (spheres, cylinders, lamellae, etc) depending on the temperature, nature, and concentration of the amphiphile and solvent properties [4].

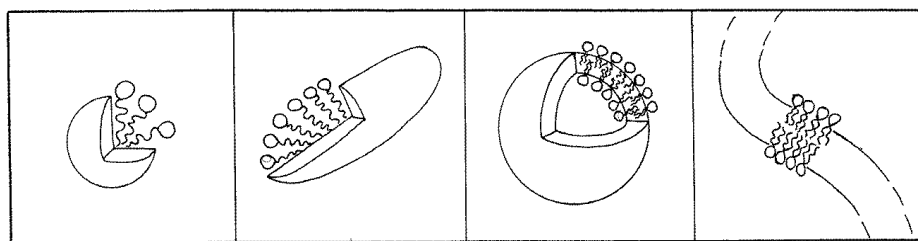


Figure 2. A sketch of four possible structures adopted by bifunctional molecules in solution: the spherical micelle, ellipsoidal micelle, unilamellar vesicle, and lamella.

The reason for the peculiar behaviour of systems consisting of solvent plus X–Y bifunctional molecules, in comparison with that of mixtures of X and Y monofunctional molecules, can be understood from the following considerations. When like–like interactions prevail, X and Y monofunctional molecules in mixtures segregate in such a way as to reduce the interface between the X-rich and the Y-rich phases. This leads to a macroscopic phase separation (large droplets) where the extent of the unfavourable interfacial region is kept as small as possible. By contrast, bifunctional molecules have vectorial binding properties (one end strongly interacts with the solvent, the other one with a nearby amphiphile), and therefore they cannot favourably pack in droplet-like aggregates; instead they form a variety of structures (lamellae, cylinders, micelles; see figure 2) where the size of the interfacial region between the dispersed amphiphile-rich phase and the bulk solvent-rich

phase is dramatically enhanced (surface-forming molecules). The solvent-rich phase is a dilute solution of amphiphiles, in monomer form, the concentration of which remains practically constant (\approx CMC) irrespective of the number of added amphiphiles. The two phases are in dynamic equilibrium—that is, they exchange monomers with a characteristic time depending on the amphiphile.

At interfaces, air–water, water–oil, etc, bifunctional molecules are not forced to form micelles or other complicated structures as in the bulk, but simply pack as a two-dimensional infinite aggregate: an oriented monolayer with the polar headgroups remaining in the aqueous medium, and the hydrophobic moieties expelling from it.

Let us now consider the more complex case of two different amphiphile molecules dissolved in a solvent. Again, above a critical amphiphile concentration, the system spontaneously demixes, forming an isotropic phase (made up of solvent and unassociated amphiphiles) and a condensed phase (e.g. micelles) where the two amphiphiles are closely packed either mixed in the same aggregate (figure 1(c)) or in different ones (figure 1(d)). The seemingly simple case of mixed aggregates of amphiphiles has a broader and more fundamental interest than is generally appreciated by colloid chemists and physicists, for the following reasons.

First, mixed amphiphile aggregates constitute a truly restricted geometry system where a pseudo-two-component, two-dimensional alloy of molecules is confined in a mesoscopic aggregate. In addition, the size and shape of the mesoscopic structure can be varied at will by making small changes in many physical parameters (ionic strength, temperature, chemical composition). Moreover, these compartmentalized structures are freely diffusing in the solvent-rich phase, behaving as isotropic or partially ordered suspensions of dispersed isolated mesoscopic phases. In addition, the multicomponent alloy made up of M different amphiphiles is not a closed system because it exchanges molecules with the solvent phase which contains a small but non-vanishing amount (\approx CMC) of amphiphiles in their unaggregated form. This concept has been overlooked by many authors, who approximated the amphiphile assembly to an isolated system, but might have some relevance in many properties of self-aggregating structures, as recently discussed in several papers (see, e.g., [5]) and repeatedly emphasized in this article.

Second, mixing properties of (pseudo-) two-component amphiphile aggregates are sensitive to a variety of agents. As we shall discuss shortly, the uneven distribution consequent on a *local* phase separation of the two amphiphiles within the aggregate has a profound influence on its size and shape; that is, aggregation provides a huge *geometrical* amplification of local mixing properties, a phenomenon that does not exist in the usual binary fluids. Geometrical variations of freely diffusing aggregates can then be easily detected by x-ray, light, and neutron scattering techniques.

In addition, many fundamental biological phenomena such as cell recognition, exocytosis (i.e. budding of small vesicles from cell membranes), and morphology of red blood cells are triggered by local coupling between non-ideal mixing of cell membrane components (which are amphiphile-like molecules) and membrane geometry.

Finally, assemblies containing amphiphilic molecules can form supra-colloidal structures giving rise, for instance, to coexisting micelle-rich and micelle-poor solutions when short-range attractive interactions cannot be neglected, as is the case for non-ionic amphiphiles (see, e.g., [6]). A non-ionic micellar solution may exhibit a phase separation when the temperature is raised above a certain value which depends on the nature of the amphiphile and its concentration in solution. The phase-separating non-ionic micellar solutions can be discussed as liquid mixtures of molecules with quite unbalanced molecular sizes, the one being the single water (solvent) molecule, and the other one the micelle (or a different

colloidal aggregate), in analogy with the case of high-molecular-weight polymer solutions which show phase separation at a critical temperature, as mentioned above.

In this paper we review the mixing properties of multicomponent amphiphile aggregates and of their supra-colloidal structures, especially for the most interesting case of non-ideal mixing of the various molecular components. Because the topic is very broad and has been recently reviewed by several authors [7–9], we focus our attention on a particular class of interesting amphiphilic molecules, the gangliosides, which show unusual features as compared with other more frequently investigated amphiphilic molecules.

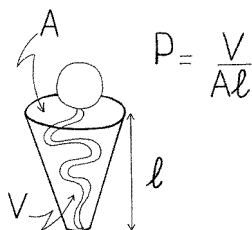


Figure 3. Geometrical representation of an amphiphile through the packing parameter P .

2. Molecular structure and properties of gangliosides

2.1. Geometrical properties of self-assembled aggregates, and the concept of the packing parameter

The type of structure formed by amphiphiles is strictly connected to the free energy of the aggregate, the calculation of which is a difficult task, as widely discussed in the literature [4]. However, simple geometrical considerations can provide a useful guide in predicting relevant properties of amphiphile assemblies. In fact, the packing of amphiphiles in an aggregate obeys some simple rules following the assumption that no water can exist inside the hydrophobic domain. The hydrophobic part is schematized as occupying inside the aggregate a region with the shape of a truncated cone, identified by three parameters: volume V , length l , and area A_0 at the interface. The dimensionless packing parameter $P = V/(A_0 l)$ (see figure 3) summarizes the geometrical properties of the amphiphilic molecule. The limiting values are $1/3$ for a true cone and 1 for a cylinder. The corresponding aggregated structures are spherical micelles and bilayers. Vesicles, liposomes, and in general membrane-like structures are formed when $1/2 < P < 1$. Micelles are formed when $1/3 < P < 1/2$. In between these values, higher P -values identify larger and non-spherical micelles. An example of an amphiphile which forms rather spherical micelles is the 12-carbon-chain surfactant sodium dodecyl sulphate (SDS) in water. Its packing parameter is quite close to $1/3$, being $P = 0.37$ with $V = 350 \text{ \AA}^3$, $l = 16.7 \text{ \AA}$, and $A_0 = 57 \text{ \AA}^2$. On the other hand, the amphiphile which typically forms vesicles and bilayers is egg lecithin, which has a packing parameter $P = 0.85$, with $V = 1063 \text{ \AA}^3$, $l = 17.5 \text{ \AA}$, and $A_0 = 70 \text{ \AA}^2$. These geometrical arguments have been shown to be rather convincing, mostly as regards the limiting values of $1/3$ and 1 for the packing parameter: the spherical micelle case with small aggregation number (< 100) and the bilayer case ($N \rightarrow \infty$), for most of the well known liposome-forming amphiphiles [4]. When the packing parameter P approaches $1/2$, the micellar size is very sensitive to slight changes of the packing parameter value, since

1/2 is the value at which the transition between globular and bilayer-type structure occurs. Changes in the local properties (as a change in the geometry of the amphiphile) can result in a change in the shape of the aggregates, and also a change in bulk properties (e.g., concentration or temperature) can be reflected in a variation of the packing properties of the amphiphile in an interplay which gives rise to the complex phase behaviour of amphiphilic solutions.

2.2. Physico-chemical properties of gangliosides

Gangliosides are a class of biologically important amphiphiles with the desirable characteristic property of having a geometrical packing parameter close to 1/2. They are discussed in this paper in connection with the interesting physical effects that they show while self-aggregating and adopting different structures. Gangliosides are natural glycosphingolipids occurring in plasma membranes where they play a key role in modulating relevant biological processes. For instance it has been shown that gangliosides have a pronounced tendency to form clusters—that is, regions of high ganglioside density—on membranes [10]. Domain formation may contribute to establishing interactions with specific proteins belonging to the same microenvironment and to modulating their properties [11]. They are double-tailed amphiphilic molecules, like phospholipids, in which a hydrophobic lipid portion, constituted by a sphingosine and a fatty acid with roughly 20 carbons each, carries a rather bulky headgroup made up of several sugar rings, some of which are sialic acid residues. The sugars in the main chain are ordered according to a fixed sequence, and also the positions available for branching of sialic acid residues are fixed, so each molecule can be obtained from each other molecule by adding or subtracting a given sugar ring. This means that not only the number of sugar units, but also the intramolecular interactions can be changed when considering different gangliosides.

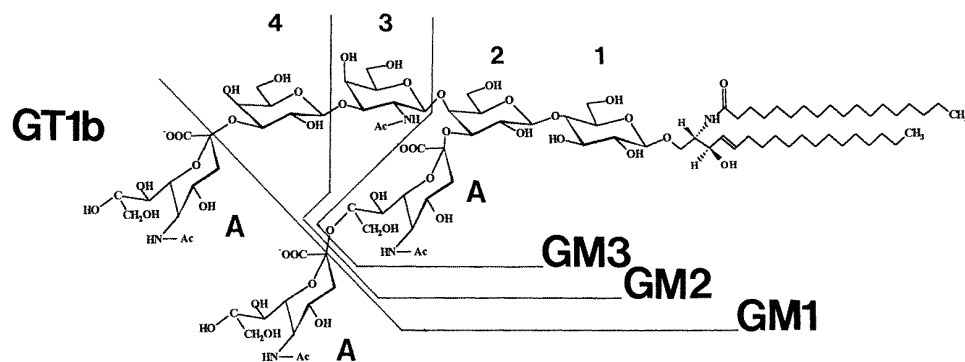


Figure 4. Chemical structure of different gangliosides.

The chemical structure of different gangliosides is given in figure 4 [10]. For GM1 a sialic acid A (*N*-acetylneuraminic acid) is attached to the sugar 2 in the oligosaccharide chain 1(glucose)-2(galactose)-3(*N*-acetylgalactosamine)-4(galactose). Sugar 4 is missing in GM2, and sugars 4 and 3 are missing in GM3. GT1b is like GM1 with a second sialic acid attached at A and a third sialic acid attached in 4. All of these gangliosides have the same hydrophobic part. So, their different properties in solution can be attributed to the hydrophilic headgroups.

Of course, gangliosides, being amphiphilic compounds, are present in dilute solutions as aggregates of high molecular weight above the CMC. Thermodynamics predicts CMC values in terms of the chemical potential difference for an individual molecule to be free in solution or inside an aggregate. The higher this difference, the lower the CMC, and also the slower the exchange process of individual molecules from aggregates to solution [4]. Therefore gangliosides, being double-tail amphiphiles with a large hydrophobic volume and a large chemical potential difference, self-aggregate in solution with a very low CMC, of the order of 10^{-8} – 10^{-9} M [12, 13]. Consequently the monomer residence time in the aggregates and micellar lifetimes are also very long, of the order of hours [14], as compared with those of normal synthetic amphiphiles.

In principle, the extended double-tailed hydrophobic part assigns gangliosides to the family of membrane-forming amphiphiles. In fact GM3, with the smallest headgroups in the series, has been seen to form vesicles [12]. Nevertheless, for gangliosides of higher complexity than GM3, the role played by the hydrophilic part of the molecule becomes very important. In fact, from GM2 on, the sugar headgroup is so extended that, despite the presence of a double hydrophobic tail, like a phospholipid, micelles are formed instead of vesicles.

Table 1. Physico-chemical parameters of different gangliosides: the monomer molecular weight MW, number of sugars in the headgroup S , aggregation form, average aggregation number N , average area per headgroup A_0 (\AA^2), packing parameter P , hydrodynamic radius R_H (\AA), micellar charge Q (electrical units), and axial ratio as deduced from light (a.r.) and x-ray (XR a.r.) scattering experiments.

| | MW | S | Form | N | A_0 | P | R_H | Q | a.r. | XR a.r. |
|------|------|-----|---------|--------|--------------|---------|-------|-----|------|---------|
| GT1b | 2142 | 7 | Micelle | 176 | 100.8 | 0.405 | 53.2 | | 1.78 | 1.4 |
| GM1 | 1560 | 5 | Micelle | 301 | 95.4 | 0.428 | 58.7 | 48 | 2.35 | 1.7 |
| GM2 | 1398 | 4 | Micelle | 451 | 92 | 0.440 | 63 | 100 | 3.03 | 2.3 |
| GM3 | 1195 | 3 | Vesicle | 14 000 | ≈ 80 | > 0.5 | 250 | | | |

The aggregate physical parameters of different gangliosides are reported in table 1. They have been deduced by static and dynamic laser light scattering observations, and sometimes confirmed by neutron and x-ray scattering experiments in the millimolar range of concentration [15–18]. The gangliosides in table 1 are ordered according to the number of sugar rings in the headgroup. On progressively reducing the number of sugar rings, going from GT1b to GM3, one observes an increase of the aggregation number and aggregate size, and even a drastic change from micellar to vesicular structure. The transition from micelle to vesicle takes place when the packing parameter goes through the value $1/2$. Table 1 shows that, as the number of sugar groups decreases, a smaller interfacial area A_0 is required by the molecule in the aggregate, which becomes bigger with larger aggregation number. Indeed, it can be seen that in the vicinity of the micelle-to-vesicle transition, the micellar aggregation number is very sensitive to small changes in the interfacial area. From GM1 to GM2 the aggregation number increases by almost 50% for only a few per cent variation in interfacial area. As ganglioside micelles get larger, they become less and less spherical, taking on an oblate ellipsoid shape and increasing axial ratios. More and more monomers are accommodated in the ganglioside micelles by keeping the minor axis practically constant and increasing the major axis of the ellipsoid of revolution. This is consistent with the understanding that in the self-aggregation process one dimension of the hydrophobic core

cannot exceed twice the extension of the hydrophobic part of the amphiphilic molecule itself.

In normal experimental conditions, a GM1 micellar solution at a concentration of 1 mM, that is 1.56 mg cm^{-3} , contains a large number of micelles, about 2×10^{15} per cm^3 , which are in dynamic equilibrium in solution with a small number of monomers, three orders of magnitude less, and have a long lifetime, of the order of hours. GM1 micelles are also rather similar in size, $R_H \approx 60 \text{ \AA}$, with a small polydispersity index, of the order of a few per cent [19], as a consequence of their large aggregation number [20], about 300 at room temperature. Ganglioside micelles have thus the interesting feature of being similar to well defined latex colloids, as regards particle stability, but at the same time they are still aggregates in slow dynamic equilibrium with monomers in solution.

In addition to the above-reported properties, let us emphasize two more aspects which are peculiar to gangliosides and their supra-molecular assemblies.

First, at variance with the case for most amphiphiles, the extended ganglioside polar headgroups may assume different low-lying molecular conformations as is evident from experimental data [21] and theoretical calculations [22, 23]. Since ganglioside headgroups are very bulky and their reciprocal distance within the aggregate is short, the conformational changes of the heads are expected to be strongly correlated, and thus a cooperative behaviour in the polar region is likely to be observed.

Second, the hydrocarbon chains of all amphiphiles (including gangliosides) may exist in more or less stretched, or ordered, conformations with a difference in length which can be of the order of 20%. When amphiphiles are embedded in a sufficiently large aggregate, both of these conformations are allowed, the ordered one being favoured at low temperature and the disordered one at high temperature. In a bilayer structure like that of a vesicle or of a lamellar phase, they give rise to the ordered L_β phase and to the disordered fluid-like L_α phase, between which an entropy-driven and highly cooperative transition occurs as the temperature is raised (see, e.g., [24]). This can be explained by considering that, as the hydrocarbon–water interfacial tension is high (roughly 50 mJ m^{-2} [4]), it follows that the order–disorder transition must be a cooperative phenomenon in order to avoid the formation of energetically unfavourable valleys and protrusions connected with the transition of isolated and uncorrelated chains. Furthermore, since the $L_\alpha \rightarrow L_\beta$ transition is basically controlled by the chain conformational entropy, it is triggered by temperature changes but is rather insensitive to other parameters. Ganglioside micelles are sufficiently large for this transition of the hydrocarbon chains to be observed [25] also in a non-bilayer structure. Of course it has a reduced sharpness due to the small hydrophobic volume of a micelle, where the degree of order which the hydrocarbon chain can reach is somewhat reduced.

On the other hand, the ganglioside cooperative transition of the polar headgroup region is independent of the water interfacial tension (in practice, there is no interfacial energy between the water and ganglioside sugar heads because both belong to the hydrogen-bond-forming molecular class) but it is very sensitive to a variety of effects such as hydrogen bond interactions among the heads or external substances dissolved in the aqueous medium or, finally, to forces originating from the interactions among the aggregates. In this respect, ganglioside-containing aggregates behave, to some extent, like small-sized ‘polymer brushes’ (polymer chains anchored at one end to a rigid substrate), a topic which has been extensively investigated in recent years mainly in connection with their technological applications (e.g., [26]). A significant difference is that ganglioside monomers, however, are freely moving along onto the aggregate surface, a property which introduces new features which are absent for conventional polymer brushes.

Hence the simultaneous presence of apolar and polar regions, both of them undergoing

conformational transitions, in addition to the lateral mobility onto the membrane plane, adds further degrees of freedom, determining a rich behaviour which may explain the unique receptorial properties of ganglioside molecules characterized by a very specific response able to discriminate among rather similar small external perturbations.

3. Coupling between lateral distribution and geometrical deformations in amphiphile assemblies

Amphiphile aggregates made up of two or more different molecules may undergo dramatic variations of their packing properties as a consequence of uneven distribution of their own components. Because of the complexity of the topic we will discuss the following cases separately: (a) non-ideal mixing in monolayers; and (b) non-ideal mixing in bilayers, micelles, and other amphiphile aggregates.

3.1. Non-ideal mixing in monolayers

Multicomponent monolayers represent a relatively simple system as regards understanding the physics of two-dimensional lateral phase separation. A simple phenomenological approach to describing the coupling between shape and segregation is based on a Landau–Ginzburg picture which decomposes the effective Hamiltonian of an amphiphilic two-component monolayer into three main contributions [27–34].

The first, F_1 , arises from the monolayer deformation from the reference planar shape, irrespective of its single- or multicomponent composition. It contains a term proportional to the water–monolayer interfacial tension γ as well as a term quadratic in the curvature, related to the monolayer bending elasticity χ . For small displacements $h \equiv h(r)$ from the reference shape, F_1 takes the form

$$F_1 = \int_S \left(\frac{1}{2} \gamma (\nabla h)^2 + \frac{1}{2} \chi h (\nabla^2 h)^2 \right) dS \quad (1)$$

The second contribution, F_2 , is typical of an inhomogeneous mixture of two amphiphiles A and B. Letting $\Phi \equiv \Phi(r)$ be the locally varying concentration of component A and $1 - \Phi$ that of component B, and introducing the variable ε defined as $\Phi = (1 - \varepsilon)/2$, F_2 can be expressed in standard form:

$$F_2 = \int_S \left(\frac{1}{2} \chi_\varepsilon (\nabla \varepsilon)^2 - \mu(\varepsilon) + f(\varepsilon) \right) dS \quad (2)$$

where μ is the chemical potential and the term $\frac{1}{2} \chi_\varepsilon (\nabla \varepsilon)^2$ takes into account the energy associated with the (diffuse) interface between A-rich and A-poor regions. The free energy $f(\varepsilon)$ contains both self-energy and interaction contributions

$$f_{inter}(r) \equiv \int_{S'} \varepsilon(r) \varepsilon(r') G(|r - r'|) dS'$$

which is related to the forces $G(|r - r'|)$ among the amphiphiles.

It is worth noting that $f_{inter}(r)$ is local if the A–A, B–B, and A–B pair interactions are limited to nearest neighbours, while it is non-local when long-range forces are present. The presence of long-range forces (e.g. dipolar interactions) may give rise to an incredible variety of different phases even in the absence of coupling between segregation and aggregation geometry, as thoroughly investigated by McConnell [35], Mohwald [36], and other authors [37].

On the basis of previous investigations on the properties of ganglioside micelles at different salt concentrations [38], we believe that the interactions among gangliosides are mainly local, due to steric and hydrogen bond forces. Then, taking into account only nearest-neighbour interactions, we may expand the free energy $f(\varepsilon)$ in the usual mean-field picture:

$$f(\varepsilon) \cong \frac{1}{2}(T - T_c)\varepsilon^2 + (1/4)b\varepsilon^4 + O(\varepsilon^6) \quad (3)$$

which, even in the absence of any coupling with geometrical parameters, exhibits a lateral phase separation for $T_c > T$.

Finally, a third contribution, F_3 , describes the coupling between deformation from the reference structure (e.g., planar) and lateral segregation:

$$F_3 = \int_S \lambda \varepsilon (\nabla^2 h) \, dS + O((\nabla^2 h)^2). \quad (4)$$

The phenomenological parameter λ measures the strength of the coupling and can be related to the spontaneous curvature of the monolayer.

Some constraints have to be imposed, such as volume incompressibility of the monolayer and/or a constant composition. This can be done by the well-known Lagrange multiplier technique, leading to the standard minimization procedure via Euler's equation. Above certain critical values, the model predicts a sinusoidal deformation of the monolayer, accompanied by a periodic modulation of the composition.

3.2. Non-ideal mixing in bilayers, micelles, and other complex amphiphilic aggregates

In more complex structures than the infinite two-dimensional monolayer, the uneven distribution of the amphiphile components may give rise to new and interesting effects. For instance, the existence of lateral heterogeneities gives rise to a complex interplay between the bilayer topology and the heterogeneity distribution between the two back-to-back monolayers building up the bilayer.

Let us first consider a two-component 'isolated' bilayer, for example in dilute solution, built up by amphiphiles with different packing parameters. Energy minimization with respect to the composition and shape of *each* monolayer determines an asymmetric distribution of the amphiphiles between the two opposite monolayers. The competition is between the bending elasticity and mixing entropy on one hand (both tend to maintain flat and homogeneous structures) and the preferential insertion of the amphiphile with the smaller packing parameter in the high-curvature regions on the other hand. The result of inhomogeneous distribution of components between the two leaflets is that the bilayer acquires a spontaneous curvature, and so becomes unstable in the planar shape and spontaneously vesiculates (closes up) forming unilamellar liposomes, structures where a bilayer shell envelops a solvent drop [5, 30–33].

For an *isolated* bilayer the scenario described so far is the most favoured one from an energetic standpoint. However, when the volume fraction of amphiphiles is progressively increased, there is not space enough to accommodate vesicles (curved bilayers) owing to the strong excluded-volume repulsion among them—whence, at high concentration, the bilayers must retain a flat structure. In this case, the forced bilayer topology induces the composition heterogeneities in the two back-to-back opposite monolayers to follow in-phase modulation accompanied by periodic dilation and compression of the bilayer (see figure 5). The amplitude of the deformations depends on the amphiphile composition, mixing properties and rigidity of the aggregate. Because of thermally induced mechanical fluctuations of amphiphilic assemblies, what is observed in reality is a periodic array of

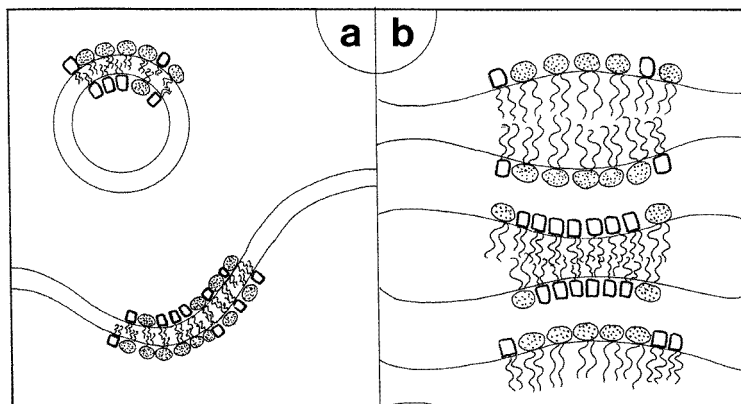


Figure 5. Coupling between non-ideal mixing and the local curvature radius in dilute (a) and concentrated (b) suspensions of two-component amphiphile lamellae.

broken lamellae [39], the rims of which are stabilized by a higher concentration of the amphiphile with a smaller packing parameter (i.e., short tails or bulky heads).

3.3. Experimental observations in mixed ganglioside assemblies

3.3.1. Ideal mixing. When the non-ionic surfactant, *n*-dodecyl octaoxyethylene glycol monoether ($C_{12}E_8$), bearing a small, elongated hydrophilic headgroup, is added to a GM1 solution, mixed micelles are formed with a molecular weight which depends on the molar ratio of the two amphiphiles. The molecular weight monotonically decreases from the value for pure GM1 micelles to that for pure $C_{12}E_8$ micelles [40] in a fashion which is in good agreement with the prediction of a thermodynamic model which involves regular mixing of the amphiphiles in the micelle.

3.3.2. Non-ideal mixing in vesicles. Spontaneous vesiculation of stable small unilamellar vesicles has been observed for the mixed system of gangliosides GM1 and GM3, which have different packing parameters, lower and higher than $1/2$, respectively [41]. As shown in table 1, GM3, the ganglioside with only three sugars in the headgroup, self-aggregates in solution adopting bilayer-type structures [16] rather than micellar ones. At first, this is not surprising since one sugar less than GM2 makes the headgroup smaller, and therefore the packing parameter can become larger than $1/2$, the upper value for micellar aggregation. What is unexpected, following the above considerations about the interplay between heterogeneity distribution and bilayer topology, is that the ganglioside GM3, as a single amphiphile, spontaneously forms vesicles, of average radius of about 250 \AA , in equilibrium with a small number of large aggregates, mostly of lamellar type [42]. It has been found that only a few per cent of the total amphiphile concentration goes into the large aggregates, considered as sheets of average diameter about 10^4 \AA . Spontaneous vesiculation in the single-amphiphile GM3–water solution will be discussed later on, in section 6.

The addition of a second amphiphile, the ganglioside GM1, to GM3 leads to a gradual decrease of the number of large lamellar aggregates coexisting with vesicles [41]. At a GM1 mole fraction of 0.35, only vesicles are present in solution, with a radius of 205 \AA . This means that the additional degree of freedom due to the composition of each monolayer in

the bilayer allows for the formation of an equilibrium phase of vesicles with a lower energy than the lamellar phase. In fact, it can happen that the lowering of the free energy arising from the outer layer matching its curvature to the spontaneous curvature can compensate the cost due to frustration of the inner monolayer, mostly when vesicles are not too large, like in the present case. Since the GM1 ganglioside has a larger headgroup than GM3, in a GM3–GM1 vesicle, GM3 should be more abundant in the inner layer, where headgroup lateral hindrance has to be lower, as discussed in previous sections. Finally, a further addition of GM1 to GM3 leads to the usual coexistence of vesicles and micelles. Although the ‘budding’ of micelles from the vesicle surface is a very interesting effect, our data are not precise enough, at the present stage, to provide evidence of peculiar properties of gangliosides; hence this region of the phase diagram will not be considered any longer.

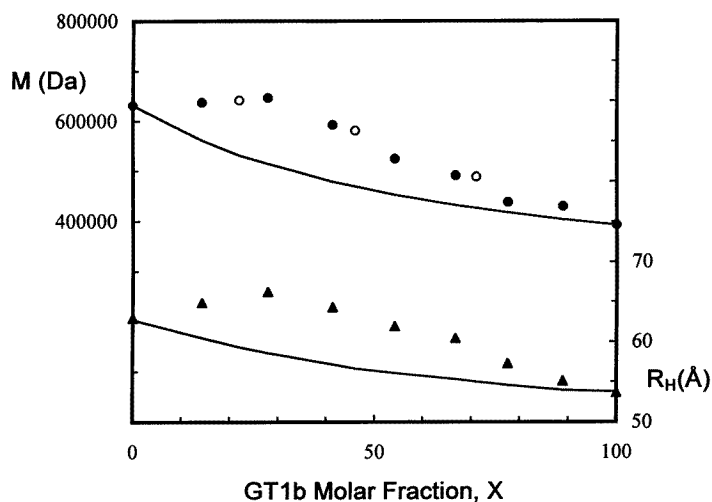


Figure 6. The molecular mass M (full dots: light scattering; open dots: neutron scattering) and hydrodynamic radius R_H (triangles) of GM2–GT1b mixed ganglioside micelles as a function of the GT1b molar ratio X . Full lines represent the predictions of an ideal mixing model.

3.3.3. Non-ideal mixing in micelles. Also on mixed micelles, segregation among different components can be detected through the geometrical variation of micelles. The effect is enhanced in large aggregates (i.e. when the packing parameter is close to $1/2$), as happens in the case of gangliosides. Non-ideal mixing within the single micelle has been observed when the two gangliosides GM2 and GT1b are mixed together. Both amphiphiles make micelles independently, with GM2 micelles larger than GT1b ones. The values of the molecular mass M of the mixed micelle, as obtained from light and neutron scattering measurements [43], are shown in figure 6 as a function of the molar fraction X of GT1b in the mixed micelle. The hydrodynamic radius R_H of the mixed micelle, as derived from dynamic light scattering data [19], is also reported in figure 6. The observed behaviour of M and R_H as functions of X constitutes clear evidence for the formation of non-ideally mixed micelles, as can be seen by looking at the discrepancy between the experimental data and the full lines, which represent the values of M and R_H as calculated in a model which assumes regular mixing of the two amphiphiles in the micelle. This indicates that GM2 and GT1b are not randomly distributed in the mixed micelle, but rather that some clustering occurs. Clustering

could either originate from some specific interaction between GM2 and GT1b headgroups, or, more simply, from geometric packing constraints. Indeed, the GM2 micelle is large and not spherical, with the shape of an oblate ellipsoid. Therefore, GT1b molecules, which have a larger headgroup, may prefer to occupy the edge of the ellipsoid where the curvature radius is small, whereas GM2 molecules would go preferentially to regions of larger radius of curvature. The increased ellipticity of the non-ideally mixed micelle implies a greater surface area per micelle and an entropic cost, both of which are unfavourable processes. This energy cost is largely compensated by a reduced repulsion energy within the clustered distribution of the two amphiphiles [44]. The balance among these conflicting demands in mixed micelles induces a segregation–geometry coupling which favours the formation of micelles that are more elliptic, accompanied by a redistribution of the amphiphiles between more curved and less curved regions.

The fact that mixed GM2–GT1b micelles are larger than both the single-component ones in a certain range of relative composition (around 75:25) gives easy experimental access to the kinetics of mixed micelle formation—by continuously monitoring the intensity of the scattered light as a function of the time delay from the instant of mixing the two single-ganglioside solutions.

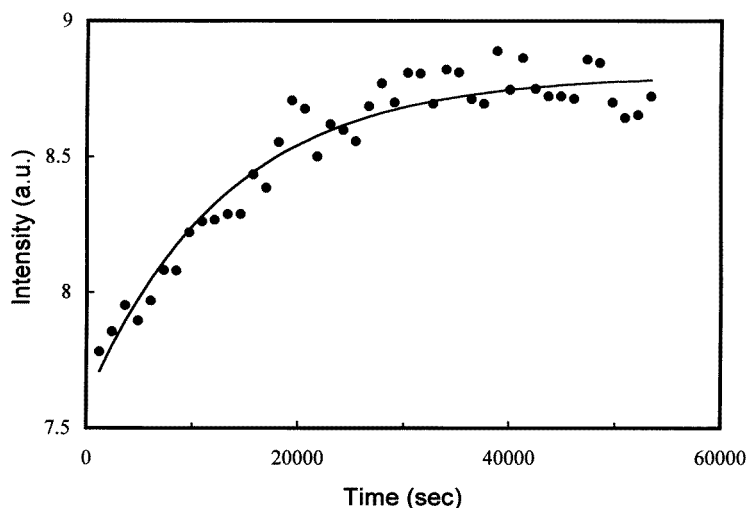


Figure 7. Scattered light intensity versus time during the formation of GM2–GT1b mixed micelles. The full line is the model prediction.

Figure 7 shows the evolution towards equilibrium of mixed micelles starting from individual micelles [14]. The final configuration is reached in about ten hours. The full line represents the theoretical behaviour calculated according to a model which attributes the mixing process to monomer transfer from one micelle to another via free molecules in solution. The equilibrium time is then dictated by the exchange rate between aggregates and the solution, and in particular it is expressed as a bilinear function of the exchange rates of the two molecular species, so the quicker one will dominate [14]. To obtain long equilibration times, it is then necessary that both the rates are slow and similar in value. Because the exchange rate is closely connected to the CMC of the amphiphiles [4], the observed equilibration time is an indirect proof that both GM2 and GT1b CMC values are quite low, of the order of 10^{-8} – 10^{-9} M.

4. Non-ideal mixing of mutually interchanging conformations

4.1. Bistability and thermal hysteresis

Non-ideal mixing in aggregates of two amphiphiles may have a profound influence on the interfacial area per molecule. Although measurements of surface area variations are in principle feasible, they are however quite difficult and subject to a number of experimental errors. Interestingly, when the amphiphile aggregates are spheroidal micelles, there is a simple relationship between the average interfacial area $\langle A \rangle$ and the aggregation number N . For the simple case of a spherical micelle, we have the obvious identity $NV = (4/3)\pi R^3$ and $N\langle A \rangle = 4\pi R^2$ (where V is the hydrophobic volume of the amphiphile molecule and R is the radius of the hydrophobic core of the micelle), and by invoking the volume incompressibility of the micellar aggregate we get

$$N = 36\pi V^2 / \langle A \rangle^3 \quad (5)$$

(a more general relationship covering the case of elliptical micelles can be easily derived; see, e.g., reference [45]). Since the aggregation number N can be easily measured by scattering techniques, the above relationship provides a useful tool for following surface small-area variations upon non-ideal mixing.

A Landau–Ginzburg picture of the coupling between the aggregation number N and mixing properties can be derived as follows. Adopting the simplest picture of amphiphile aggregation [20], the local optimal area $A(r) \equiv A$ for an amphiphile assembly arises from the balance between the interfacial energy, γA (γ being the interfacial tension at the water interface) and the repulsion among heads, which can be described as C_0/A , where C_0 measures the strength of the repulsion forces. We extend the simple model [20] by replacing C_0 with a composition-dependent interaction parameter $C(\Phi)$ which is related to the local distribution $\Phi(r) \equiv \Phi$, that is, to the fractions Φ and $1 - \Phi$ of the two-component (or two-conformer) mixture. A useful approximate expression for $C(\Phi)$ is $C_0 + \Lambda\Phi(1 - \Phi)$. On defining the new variable ε through $\Phi = \frac{1}{2}(1 - \varepsilon)$ and combining the above results, and introducing in the surface area value a gradient term accounting for spatial inhomogeneities, the free energy per molecule turns out to be

$$U_0 \cong \frac{1}{2}\chi_A(\nabla A)^2 + \gamma A + C(\varepsilon)/A + U_{MIX}(\varepsilon). \quad (6)$$

The mixing energy $U_{MIX}(\varepsilon)$ of a two-dimensional fluid containing two different species (or conformations) can be calculated as a balance of entropic and interaction forces as described in equations (2) and (3). Insertion into equation (6) and integration over the spherical micelle surface yields [45]

$$F_{TOT} \cong \text{constant} + 2\pi \int_0^\pi [\frac{1}{2}\chi_\varepsilon(\nabla\varepsilon)^2 + \frac{1}{2}\chi_A(\nabla A)^2 + \gamma A + C_0^{eff}/A - \frac{1}{2}(T_c(A) - T)\varepsilon^2 + (1/4)b\varepsilon^4 + \mu\varepsilon]R^2 \sin\theta \, d\theta. \quad (7)$$

Here $C_0^{eff} \equiv C_0 + (1/4)\Lambda$; $T_c(A) \equiv \Lambda/2A$ and $b \cong T/3$.

The coupling between the total area and the surface composition is contained in the term $T_c(A)\varepsilon^2$. From energy minimization, $\delta F_{TOT}/\delta\varepsilon = 0$ and $\delta F_{TOT}/\delta A = 0$, the unknown area A and surface composition ε can be calculated. Hence, after averaging over all of the micelles in solution, the mean aggregation number N is immediately found from (5).

Two regimes can be identified according to whether entropic forces ($T > T_c$) or like-like interactions among similar conformations ($T < T_c$) prevail. When the entropic forces overcome the interactions among identical molecular conformations ($T > T_c$), the system is nearly ideal and the aggregation number becomes a monotonic function of composition

ε . This behaviour has been predicted and experimentally supported by the observation that several properties of mixed micelles, like aggregation number, CMC, and macroscopic surface tension vary monotonically with composition (see, e.g., [46]). In this regime, non-ideality of the mixing has little effect, just slightly changing the slope and shape of the corresponding curves.

More interesting is the behaviour below the critical temperature T_c where the interactions among like conformations overcome the mixing entropy contribution. The total free energy, equation (7), exhibits two unequal energy minima with different compositions and areas; that is, two stable populations of micelles of different sizes coexist. A time-dependent Landau–Ginzburg (TDLG) analysis shows that the forward and backward interconversion rates among the two populations may differ to a large extent [45]. This fact introduces the interesting possibility of observing thermal hysteresis effects for $T < T_c$. Hence, if the backward rate is slow, the micelles are trapped in a metastable state characterized for each temperature by a conformational composition, surface area and, thus, aggregation number.

These results indicate the interesting properties which characterize the nucleation and growth phenomena of a new phase in confined media. In fact, in a boundless system, once the nucleus of a new phase has reached the critical size, no further limits are imposed on its growth, so the rate of propagation of the new phase is mainly limited by diffusion processes.

By contrast, in micellar suspensions the growth of a new phase takes place within each micelle; hence, even at high micelle concentration, the system behaves as a collection of independent objects (the micelles), each of them undergoing a phase transition. Second, the reduced micellar geometry poses severe constraints on the critical size of the nucleus, because now it cannot exceed the micelle dimension, while no limits on the nucleus size exist in a boundless system, the critical size depending upon the energy balance between bulk and surface forces alone.

4.2. Thermal hysteresis of gangliosides

For the interesting case of gangliosides, as already said, hydrophilic heads may exist in different molecular conformations [21–23]. Therefore, a micelle, made up of monomers of a single ganglioside, which is then chemically homogeneous, can nevertheless physically behave as a binary mixture. Interchange between the two conformations is possible, with the important consequence that the molar fraction of the two species in the mixed aggregate is not conserved. This can give rise to non-ideal mixing effects which are different from the ones already discussed for the mixed GM2–GT1b or GM3–GM1 systems (see section 3.3), in which the molar ratio of the two constituents was fixed.

The experimental finding of a thermotropic behaviour of ganglioside micellar solutions which is irreversible with respect to the temperature and is not due to a denaturing of the molecule suggests the presence of complex cooperative effects involving the ganglioside molecular conformations as discussed in section 4.1. In our experiments the conformational change is observed through the ganglioside aggregative properties. In fact, the micellar aggregational state of gangliosides, which have a packing parameter in the higher part of the micellar range, close to $1/2$, are very sensitive, revealing even a slight change in the ganglioside molecule conformation with no need for local probing, provided that this change modifies its geometrical hindrance, as the micellar aggregation number, or, equivalently, the micellar molecular mass is going to change. The change of micellar molecular mass is monitored in dilute ganglioside solutions by measuring the light intensity scattered at an angle of 90° . The scattered intensity is, in fact, proportional to the weight average mass of the micelles in solution, or equivalently to their average aggregation number N , since the

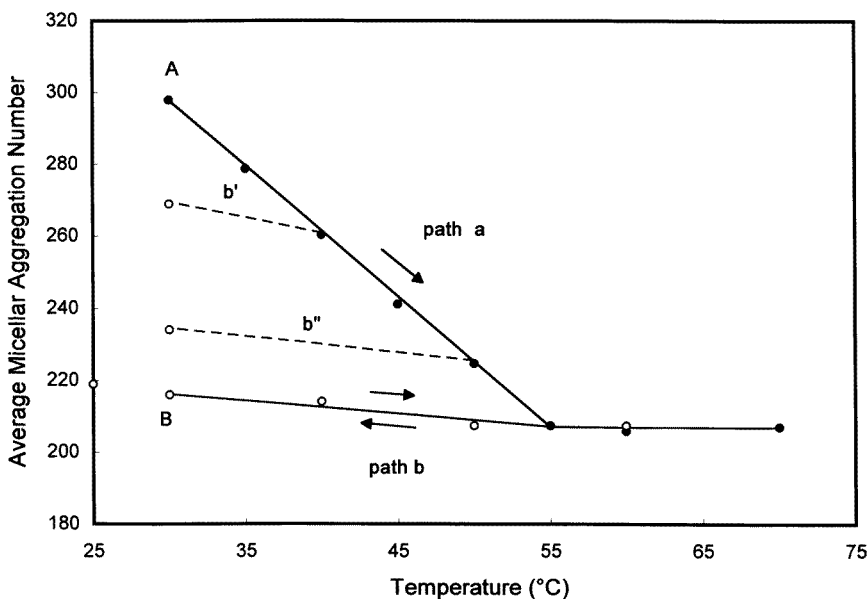


Figure 8. The variation of the micellar aggregation number N in a 1 mM GM1 aqueous solution during the temperature cycle. Upon heating up to 55 °C the system follows path a. Then it follows path b for any subsequent heating and cooling cycle. Paths b' and b'' represent intermediate hysteresis effects for $T_{max} = 40$ °C and $T_{max} = 50$ °C respectively (see the text).

molecular mass of the single ganglioside molecule is fixed. Due to the small dimension of the ganglioside micelles, the scattered intensity does not have a significant dependence on either the scattering angle or the micellar shape [18]. Figure 8 shows the behaviour of the light intensity scattered by a solution of GM1 as the temperature is changed. Point A is the value at a temperature of 30 °C. Upon heating the solution up to 55 °C, the scattered intensity decreases considerably—path a. Then, on cooling, the scattered intensity stays practically constant at its lowest value—path b of figure 8—and at room temperature it is at point B. At this stage, the scattered intensity follows path b for any subsequent heating and cooling procedure. The micellar hydrodynamic radius, as measured via dynamic light scattering [18], varies consistently with the molecular mass along the cycle—that is, decreases during the first temperature scan between 30 °C and 55 °C, and then stays practically constant. Throughout the cycle the polydispersity does not change appreciably.

Within the same temperature range, a similar behaviour is observed also for other micelle-forming gangliosides different from GM1 [47]; that is, in a temperature cycle, the scattered intensity evolves like that along paths a and b of figure 8. For each of these gangliosides two extreme conditions can be identified at room temperature: the ‘cold’ and the ‘warm’ one, characterized by a different micellar mass and a different behaviour on heating: ‘warm’ and ‘cold’ micelles are the ones which have been and have not been subjected to a temperature cycle, respectively.

Table 2 summarizes the micellar parameters for the gangliosides GM2 and GM1 relative to the two extreme conditions of unheated samples—the ‘cold’ state—and heated samples—the ‘warm’ state. The values of the packing parameter $P = V/(A_0l)$ are calculated [15, 48] from the measured values of the micellar mass using the known values of the ganglioside density and extensions of the hydrophobic tails l . The average area per headgroup A_0 is

Table 2. Micellar parameters for ‘cold’ and ‘warm’ GM2 and GM1 micelles: average aggregation number N , average area per headgroup A_0 , and packing parameter P .

| | N | | A_0 (\AA^2) | | P | |
|-----|------|------|--------------------------|------|-------|-------|
| | Cold | Warm | Cold | Warm | Cold | Warm |
| GM2 | 451 | 261 | 92 | 96.5 | 0.445 | 0.423 |
| GM1 | 301 | 205 | 95.4 | 99.5 | 0.428 | 0.411 |

reported separately, as it is the only parameter appearing in P which changes on going from the ‘cold’ to the ‘warm’ state. Thus the variation ΔA_0 in the interfacial area is responsible for the observed decrease in the aggregation number N due to the temperature cycle (see equation (5)).

Irreversible cycles like those reported in figure 8 are obtained also for intermediate temperature changes between the extreme values of 30 °C and 55 °C. After the temperature is raised from 30 °C to an intermediate value, say 40 °C, the new value of the micellar average aggregation number is smaller than the corresponding value at 30 °C, and stays practically unchanged for any subsequent cycle throughout which the temperature does not exceed 40 °C, path b' in figure 8. Then, if the temperature is raised to a new value above 40 °C, say 50 °C, the average micellar aggregation number is lowered again to a value which remains practically constant, path b'' in figure 8, as long as the system is kept below 50 °C. This happens until the system reaches the limiting ‘critical’ temperature of 55 °C, above which micelles do not show a change in their average aggregation number any more.

The experimental behaviour of figure 8 is well described by the theory developed by us [45]. Irreversibility, ‘intermediate’ thermal hystereses and critical temperatures are naturally emerging from a picture of a nucleation process in confined geometry. Indeed, the key factor for seeing thermal hysteresis is the formation of two coexisting populations of micelles with different sizes. The above effect can exist only in a cooperative system, and disappears on approaching a critical temperature, at which the micellar aggregation numbers of the two populations become identical (see section 4.1).

As far as a picture of the different conformers can be tried, either the carbohydrate residues in the oligosaccharide chain change their relative steric position, or, more simply, the oligosaccharide chain axis in the ‘warm’ state may tilt with respect to the hydrophobic portion, increasing the solid angle needed by the monomer in the aggregate and, therefore, the area A_0 . From the measured aggregation number of ganglioside micelles, one can calculate the average area-per-headgroup increase on passing from A to B, as reported in table 2. It can be noticed that, although the extent of the variation in the aggregation number is different for the two gangliosides, nevertheless the change in the monomer average surface area ΔA_0 is quite similar, around 4\AA^2 . The physical mechanism involved in the interconversion between the two conformational states of the ganglioside molecule is not definitely known yet, but is probably connected to a different degree of hydration which causes a change in the tilt angle between the hydrophobic and hydrophilic portions. Temperature could, in fact, change the relative interplay of the hydrogen bonding of the water solvent molecules with the gangliosides as well as the hydrogen bonding between sugar units belonging to the same oligosaccharide chain or to different adjacent ganglioside molecules. This hypothesis is suggested by the fact that the gangliosides GM2 and GM1, which differ in the length of the saccharidic portion but keep unchanged the hydrophilic–hydrophobic boundary region, have been observed to experience similar irreversibility effects with the

same ‘critical’ temperature of 55 °C [45].

The theory and experiments described so far in sections 4.1 and 4.2 deal with temperature-induced collective variations of headgroup conformation on going from ‘cold’ to ‘warm’ states. This model can be extended, accounting for the solvent-mediated interactions among amphiphilic assemblies which might induce the above-mentioned conformational transitions. For instance, we have observed an interesting behaviour in concentrated ordered suspensions of micelles as well as in concentrated lamellar phases. In the next section, section 5, we will describe a few consequences of non-ideal mixing for the interactions among supra-colloidal aggregates.

5. Interaction among non-ideally mixed fluid ‘supra-colloidal’ aggregates

5.1. Interaction between planar laterally inhomogeneous surfaces

The interactions between two surfaces brought into close contact and allowed to re-adjust their structure in order to minimize the total free energy is an interesting topic, especially in fluid and soft assemblies like biological ones.

In fact, it is well known that surfaces decorated with repulsive domains brought into close contact repel each other less than homogeneous surfaces, provided that the proper dephasing among the opposite domains takes place [48, 49]. More intriguing is the case of fluid-like surfaces where molecules can migrate, and the formation and growth of uneven domains arises as a consequence of repulsive interactions. This phenomenon has been recently detected in several mixed amphiphile aggregates of biological interest (including gangliosides [50]) brought into close contact [51], and has been theoretically investigated in a series of papers [52–55]. All of these investigations provide evidence that the restructuring of the interfacial region, and the consequent softening of the repulsion, can be dramatic, switching, in some circumstances, from repulsive to attractive.

By preserving the simple phenomenological approach used in the previous sections, we developed an approximate model which gives a number of qualitative predictions. Let us consider a collection of planar mixed amphiphilic assemblies, lamellae. Let us then assume that the interaction potential is mainly repulsive and that the energy per unit area between two small regions of opposite lamellae containing the two A and B amphiphiles, with local composition $\Phi(r)$ and $1 - \Phi(r)$, is described by the superposition of pure-component potentials:

$$U_I(\varepsilon) = (1 + \varepsilon)P_A \exp(-z/\lambda_A) + (1 - \varepsilon)P_B \exp(-z/\lambda_B) \quad (8)$$

($\Phi = \frac{1}{2}(1 + \varepsilon)$) where P_j and λ_j describe the strength and the decay length of the force, respectively, and $z \equiv z(r)$ is the local relative distance between the midplanes of two neighbouring lamellae. Equation (8) is purely phenomenological. Its physical origin has been widely discussed in many papers [4, 55] and this discussion is not repeated here. The assumption of volume incompressibility for the lamellae and solvent yields $Az = \text{constant}$, where A represents the lamellar surface. The total free energy can be expressed as the sum of the energy of the isolated lamellae, U_0 (described in (6)), plus an internal term, $U_I(\varepsilon)A$, the analytical expression for $U_I(\varepsilon)$ being as reported in (8):

$$F = U_0 + U_I(\varepsilon)A. \quad (9)$$

Under the above conditions and for fixed composition ε of the lamellae, the total free energy seeks a minimum under the constraint of a coupled variation of A and z :

$$\delta F|_\varepsilon = \left. \frac{\partial F}{\partial A} \right|_\varepsilon \delta A + \left. \frac{\partial F}{\partial z} \right|_\varepsilon \delta z = \left[\left. \frac{\partial F}{\partial A} \right|_\varepsilon - \frac{z}{A} \left. \frac{\partial F}{\partial z} \right|_\varepsilon \right] dA \quad (10)$$

where $\delta z = -(z/A)\delta A$, since Az is constant. Hence, from (6) it follows that the equilibrium area is obtained by solving

$$\left. \frac{\partial U_0}{\partial A} \right|_{\varepsilon} - z \left. \frac{\partial U_I}{\partial z} \right|_{\varepsilon} = 0. \quad (11)$$

When the lamellae contain two different species (or conformations) in thermodynamic equilibrium, equation (11) must be supplemented by

$$\left. \frac{\partial (U_0 + AU_I(\varepsilon))}{\partial \varepsilon} \right|_{\varepsilon} = 0. \quad (12)$$

Equations (11) and (12) allow one to obtain the optimum values of the lamellae area A and composition ε . In the small-deformation limit the final equations do not appreciably differ from those developed in section 3.1, apart from the rescaling of some coefficients modulated by the interactions among the amphiphile aggregates [56].

The model predicts that below a critical temperature the amphiphiles are arranged into domains of densely and loosely packed lamellae, the size of the domains being related to the faster growth of certain critical fluctuations, while the critical temperature crucially depends on the amphiphile/water ratio [56].

The coexistence of two different lamellae repeat distances is thus the result of non-ideal mixing in a two-component (or two-conformer) fluid of amphiphiles. This kind of stress-induced phase separation should be widespread in amphiphilic assemblies and, indeed, it has been observed in a few ternary systems (water plus two different amphiphiles [57, 58]). Evidence for its occurrence in binary systems is more recent [59–61]. Lately, x-ray measurements on concentrated (above 55% in weight) dispersion of the GM1 ganglioside in water have also shown such a coexistence of lamellar phases, with different repeat distances—about 90 Å and 100 Å [61].

The onset of two coexisting lamellar phases with different spacing poses interesting fundamental questions, because the array of staked lamellae interacting with an arbitrary potential can be seen as an example of the ideal one-dimensional gas, for which exact analytical results have existed in the literature for a long time. The interesting feature is that the one-component 1D gas (known as the Tonks gas) does not exhibit phase separation except in the case of very long-range potentials (see, e.g., [62]). Here we do not pause at the subtle and interesting problems related to the phase transitions in one-dimensional systems. However, we want to stress that the recent experimental evidence of coexisting lamellar phases with different spacing [59–61] provides new systems within which to study the physics of pseudo-1D fluids.

5.2. Interactions in bistable densely packed micelles

The above model of interacting lamellae can be extended to different geometrical arrangements—for instance, micelles packed into a regular lattice or lamella–micelle coexistence phases. These more complex geometries introduce mathematical difficulties in the calculation of the intermolecular forces (e.g., through an expansion of the potential in spherical harmonics) but do not appreciably modify the physics of the phenomenon.

Under the assumption of constant structure of the micelle surface, simple mean-field calculations predict that the presence of strong repulsive interactions for concentrated ordered arrays of micelles causes a decrease of the interfacial area and then an increase of the average aggregation number (see (5)). Recent x-ray scattering data have been obtained for GM1 micelles in concentrated solutions, where they assume an ordered crystalline-like structure referred to as a $Pm3n$ cubic phase [61]. Following the picture given by Fontell *et*

al [63], the $Pm3n$ conventional unit cell, the length parameter of which is experimentally determined, contains a number of disconnected globules to a total amphiphile volume equivalent to eight non-spherical micelles. The calculated average aggregation number of GM1 micelles in the $Pm3n$ cubic phase turns out to be about 200 [61], which is lower than the value of 300 which is measured at low concentration where intermicellar interactions can be neglected. This behaviour is just the opposite to what is predicted by the mean-field calculations in the case of strong repulsive interactions among micelles. This discrepancy can be reconciled only by guessing a bistable conformational population of the headgroups, where one conformation (e.g., the extended one) is more sensitive to the external pressure induced by the surrounding micelles than the other (e.g., the bent conformation).

6. Elastic properties of non-ideally mixed bilayers

Several areas of direct [64] and indirect [16, 42, 65, 66] evidence suggest that the elastic constants of glycolipid bilayers are much smaller than the elastic constants of the membranes made up of the usual membrane-forming amphiphiles, such as phospholipids. It is surprising that bilayers in which the hydrophilic part is as extended as the hydrophobic part exhibit such a low rigidity. In fact the classical theory of elasticity predicts a dramatic increase of the bending rigidity on increasing the equilibrium thickness h_0 (roughly as h_0^3 [67, 68]) of the bilayer. Furthermore, recent theoretical calculations for membranes decorated by polymer 'brushes' predict that the membrane gets stiffer and stiffer when either the polymer length and/or its surface density σ are increased [5, 69]. However, if the calculations are performed at *constant chemical potential* (that is, by allowing a redistribution of the surface density of brushes both in the inner and the outer surface of the membrane), the result is just the opposite: the membrane becomes softer, developing an asymmetrical polymer distribution between the two membrane leaflets. This could be the case for ganglioside bilayers. Indeed, as discussed in section 2.3 and further confirmed by the indirect evidence reported in sections 4 and 5, gangliosides undergo a conformational transition between two (or more) low-lying molecular conformations with different surface areas. Hence, even in the case of pure ganglioside bilayers ($\sigma = 1$), as a consequence of bending, the system readjusts its local conformational composition in order to minimize the internal stresses. This effect can be easily modelled following the ideas developed in section 3.1 for the coupling between local composition and deformation of a lamella, leading, as expected, to a softening of the bending energy for bilayers built up from amphiphiles carrying bulky headgroups. Several unusual features of gangliosides—for instance, the spontaneous vesiculation of pure GM3 in water [16, 42]—may thus be tentatively explained in terms of the low bending rigidity connected with the conformational changes in the headgroup region.

Recently we have undertaken a dynamical analysis of the above problem by adding an inertial term (related to the bilayer density) and a relaxation time for the local population of conformers to a model similar to the one developed in section 3.1. This picture describes the bilayer as two back-to-back elastically coupled and deformable monolayers [65], and allows one to calculate both the bending and squeezing modes of the bilayer. The model is aimed at explaining the unusual thermotropic behaviour of GM3 ganglioside bilayers. Combined x-ray and neutron scattering experiments, in fact, reveal typical features of such bilayers, which can be interpreted as a decrease of thickness fluctuations with temperature [66], which is somewhat unexpected.

The theory takes into account two opposite contributions: on one side, the usual increment of the mean amplitude of the vibration with temperature ($\langle (h - h_0)^2 \rangle \propto T$), and on the other side, the levelling effect of temperature on the headgroup conformational

population, which leads to a dampening of the vibration amplitudes. Since deformation–conformational change coupling is responsible for the bilayer softening, the thermotropic thickness variation of the amphiphile lamellae deviates considerably from the ideal one at low temperatures, while at high temperatures the disappearance of the coupling (which is strongly temperature dependent) restores the ideal behaviour for $\langle(h - h_0)^2\rangle$.

Although the above results deserve further theoretical and experimental work, once again gangliosides have shown peculiar properties when compared with other more commonly investigated amphiphiles.

7. Conclusions

Mixing of amphiphilic molecules has been shown to be an interesting physical problem. The typical landscape of like–like and like–unlike interactions in the mixing process is enriched by the fact that amphiphilic molecules by themselves generate confined structures, the topology of which can be strongly dependent on such interactions.

The discussion of the pseudoternary system constituted by a water solution of a single amphiphile whose molecules can assume two different conformations is rather new and stimulating. The peculiarity of such a system is the fact that the mutual interchange of conformations is possible. This can give rise to interesting cooperative effects in the confined structure of the aggregate, such as the observed bistability and intermediate hysteresis in micellar solutions of gangliosides.

Acknowledgments

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