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Formation and stability of micelles and vesicles

Jan BFN Engberts* and Jan Kevelam†

Recent studies on the self-assembly of novel catanionic, bolaform and gemini surfactants provide evidence that the Israelachvili packing parameter approach can often be successfully used to predict the morphology of surfactant aggregates on the basis of the geometrical properties of the surfactant molecules. Furthermore, combined theoretical and experimental efforts have provided a consistent picture of the requirements for spontaneous vesicle formation which, in addition to a favorable packing parameter of the individual surfactant molecules, calls for a nonideal mixing of the bilayer components in order to provide the bilayer with a nonzero spontaneous curvature.

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Abbreviations

CTAB	cetyltrimethylammonium bromide
DOAB	dioctadecyldimethylammonium bromide
DPPC	dipalmitoylphosphatidylcholine
EO	ethylene oxide
MD	mean aggregate diameter
OG	octyl glucoside
QELS	quasi-elastic light scattering
SDS	sodium dodecylsulfate
SLS	static light scattering
TEM	transmission electron microscopy

Introduction

Vesicles are closed bilayer structures that enclose an aqueous pool and are surrounded by an aqueous solution [1•]. Vesicles are not only interesting because they mimic biological membranes, they have recently also been used in applications such as drug delivery, nanotechnology [2] and two-dimensional-crystallization of proteins. On the other hand, micelle-forming surfactants are widely used in enhanced oil recovery, rinse-off preparations and as detergents. Aqueous solutions containing entangled rod-like micelles often exhibit interesting rheological (visco-elastic) properties [3].

Both micelles and vesicles have amphiphilic molecules as building blocks, and the issue is to tune the structures of these building blocks so that aggregates of the desired morphology and properties are obtained. Another important factor is the stability of the aggregates that are formed. In the case of micelles, the aim is to predict and tune the CMC, whereas for vesicles the phase transition temperature of the bilayer is of particular

importance. In this review, we will show that the interplay of theory and biochemical, physical, and (physical) organic experiments can afford a pleasingly consistent picture of the factors determining the stabilities and morphologies of the aggregates (spontaneously) formed from amphiphilic molecules in aqueous solutions.

Morphology of surfactant aggregates: the packing parameter

In a first approximation, the ability of amphiphiles to form aggregates of a well defined morphology depends on the molecular architecture of the surfactant molecule. The packing properties of surfactants depend on the balance between the optimal cross-sectional surface area of the headgroups a_0 , the volume v of the hydrocarbon chains, and the maximum length l_c that the chains can attain. The dimensionless packing parameter $P = v/a_0l_c$ characterizes the ability of the surfactants to form spherical micelles (cone-shaped surfactant molecules, $P < 1/3$), rod-like micelles (truncated cone, $1/3 < P < 1/2$) or bilayers ($1/2 < P < 1$) [4]. Although this is not a novel approach, there are many recent examples of a wide variety of surfactant molecules that obey these geometrical rules. For instance, $C_{12}EO_3$ forms vesicles, whereas $C_{12}EO_7$ having a larger head group, forms rod-like micelles [5•]. Even polystyrene (PS)-*b*-poly(acrylic acid) (PAA) diblock copolymers form vesicles (PS-*b*-PAA=400-*b*-16) or rod-like micelles (200-*b*-15) depending on the hydrophobic chain length (i.e. PS content) at constant head group area (i.e. PAA content) [6]. Shinkai and coworkers [7•] synthesized some remarkable tetracationic amphiphiles bearing a calix[4]arene core. Their working hypothesis was that, inferring from the monomer shape, the cone isomer favorably forms micelles, whereas the 1,3-alternate isomer preferably aggregates as two-dimensional lamellae, see Figure 1. This is borne out in practice. The bilayer thickness of the lamella, however, is about twice the predicted value [7]. Therefore, it seems that the proposed bolaform (membrane-spanning) structure is incorrect. In our opinion, one could envisage a model in which the cylindrical 1,3-alternate is turned 90 degrees in-plane so that it can form a monolayer characterized by a rectangular headgroup and four hydrophobic tails per headgroup. The monolayers can be stuck together at the hydrophobic interface to form a bilayer having twice the thickness of the bolaform bilayer originally proposed.

Double-tailed surfactants can form bilayers even if the tails have different lengths, as in the case of sodium sulfopropyl alkyl maleates. In that case, interdigitated bilayers are formed [8], where the combined effects of intertwined surfactants at opposite sides of the bilayer produce an average packing parameter of between 0.5 and 1 [9] (Fig. 2).

Figure 1

Expected aggregation modes of (a) cone-shaped cone-1 and (b) cylindrical 1,3-alternate-1 (or 2n). The structural formulas of 1 and 2n and their geometric isomers are presented at the top left hand and bottom left hand corners of the figure, respectively. Published with permission from [7•].

Figure 2

Idealized unit of two interdigitated molecules of sodium sulfopropyl octadecyl maleate. Reproduced with permission from [9].

The influence of the counterion on aggregate morphology has been addressed by Sein and Engberts [10•]. The borderline between aggregation in micelles or in lamellar aggregates is sometimes thin, as shown by the hesitant amphiphile sodium dodecylbenzenesulfonate—this surfactant does not have a strong tendency to form aggregates of a single morphology, but it does form either micelles or vesicles depending on subtle factors such as salinity or the presence of additional counterions. In pure water, this surfactant forms micelles. The addition of salt, however, induces the formation of lamellae by increasing the counterion binding which lowers the headgroup hydration and therefore the cross-sectional headgroup area. Moreover, the capacity of larger cations such as cesium and tetramethylammonium to induce this transition is stronger than that of the smaller lithium and sodium counterions. The reason simply involves the increased

counterion binding of the larger cations which are less strongly hydrated than their smaller counterparts.

Surfactant aggregation and hydrophobic interactions

The driving force for surfactant aggregation in water is generally assumed to be hydrophobic interactions. Indeed, there are numerous examples showing that the CMC decreases upon increasing the alkyl chain length. By studying the introduction of mesogenic units into ammonium amphiphiles and the effect on the aggregation behavior in water, Sudhölter and coworkers [11•] concluded that, in the case of azobenzene mesogens incorporated in the tail, favorable enthalpic interactions through pi-stacking dominate the surfactant aggregation. This observation might suggest that the hydrophobic effect may not always be a prerequisite for surfactant aggregation. Indeed, it is well known that aggregation of amphiphiles is not limited to (pure) water as a solvent. Micelle formation can occur in a wide variety of polar organic solvents of high cohesive energy density, such as formamide and alkane diols, although the aggregate stability is generally lower than that in water [12]. The issue of the stability of lamellar phases in water/alkane diol mixtures has recently been addressed by Martino and Kaler [13]. Lamellar phases of nonionic ($C_{12}E_5$) surfactants disappear as water is gradually replaced by propylene glycol. As the d-spacing and area per headgroup are unaffected by the diol, a first hypothesis is that the intrabilayer properties remain the same. Finally, however,

it is argued that added propylene glycol lowers the Van der Waals attractive forces between the bilayers, so that the ordered lamellar phase is transformed into a disordered fluid [13].

Propylene glycol also lowers the stability of lamellae built up from ionic amphiphiles [13]. In this case, however, the bilayer spacing is reduced. This is explained in terms of a lowering of interbilayer electrostatic forces due to a reduction of the mixed solvent dielectric constant, which leads to a decreased surface charge. Also, the headgroup area is decreased upon adding propylene glycol. The authors explain this in terms of a break-up of hydration structure around the headgroup. This would be consistent with the observed concomitant decreased curvature in vesicular bilayers [13]. The lowering of the stability of the lamellar phase is tentatively explained by the alleged analogy of the action of propylene glycol on the hydration structure with that of temperature. We have difficulty with this explanation because in our opinion the effect of temperature is mainly to disturb the packing of the hydrophobic chains in the bilayer. Moreover, the effect of temperature on headgroup hydration is strongly dependent on composition and is not easy to predict, as has been shown by water self-diffusion NMR experiments [14].

Finally, high concentrations of urea are found to hamper surfactant aggregation. There is quite strong evidence that added (0.5 M) urea decreases the counterion selectivity in surfactant aggregates. For instance, in pure water the counterion binding of bromide to micelles composed of cetyltrimethylammonium surfactants is larger than that of chloride, as chloride ions are more strongly hydrated. Upon adding urea, however, the counterion binding (β) decreases to a lower value which is similar for both bromide and chloride. This is interpreted in terms of preferential head group solvation by urea, and the squeezing out of nearby counterions [15]. Similar effects were observed for dioctadecyldimethylammonium monolayers. The minimum headgroup area in the monolayer is directly related to the degree of counterion binding, and by measuring a_0 as a function of β in the presence

and absence of urea, it was found that the counterion specificity is strongly diminished upon addition of urea [16]. We consider the proposed extrapolation of these observations to the protein denaturing effect of urea as highly tentative.

Gemini surfactants

Gemini surfactants are dimeric surfactants which can be considered as amphiphiles that are linked at the level of the headgroup by a polymethylene chain (see Fig. 3).

Figure 3

General chemical formula of a dimeric surfactant. $s=2-20$; $m=8,10,12,14,16$. Published with permission from [18••].

The idea underlying the use of dimeric surfactants is the following. In conventional surfactants, the headgroups are randomly distributed on the surface separating the aqueous phase and the micelle hydrophobic core, with a distribution between headgroups that peaks at the thermodynamic equilibrium distance d_T (see Fig. 4).

On the basis of the reported values of the surface area per head group, d_T is about 0.7-0.9 nm. When using dimeric amphiphiles, the distribution of distances may become bimodal. If the spacer is short enough ($s < 6$), then it is probably fully stretched at the water/core interface [17]. The headgroup distance distribution function then exhibits a narrow maximum at the distance d_s , corresponding to the extended length of the spacer, and another maximum at the thermodynamic equilibrium

Figure 4

Schematic representation of the distribution of distances, $[P(d)]$, between headgroups in micelles of conventional (a) and dimeric (b) surfactants. d denotes distance between the headgroups in micelles, d_T denotes the thermodynamic equilibrium distance and d_s denotes the extended length of the spacer. Reproduced with permission from [18••].

distance d_T . This modification of the distribution of headgroup distances, as well as the effect of the headgroup spacer on the packing in the micelle core, is expected to affect strongly the curvature of surfactant layers, and thus micellar shape [18••]. Light scattering and cryo-TEM revealed the following features [18••]. For intermediate spacer lengths ($3 < s < 10$) and at constant tail length, the geminis form spherical micelles in aqueous solution, having aggregation numbers that are almost the same as those of micelles formed by the corresponding single-tailed ammonium amphiphile, viz 40-50 dodecyl chains per micelle. For short spacer lengths ($s = 2$) peculiar behavior was observed; micellar growth was strongly promoted by increasing surfactant concentration, and long, thread-like micelles were observed at 1.3% (w/w) surfactant. Apparently, the short spacer promotes the formation of aggregates of smaller curvature due to a reduction of the headgroup area. Finally, if s is increased to 16 or 20, the spacer itself is expected to become part of the hydrophobic section of the amphiphile. For example, the spacer might become membrane-spanning, so that the amphiphile becomes bolaform. Indeed, vesicles and lamellar structures are formed by the 12-16-12 or 12-20-12 surfactants in aqueous solutions. We contend, however, that the observation of double lamellar structures does not agree with a bilayer composed of a single membrane-spanning surfactant.

For more examples of (mainly) diammonium gemini surfactants, the reader is referred to the original literature [19-25]. Also, new types of bolaform surfactants have recently been synthesized that are based on glycosyl ethers [26], or carry bipolar phosphate headgroups [27], or have perfluoroalkylated carbon chains [28], or are based on dimeric glycerophospholipids [29].

Vesicle solubilization by surfactants

The understanding of the phase behavior of phospholipid-surfactant systems in excess water is important for the elucidation and the control of biological membrane component reconstitution [30], as well as the optimization of vesicular drug delivery [31]. In addition, features of the transition of lipid bilayers into micelles upon addition of a micelle-forming surfactant, which has been termed solubilization, provide another critical test for the packing parameter approach.

Dilute lipid-surfactant mixtures can be treated as pseudo-binary systems, and the liquid crystalline lamellar→micellar phase transition has been successfully described by the Lichtenberg model [32]. Accordingly, surfactant molecules are incorporated into lipid membranes up to a critical effective surfactant/lipid ratio producing saturation ($R_{e,SAT}$). Then, lipid-saturated micelles with composition $R_{e,SOL}$ start to be formed in coexistence with surfactant-saturated vesicle bilayers of composition $R_{e,SAT}$, with varying proportions. Increasing the total surfactant content

to values above $R_{e,SAT}$ destroys all bilayers, and only micelles remain.

$R_{e,EVENT}$ can be obtained by plotting the total surfactant concentration needed ($S_{T,EVENT}$) for a certain event to occur against the total lipid concentration (PL) [33]:

$$S_{T,EVENT} = S_w + R_{e,EVENT} \cdot PL \quad (1)$$

where S_w is the concentration of surfactant in the aqueous phase (i.e. unbound surfactant).

An important question arises as to the methodology needed to determine $S_{T,EVENT}$. The original approach, which is still popular [34•-36•], is to define $R_{e,SAT}$ and $R_{e,SOL}$ as the surfactant/lipid ratios where light scattering starts to decrease from the original value, and where light scattering shows no further decrease, respectively. We contend that there is some danger if one relies upon these experiments alone, as the transition points are hardly detectable with sufficient accuracy. Moreover, the method assumes that the solubilization process follows the simple lines described above, which would not necessarily be the case, *vide infra* [36•]. Therefore, it appears recommendable to determine R_e values by at least two independent methods. This has been made possible by Heerklotz *et al.* [37••] who characterized dilute lipid-surfactant mixtures of POPC and $C_{12}EO_8$ by means of isothermal titration calorimetry. In a thermodynamic analysis, the authors were able to construct a consistent set of transfer enthalpies and entropies for the system of monomers, micelles and bilayers, which is shown in Table 1.

Firstly, the substantial ($10-15 \text{ kJ mol}^{-1}$) increase of the enthalpy for the transfer of surfactant from micelles into bilayers might be explained in terms of headgroup hydration and conformation, because the different chain conformations in micelles and bilayers have a minor contribution to the total enthalpy [38]. In fact, a dehydration of the ethylene oxide headgroup upon transfer from the micelles into the bilayers should be involved, in agreement with the geometrical argument that the space available for a surfactant headgroup is significantly reduced within the lamellar structure. Dehydration results in a volume decrease of the headgroup, reducing the packing parameter of the truncated, cone-shaped nonionic. The dehydration effect is also the origin of the large enthalpic nonideality parameter ($+10 \text{ kJ mol}^{-1}$). The entropic gain upon insertion of $C_{12}EO_8$ into the bilayer could be tentatively accounted for by a possible concomitant fluidization of the bilayer [39,40]. Moreover, the liberation of hydration water is expected to be even more important.

In contrast, the transfer of a lipid molecule from a bilayer into a micelle does not change its enthalpy. This appears to indicate that the headgroup of the lipid is almost fully hydrated even in the bilayer, and

Table 1

Enthalpy and standard chemical potential differences h and μ^0 , respectively, for the transfer of detergent and lipid between various phases of the ternary system water/POPC/C₁₂EO₈ at 25°C*.

Experiment being measured	Δh		$\Delta\mu^0$		$-T\Delta s$	
	Det	Lipid	Det	Lipid	Det	Lipid
Bilayer-water (partition)	31.5 ± 1 [†]		-30.4 ± 0.2 [†]		-62 ± 2 [†]	
Micelle-water (demicellization)	16 ± 1 [‡]		-33.1 ± 0.3 [‡]		-49 ± 2 [‡]	
Bilayer-micelle transition	15.5 ± 2.5 [†]		+2.7 ± 0.5 [†]			
Bilayer-micelle (solubilization)	15 ± 1 [§] , 10.0 ± 0.5 ^{sat}	-5 ± 1 [#] , -2.5 ± 0.5 ^{sol}	+1.5 ± 0.5 ^{sat/sol}	-1.5 ± 0.3 ^{sat/sol}	≈ -8	≈ +1

*All data are presented in kJ mol⁻¹. The phase compositions that the values refer to are given as superscripts. †=mixed bilayer; ‡=pure detergent micelles; §=pure lipid bilayer; sat=detergent-saturated bilayer; #=mixed micelles; sol=lipid-saturated micelles. The first row corresponds to the partition experiment, the second to the demicellization experiment, the third gives the estimation for the bilayer→micelle transfer values using the data of the demicellization and partitioning experiment. This is for comparison with the values determined directly by the solubilization experiment (fourth row). Published with permission from [37•].

more headgroup space in the micelle does not affect its hydration. Equally interesting is the observation that the mixing nonideality parameter for lipids in micelles (+5.5 kJ mol⁻¹) is much less than that for surfactants in lipids (+10 kJ mol⁻¹). The rationale is as follows: for micelles, a nonrandom arrangement of the molecules should be considered, namely, the cone-shaped surfactant molecules are assumed to be located preferentially at the highly curved edges of the mixed micelles, whereas the lipid prefers the less curved regions. This should afford a disk- or rod-like morphology, which is consistent with cryo-TEM evidence [34•].

It is clear that cryo-TEM is an effective tool in solubilization studies, in particular when light scattering data are hard to interpret. This is clearly the case for the solubilization of sonicated mixed diglycerol hexadecyl ether-cholesterol (1 : 1 wt) nonionic surfactant vesicles (NSV) by added octyl glycoside (OG) surfactant [36•]. Figure 5 shows the changes of the optical density (OD) at 350 nm, and the mean aggregate diameter (MD) as determined by quasi-elastic light scattering (QELS), as a function of total OG concentration.

The size of the initial NSV, at 0 mM OG, appears to be small, and the vesicular shapes range from spherical to elongated. It has been previously shown [41] that the NSV membrane is mainly composed of major ordered domains with tightly associated diglycerol hexadecyl ether and cholesterol regions. This might be responsible for the irregularity of the bilayer curvature and consequently the irregular vesicular shape. At point E of the curve in Figure 5 ([OG] = 10 mM), cryo-TEM reveals the presence of long, tubular structures. The authors suggest that the aggregate growth might be attributed to vesicle fusion. Indeed, it has been previously shown that, due to its edge-activity, the addition of surfactant might induce the transient formation of disk-like micelles, which could combine to form fused vesicles [42]. The possibility also exists, however, that vesicle growth is induced by increasing amounts of surfactant in the bilayers due to the incorporation of OG. At 15 mM OG, close to point D, the vesicles are spherical and their size has further increased. The passage to large spherical structures indicates the homogenization of the bilayer curvature. This is likely to be due to a lowering of the packing

Figure 5

Variations of OD at 350 nm (closed circles, curve I) and QELS MD (closed squares, curve II) from the overall NSV-OG mixtures. Also, QELS MD (open squares, curve III) from the upper phase alone, as a function of total OG concentration. The total lipid concentration is constant and equal to 2.4 mM. Reproduced with permission from [36•].

constraints due to the surfactant enrichment of the bilayer. Between D and C, the system exhibits a macroscopic phase separation characterized by a turbid upper phase and a fleecy precipitate. Between D and A, the precipitate is only sparse: the upper phase contains spherical vesicles whose diameters are increasingly lowered. At point A, clusters of intact vesicles are observed which appear as honeycomb structures: the vesicle shape appears to be hexagonal. From A to B, the concentration of vesicles is reduced with increasing OG concentration, and the amount of the precipitate increases. At B, maximum turbidity is observed. The authors make no attempt to explain these phenomena. To us it seems likely that the aggregation process is due to depletion flocculation. From 20 mM OG, micelles are observed in coexistence with vesicles. Increasing the OG concentration results in an increase in the number of micelles (at the expense of the vesicles), implying increased crowding of (small) micelles in the volumina left by the (bigger) vesicles, causing the vesicles to pack together. Indeed, the honeycomb shape strongly suggests the presence of osmotic forces, as we have observed recently [43]. From C onwards (Fig. 5),

only micelles are observed. In conclusion, cryo-TEM is a prerequisite in order to establish the limit of the vesicular domain at D ($R_{e,SAT}$) and of the micellar domain at C ($R_{e,SOL}$).

These results shed some doubt upon the work by De la Maza and Parra [35•]. These authors only employed light scattering to determine $R_{e,SAT}$ and $R_{e,SOL}$ in their study of alterations of phosphatidylcholine bilayers by SDS-Triton X-100 mixtures. The experimental data clearly show that the light scattering intensity as a function of added surfactant concentration goes through a maximum before the 100% value is reached at the alleged (Lichtenberg) value of $R_{e,SAT}$. In analogy with [36•], however, $R_{e,SAT}$ might well be located at the maximum of the curve. Moreover, the authors tried to obtain a $R_{e,CF}$ at subsaturating surfactant/lipid ratios. This value refers to the effective surfactant/lipid ratio where vesicles become leaky due to the incorporation of surfactant, as monitored by the release of encapsulated carboxyfluorescein (CF). One should bear in mind, however, that the concept of $R_{e,CF}$ is only meaningful if one understands why the vesicles become leaky. Is it because the bilayers become holey as suggested in [34•] or because fusion [36•] occurs via disk-like micelles [34•], whereby the contents of the vesicles are released? Nevertheless, this work [35•] contains some very interesting aspects. The authors not only studied static light scattering (SLS) intensities as a function of total surfactant (and lipid) concentration, but they also varied the mole fraction of SDS. This allows a study of the relative efficiency of SDS and Triton X-100 in making the vesicles leaky, and in saturating and solubilizing the membrane. Thus, SDS is more effective at the sublytic level, where it induces vesicle leakage, whereas the nonionic surfactant is more effective at higher surfactant/lipid ratios, inducing saturation and solubilization of the bilayer. The influence of coulombic repulsions between bound SDS molecules, in particular at higher surfactant concentrations, may well account for their lower capacity to saturate and solubilize the bilayers relative to the nonionic surfactant. We postulate that the high efficiency of SDS (relative to Triton X-100) to induce vesicle leakage might be explained either by its higher edge-activity (in the fusion pathway) or due to its tendency to create holes (as in lecithin vesicles [34•]). Finally, the authors provide conclusive support for the general assumption [32] that the concentration of free surfactant must reach the CMC for solubilization to occur: $S_{w,SAT}$ and $S_{w,SOL}$ are always comparable to the CMCs of the surfactant mixtures in the absence of lipid.

Edwards and coworkers [34•] employed SLS and turbidity measurements in combination with cryo-TEM to study lecithin vesicle solubilization by n-alkyl sulfate surfactants (n-alkyl = C₁₀, C₁₂, C₁₄). A remarkable feature in the turbidity versus [surfactant] plot is the initial minimum at low amounts of incorporated surfactant. This can be attributed to disintegration of small vesicle clusters due

to electrostatic repulsion; the real vesicle size does not necessarily change.

Interestingly, the maximum number of surfactant molecules per lipid increases from 1.3 to 2.0 as decylsulfate is replaced by dodecylsulfate. This can be understood in terms of the packing parameter for two-component aggregates [44]:

$$(v/a \cdot l)_{eff} = (V_{lip} \cdot X_{lip} + V_{surf} \cdot X_{surf}) / (a_{lip} \cdot X_{lip} + a_{surf} \cdot X_{surf}) \cdot l_{lip} \quad (2)$$

where X_{lip} and X_{surf} are the mole fractions of lipid and monomer within the aggregate. Equation 2 thus indicates that the formation of cylindrical micelles will take place at a lower surfactant/lipid ratio when the surfactant hydrophobic volume is decreased. The addition of C₁₀SO₄⁻, however, leads to a direct transformation of the bilayers into thread-like micelles, whereas addition of C₁₂SO₄⁻ and C₁₄SO₄⁻ induces the formation of a holey lamellar phase prior to the formation of mixed micelles. Furthermore, addition of C₁₂SO₄⁻ and C₁₄SO₄⁻ results in vesicle growth, but only in the presence of 150 mM NaCl. The idea is that vesicle fusion might occur through open vesicle structures which combine only if there are no substantial electrostatic barriers.

Thermal stability of vesicle membranes

Membranes consist of amphiphilic molecules which, at low temperatures, are highly ordered into a lamellar phase (L_β); the hydrocarbons are stiff and lie parallel to each other. By increasing the temperature to T_m , a discontinuous main phase transition from the gel state to a less ordered, liquid crystalline lamellar phase (L_α) is observed. The phase transition temperature T_m is experimentally accessible by several techniques, including the dependence of the differential heat capacity δC_p on temperature. At the melting temperature, a peak is observed in the differential scanning calorimetry (DSC) enthalpogram. In general, the shape of the dependence of δC_p on temperature does not obey an ideal bell shape, and the notion of groups (patches) of monomers undergoing the transition cooperatively was invoked to fit the recorded scan to the Van't Hoff equation [45]. The details of the features of the phase transition are difficult to determine experimentally, but this issue has received recent theoretical interest. For instance, the patch concept has been confirmed by Peters, who studied the melting of a hexane bilayer by molecular dynamics simulations [46].

Blandamer *et al.* [47] have employed differential scanning microcalorimetry to monitor the influence of micelle-forming surfactant on the phase transition temperature of vesicle bilayers at surfactant/lipid ratios below $R_{e,SAT}$. Two features are clear-cut. First, addition of CTAB to dioctadecyldimethylammonium bromide (DOAB) bilayers removes the pretransition, indicating a marked decrease

in vesicle-vesicle interactions. It has been mentioned above that vesicle aggregation is suppressed when small amounts of ionic surfactants are added. Second, T_m is decreased upon addition of CTAB. Not only headgroup packing frustrations but in particular the mismatches in hydrophobic chain lengths are held responsible for this effect. Indeed, Kacperska [40] provided additional evidence for hydrophobic mismatch by showing that the T_m of DOAB vesicles is lowered more (1-7 K) by the addition of dodecyltrimethylammonium bromide than by the addition of octadecyltrimethylammonium bromide (0.5-3 K).

There is a small but important detail to be noticed in the two previous studies, that is, the addition of surfactant to the preformed vesicles having a T_m above room temperature yields systems that are not at equilibrium: subsequent scans, which are equal one after another, clearly differ from the first scan. Apparently, the system after the first scan is a metastable state, which suggests that surfactant penetration only occurs rapidly when the bilayers are in the liquid crystalline state. This is an important issue in solubilization experiments, which should be performed above the main phase transition temperature of the bilayer.

Finally, we note that the addition of SDS to DOAB vesicles results in an increase of T_m by approximately 20 K, but the eventual melting process is much less clearly defined [47]. The authors presume that the incorporation of the dodecyl chains into the vesicle, containing octadecyl chains, leads to some disruption of the chain packing, but the negatively charged headgroups of SDS diminish the electrostatic repulsion between the ammonium headgroups. This will raise the stability of the gel state.

Another approach has been adopted by Lissi and coworkers [39], who determined the amount of alkanol needed to lower the temperature of the main phase transition of DPPC large unilamellar vesicles (LUVs) to the main temperature of the experiment, as monitored by the fluorescence anisotropy of a fluorescent probe (diphenyl-hexatriene). We feel that the argument presented above sheds some doubt on this method, because the alkanol has been added to the vesicle dispersion at $T < T_m$. Although the problems might be less severe because the long chain alkanols are only sparsely soluble in water and might be forced to bind into the vesicle bilayer, this should be checked by DSC. The results, however, are consistent with those obtained by Kacperska [40] in the sense that the effect of a hydrophobic mismatch was again demonstrated: for example, one needs less 1-hexanol than 1-decanol to disturb the DPPC bilayer. Furthermore, the authors suggest that solute topology is important. By geometric considerations, one would expect the bilayer packing to be more severely disturbed by a branched

alcohol with the same number of carbon atoms as its linear counterpart; this is borne out in practice.

Spontaneous vesicle formation

A bilayer is composed of two monolayers (bilayer leaflets), stuck together via their hydrophobic interfaces. Each monolayer is characterized by an equilibrium spontaneous curvature which, in general, is not zero. We note, however, that a bilayer composed of identical monolayers is frustrated because the monolayer curvatures are of equal magnitude, and are of opposite sign. In terms of symmetry, the bilayer is least frustrated, that is, has the lowest Gibbs energy, when its spontaneous curvature is zero! This explains why, generally, vesicle formation needs the input of mechanical energy.

If the compositions of the two monolayers are allowed to differ, however, the spontaneous bilayer geometry will possibly be nonplanar, thus favoring the formation of finite size vesicles. Indeed, spontaneous vesicle formation has been observed in dilute aqueous mixtures of cationic and anionic surfactants (*vide infra* and [48]), and also mixtures of ionic surfactants and alcohol cosurfactants with brine [49]. This enthalpic stabilization mechanism was originally proposed by Safran *et al.* [50] who showed theoretically that the Gibbs energy of a two-component bilayer may sometimes be minimized in a state of nonzero curvature due to the spontaneous formation of different chemical compositions in the outer and inner leaflets. Recently, Porte and Ligoure [51] have emphasized the interplay between bilayer curvature and the composition difference between both monolayers. The importance of nonideal mixing was independently demonstrated by May and Ben-Shaul [52], who showed that addition of short chain amphiphiles to a bilayer composed of long chain lipids does reduce its bending rigidity, but, in the case of random (ideal) mixing, this lowering is not sufficient to enable spontaneous vesicle formation.

The matter becomes even more complicated when electrostatics come into play, for example, in mixtures of ionic surfactants and alcohol cosurfactants with added salt. It is well established that pure SDS forms (almost) spherical micelles in aqueous solutions, and by addition of salt, structures with less-curved surfaces are formed. One could imagine the possibility that such a large amount of salt could be added to favor the formation of planar bilayers. Bergström and Eriksson [53], however, calculated that bending of this bilayer into a closed vesicle would be disfavored because electrostatic forces render the bending parameter too large. Only the addition of a long chain alcohol to the SDS-brine mixture brings about the necessary reduction of the bending energy, thereby facilitating the formation of mixed vesicles.

An alternative approach has been followed by Morse and Milner [54•] who suggest the possibility of entropic

stabilization. The concept is that the internal Gibbs energy of the bilayer is lowest in a flat conformation, but the assumption is that vesicles can nevertheless be formed at sufficiently low surfactant concentrations due to the combined effects of translational entropy and renormalization of the bending elasticity.

The two mechanisms can be discerned through the dependence of the vesicle size distribution on the total surfactant concentration. For entropically stabilized vesicles, the size should be small enough to maximize the translational entropy. On the other hand, a reduction of the vesicle size increases its bending rigidity so that the number of undulation wavelengths decreases (in a sense, the entropy associated with vesicle size fluctuations is diminished). The result is that the average vesicle radius should increase with increasing total surfactant concentration. In the case of enthalpically stabilized vesicles, the average radius should depend only on the relative concentration of both types of surfactant, not on the total surfactant concentration. In practice, the latter is observed both for catanionic surfactants [48] and for vesicles composed of SDS/octanol/brine [49]. These results support the Safran model (enthalpic stabilization).

Finally, bilayers sometimes not only spontaneously close to form vesicles, but also even in dilute amphiphile solutions where the system could easily form vesicles, a certain number of disk-like aggregates can be detected. Raudino [55] explains this by including the nonharmonic term in the bending elasticity, which leads to a (temperature-dependent) shrinkage of the bilayer edge. The reduction of unfavorable edge energy leading to the formation of finite size disks is particularly pronounced for very flexible bilayers bearing bulky hydrophilic head groups. Indeed, clustering of surfactants with large heads or short tails should stabilize the curved region of the rim [56].

Catanionic surfactants

Upon mixing micelle-forming (single-tailed) anionic and cationic surfactants, the spontaneous formation of closed bilayers is often observed. Catanionic surfactant mixtures are therefore of immediate theoretical importance. Firstly, the mean ionic head size in an organized catanionic assembly is greatly reduced as compared to that of its pure individual constituents because of the strong coulombic interaction between the oppositely charged surfactant ions and the removal of hydration water as charges become neutralized. Thus, the packing parameter of a catanionic surfactant is higher than that of the individual surfactant ions, enabling the formation of lamellar phases. Secondly, as has been outlined above, it is likely that nonideal mixing of the surfactant in the bilayer occurs such that the bilayer spontaneous curvature becomes nonzero.

Huang and Zhao [57•] have studied mixtures of sodium alkylcarboxylate and alkyltrimethylammonium bromide at different pH, using light scattering and TEM. For

1 : 1 mixtures, the pH should be about 9 for optimum spontaneous vesicle formation. At lower pH, protonation of the carboxylate occurs, thereby the strong electrostatic headgroup attractions are lost, resulting in an increase of headgroup size and a destabilization of the lamellar phase through a reduction of the packing parameter. The lamellar phase is also destabilized at $\text{pH} \gg 9$. As the pK_a of a carboxylic acid is approximately five [58], at pH 9, almost all of the anionic surfactant is present as the carboxylate. Therefore, the authors suggestion that the ratio [carboxylic acid] : [carboxylate] would be optimal at pH 9 and would be decreased to an unfavorable value at pH 13, seems unlikely. Instead, at pH 13, the concentration of sodium and hydroxide ions becomes so large that the surfactants are salted out, in accord with the formation of a precipitate at high pH.

At pH 9, spontaneous vesicle formation was observed (by EM) for 1 : 1 mixtures of $\text{C}_{11}\text{H}_{23}\text{COONa}$ and (octyl-dodecyl)trimethyl ammonium bromide. On the contrary, 1 : 1 mixtures of heptylcarboxylate with any alkyltrimethyl ammonium bromide did not yield vesicles at all, even after sonication. The authors did not make an attempt to explain this observation [57•]. We suggest that the rigidity of the vesicle bilayers composed of the short chain alkylcarboxylate is too low, thus, the lamellar phase is transformed into an isotropic phase. Indeed, Kaler and coworkers [59•] found that catanionic vesicles are more rigid if the fraction of long chain surfactant is relatively higher, whereas the opposite is true for high intralamellar concentrations of the short chain surfactant.

The effect of head group size was studied by replacing alkyltrimethylammonium bromide for alkyltripropylammonium bromide [57•]. In agreement with the packing parameter approach, vesicle formation is hampered by the larger headgroups. Here we need to refer to the study of Yaacob and Bose [60] on the influence of the counterion. The interesting observation was made that for a 64/36 cationic/anionic surfactant molar ratio, the tosylate counterion promotes the formation of larger vesicles as compared with the bromide counterion. It was suggested that tosylate counterions bind more strongly to the cationic headgroups than bromide, leading to more effective charge screening.

Finally, by monitoring the light scattering of the clear solutions that formed the bottom layer of centrifuged samples of mixed surfactants, Huang and Zhao [57•] concluded that rod-like micelles are present in coexistence with vesicles if the surfactant concentration is high enough (5-10 times the CMC) (compare to [61•]). In their study of the phase behavior of aqueous mixtures of CTAB and sodium octyl sulfate, Kaler and coworkers [59•] observed a similar phenomenon. These authors attributed the limit of the vesicle phase at higher surfactant concentrations by the close packing of the vesicles. The formation of micelles not only depends

upon the total surfactant concentration, but also on the relative surfactant concentrations in the catanionic mixture. Yaacob and Bose [60] observed (by cryo-TEM) the formation of tubular membranes in 3/7 sodium dodecylbenzenesulfonate/cetyltrimethylammonium tosylate mixtures. Indeed, features of catanionic surfactant mixtures characterized by ratios deviating strongly from 1 : 1 should be comparable to those features commonly observed in structures that are formed when vesicle bilayers are solubilized by micelle-forming surfactants.

The thermal stability of sodium 10-undecenoate/decyltrimethylammonium bromide catanionic vesicles has been studied by Zhao and Yu [61]. As anticipated, T_m is highest for the 1 : 1 surfactant mixture, where the packing is closest. We wish to remark that T_m of the 1 : 1 mixture is relatively high compared with that for the conventional double-chained surfactant bilayers, viz 70°C. For example, T_m for dioctadecyldimethylammonium bromide is about 45°C, despite its larger chain length and the fact that the alkyl chains are fully saturated. This emphasizes the truly efficient chain packing in catanionic surfactant bilayers.

We feel that it is very unfortunate that the question concerning enthalpy versus entropy-driven spontaneous formation of vesicles has not been addressed by a proper analysis of the vesicle size distribution as a function of surfactant concentration. These gaps should be filled in the near future. Yaacob and Bose [60], however, determined the vesicle size distribution of a 64/36 CTAB/HDBS mixture to be log-normal instead of the Gaussian distribution, as predicted in the original model by Safran *et al.* [50]. This observation in itself does not exclude the possibility of an enthalpy-driven process, as it could simply indicate that composition differences among vesicles themselves might have to be accounted for.

Conclusions

The synthesis of novel amphiphilic molecules continues to be an active field in chemical research, providing a variety of compounds for potential applications and for fundamental research. Moreover, it is again convincingly shown that the morphologies of the aggregates formed from surfactants can often be traced down to the geometrical properties of the constituent monomers. For example, by mixing cone-shaped (micelle-forming) surfactants with (bilayer-forming) surfactants of cylindrical shape, a variety of aggregate morphologies may be obtained. The thermal stability of vesicle bilayers is mainly dependent on the efficiency of chain packing. The phase transition temperature of the bilayer can be lowered by the addition of long chain alcohols or surfactants, and many effects can be understood in terms of the concept of the hydrophobic mismatch. Finally, the interplay of theory and (physical-organic) experiments might yield important novel insights into the origin of spontaneous vesicle formation.

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