Phase Behavior and Microstructure in Aqueous Mixtures of Cationic and Anionic Surfactants

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1. SYNOPSIS

Mixtures of anionic and cationic surfactants in water display interesting phase behavior and a range of microstructures, including small micelles, rod-like micelles, lamellar phases and vesicles. This chapter reviews the properties of these mixtures with a focus on the experimental and theoretical aspects of vesicle formation and stability.

I. INTRODUCTION

Electrostatic forces are typically the dominant interaction in colloidal systems, and application of surfactants in solution or at surfaces calls for early consideration of the desired sign of the surface charge. It is no surprise then that oppositely charged surfactants in solution mix in a highly non-ideal way, and in fact are able to form spontaneously structures (in particular, unilamellar vesicles) that are uncommon in other surfactant mixtures. The range of self-assembled microstructures in mixtures of cationic and anionic surfactants also includes small spherical micelles, cylindrical or worm-like micelles, and other bilayer lamellar or L3 phases. The state of equilibrium of all of these microstructures has not yet been fully established, but some vesicle phases have been observed to be stable for well over a decade. Although the folklore in surfactant formulations suggests that oppositely charged surfactants should never be mixed because of the potential for precipitation of the insoluble surfactant ion pair, precipitation is generally found only near equimolar compositions or in samples below their Krafft temperature, and even this precipitation can sometimes be blocked.

Of particular interest in this review are mixtures of oppositely charged surfactants from which the companion co-ion simple salt has not been removed. This is in contrast to true ‘catanionic’ surfactants, as first described by Jokela and Wennerstrom [1], in which solutions contain only water and the two oppositely-charged surfactant ions. These catanionic
surfactants are also called “ion-pair amphiphiles” (IPAs), and they are most easily formed by combining an organic acid and an organic hydroxide, so the common ancillary product is water rather than a salt. IPA properties have been reviewed recently by Tondre and Caillet [2]. A spectacular example of the assembly of IPAs is the observation by Zemb et al. [3] of self-assembled structures about 1 μm in dimension having a regular hollow icosahedral form. The icosahedral particles are stabilized by pores located at their vertices, and they dissolve when salt is added.

Historically the addition of oppositely charged moieties has been used most frequently to control the growth of rod-like or worm-like micelles. This is typically done by adding an anionic hydrotrope (such as salicylate or tosylate salt) to a solution of cationic surfactant [4]. The hydrotrope partitions into the surfactant headgroup region and results in dramatic micellar growth. Similarly, a cationic hydrotrope can drive the growth of anionic micelles [5]. As the molecular weight (or length) of the hydrophobic portion of the hydrotrope is increased, the hydrotrope begins to behave more like a second surfactant and can alter the self-assembly in solution, leading to the formation of vesicles. For example, when alkyl sulfates (CₙSO₄⁻-Na⁺) with n greater than six are added to alkyl ammonium halide surfactants, vesicles can form on both the cationic and anionic-rich side of the phase diagram [6]. Such vesicles form spontaneously and without the input of shear, and their structural and thermodynamic properties are discussed below. Both the static and dynamic properties of these mixtures have recently been reviewed [7].

Vesicles are, of course, not newly discovered. Nearly forty years ago, Bangham et al. showed that phospholipids dispersed in water formed closed, multilayer aggregates called liposomes, capable of separating an internal compartment from the bulk solution [8]. As bilayers are relatively impermeable to many ions and nonelectrolytes, it became possible to create small domains of different composition and to explore some of the properties that nature has designed into cells and organelle membranes. Unilamellar vesicles are distinguished from multilayer liposomes by their single-bilayer closed shell
structure that encapsulates a single aqueous compartment. While vesicles often form spontaneously in vitro, they have only rarely been observed to form in vitro without the input of considerable mechanical energy or elaborate chemical treatments. Hence, a variety of methods have been developed to create vesicles with sizes ranging from about 20 nm to more than 20 μm [9].

The stability of such vesicles is limited because the bulk lamellar phase, which at high water fractions can exist as a colloidal dispersion of multilamellar liposomes, is the equilibrium form of aggregation under typical conditions. Hence, unilamellar vesicles formed from lamellar phases are metastable and eventually will revert to multilamellar liposomes. This reversion is invariably accompanied by a release of the vesicle contents and failure of the vesicle carriers. For multilamellar liposomes to be stable at high water fractions, the bilayers must have a net attractive interaction. The stability of mechanically formed vesicles against aggregation can be enhanced in many of the same ways that other colloidal systems are stabilized. These include the incorporation of bulky, polymer-like head groups on some fraction of the lipids [10–12], or the addition of charged lipids or surfactants to the bilayers to enhance electrostatic repulsion [13–15]. Both additives decrease the net bilayer attraction and help vesicles form spontaneously or with little energy input [16]. Once formed, unilamellar vesicles can also be made mechanically stable by polymerization of the head or tail groups [17,18]. These methods limit the kinetics of reversion, but leave the question of equilibrium stability unanswered.

There have been surprisingly few demonstrations of equilibrium vesicle phases in the literature, in comparison to the immense body of literature on vesicles and liposomes in general. By equilibrium, we refer to the following three criteria:

1. Unilamellar vesicles are formed spontaneously upon dispersing dry surfactant into water without mechanical or chemical perturbation;
2. Vesicles do not aggregate with time; and
3. Any physical or chemical process to which the vesicles are exposed will result in spontaneous reformation of unilamellar vesicles on reversing the process.

Surprisingly, nearly all of the several reports of spontaneous vesicle formation in the literature have involved surfactant mixtures. Single component bilayers usually form multilamellar phases, although there are reported exceptions [19]. Nonetheless, the question of equilibrium is a difficult one to address in these mixtures because the state of aggregation changes in some cases only on the time scale of years, and may thus reflect chemical degradation of the surfactant (e.g., hydrolysis) rather than a physical progression to a more thermodynamically stable structure.

The fundamental questions are why vesicles form in mixtures of oppositely charged surfactants, and why, once formed, do they remain stable? The formation and stability of any surfactant aggregate depends on whether that aggregate represents the global minimum in free energy for a given composition, and there are different approaches to describing the thermodynamics of these mixtures. As a way to begin, it is useful to start not with a molecular-level description, but instead with the more mechanical description of the properties of the bilayer first given by Helfrich [20]. In this case the elastic free energy of a bilayer is written in terms of its local state of curvature, described by the two principle curvatures \(c_1\) and \(c_2\).

For spherical vesicles, \(c_1 = c_2 = 1/R\), in which \(R\) is the vesicle radius. To terms second order in curvature, the free energy of a bilayer, per unit area, is

\[
E/A = \frac{1}{2} \kappa (c_1 + c_2 - 2c_0)^2 + \tilde{\kappa} c_1 c_2
\]

in which \(\kappa\) is the bending modulus, \(\tilde{\kappa}\) is the saddle splay or Gaussian modulus, and the spontaneous curvature of the bilayer is \(c_0\). By definition, \(\kappa > 0\) but \(\tilde{\kappa} > 0\) for surfaces that prefer hyperbolic shapes (saddle shaped surfaces in which the centers of curvature are on opposite sides of the surface, \(c_1 c_2 < 0\)) and \(\tilde{\kappa} < 0\) for surfaces that prefer elliptical shapes (spheres, ellipsoids, etc., in which the centers of curvature are...
on the same side of the surface, \( c_1 c_2 > 0 \) \[21\]. For a chemically
and physically symmetric bilayer, the spontaneous curvature
\( c_0 = 0 \). Hence, a single component bilayer cannot have a
nonzero spontaneous curvature. It is necessary for the bilayer
or its local environment to be chemically asymmetric for
spontaneous curvature to exist \[22\].

Thermal fluctuations of the bilayers lead to a repulsive
interaction as the bilayers come into contact and the are
damped \[23\]. For bilayers separated by a distance, \( d \) \[23\], the
so-called undulation interaction energy is

\[
E_{\text{fluct}} = \frac{3\pi^2 (k_B T)^2}{128 \kappa d^2}
\]  

(2)

The repulsive undulation interaction can overwhelm the van
der Waals attraction between bilayers (which is also propor-
tional to \( d^{-2} \)) when \( \kappa \) is small, leading to a net repulsive inter-
action between bilayers and hence, stable unilamellar vesicles,
especially when combined with electrostatic repulsion in
charged systems \[24\].

Within this Helfrich framework, vesicles may be made
stable by either an entropic or an enthalpic mechanism.
Entropically stabilized vesicles have a low bending constant
(\( \kappa \sim k_B T \), where \( k_B \) is Boltzmann’s constant). The bending
energy is therefore low and the population of vesicles is
stabilized both by the entropy of mixing and the undulation
interaction. The resulting size distribution is broad.
Enthalpically stabilized vesicles, on the other hand, require a
non-zero spontaneous curvature and a larger value of the
bending constant (\( \kappa > k_B T \)). In this case the vesicles are
narrowly distributed around a preferred size set by the
spontaneous curvature. These theories are discussed in more
detail below.

Work in our laboratories has demonstrated the existence
of spontaneous, apparently equilibrium vesicles with both
narrow \[25–27\] and broad size distributions \[6,25,28–32\] in
aqueous mixtures of a wide range of surfactants. The most
common cationic surfactant used has been an alkytrimethyl-
ammonium bromide or tosylate, e.g., cetyltrimethylammonium
bromide (CTAB) or tosylate (CTAT) as well as cetylpyridinium chloride (CPCl), while the common anionic surfactants are the sodium alkylsulfates, e.g., sodium octyl (SOS) or decyl sulfate (SDS), or dodecylbenzene sulfonate (SDBS), which may have either a branched or comb structure [6,33]. In addition, vesicles form with CTAB when the anionic surfactant is a salt of perfluorinated carboxylic acid such as sodium salts of perfluorohexanote (FC₅) or perfluorooctanoate (FC₇) [25,34]. Unilamellar monodisperse equilibrium vesicles also form spontaneously when cholinergics (for example, choline chloride) are added to aqueous solutions of sodium bis[2-ethylhexyl] sulphosuccinate (AOT) [35]. In this case, the cationic “surfactant” is actually a hydro trope and is water-soluble while the anionic surfactant alone forms bilayers in water. Thus, vesicle formation in this case is due to a change in curvature of the AOT bilayer induced by addition of the cholinergic compound.

A substantial number of other researchers have also explored vesicle formation in other mixtures of oppositely charged surfactants, and the recent review of Gradzielski [7] is especially comprehensive. Vesicles also form when a single-tailed anionic surfactant (SDS) is added to a double-tailed cationic surfactant (DDAB) [36], and these vesicles also form interesting phases and complexes in the presence of DNA and other polyelectrolytes [37]. Certain bacterial surfactants known as siderophores can undergo micelle to vesicle transitions on complexation of multivalent ions [38], which may be important for sequestering sufficient iron in marine environments. A range of zwitterionic/ionic pairs have been studied, as have surfactant pairs when one of the ionic species is produced by a chemical reaction [39]. The kinetics of formation of vesicles in these mixtures has been probed by light and small angle scattering as well as by cryo-TEM [40–42]. A variety of unstable rod and disk-like structures are found as the aggregate morphology progresses from simple micelles to vesicles.

This chapter is organized first around experimental observations of the surface chemistry of dilute mixtures, phase diagrams, and microstructure characterization. This
section is followed by a review of the relevant theories and a
discussion of the origin of stability of vesicles in cationic/anionic
mixtures.

II. EXPERIMENTAL OBSERVATIONS

A. Surface Tensions and Nonideal Mixing

The first striking observation about cationic–anionic mixtures
is their high degree of nonideality. This is clearly shown by the
strong variation in critical aggregation concentration given by
surface tension measurements [33]. For example, mixtures of
CTAT and SDBS display critical aggregation concentrations
($c_{acs}$) orders of magnitude lower than the individual Critical
Micellization Concentration (CMC) values (Fig. 1). Mixtures of
CTAT and SDBS also produce a lower surface tension than is
observed for either surfactant alone. Visual and light scattering
observation of samples with intermediate concentration a few
times the $c_{ac}$ are revealing. The samples appear somewhat blue
in color, suggesting the presence of colloidal structures larger
than micelles. This is borne out by light scattering measure-
ments of dimensions of $ca. 100 \text{ nm}$, and, as described below,
unilamellar vesicles are the first aggregates to form in these
highly dilute solutions.

Similar results hold for mixtures of SOS and CTAB. The
CMC of pure SOS is 3 wt% (120 mM) and that of pure CTAB is
0.03 wt% (0.88 mM). The critical aggregation concentrations
for mixtures of CTAB and SOS are significantly lower and
range from 0.001 wt% to 0.002 wt% ($3-7 \times 10^{-5} \text{ M}$) [31], and
mixtures of CTAB and SOS also produce a lower surface
tension than either pure surfactant. Precipitate appears in
samples in the vicinity of the $c_{ac}$ after equilibration for several
days, thus the $c_{ac}$ for mixtures of CTAB and SOS corresponds
to the solubility of the equimolar precipitate. The value of the
solubility product [$= (a_{CTA+}) (a_{SOS-})$] for this salt, calculated
from the dependence of the $c_{ac}$ on bulk mixing ratio, is
$9 \times 10^{-10} \text{ mol}^2/\text{l}^2$, and similarly low values are expected for
other surfactant ion pairs.
B. Phase Behavior

Aqueous mixtures of cationic (R\(^+\)X\(^-\)) and anionic surfactants (R\(^-\)X\(^+\)) are actually five-component systems according to the Gibbs phase rule: R\(^+\)X\(^-\), R\(^-\)X\(^+\), R\(^+\)R\(^-\), X\(^+\)X\(^-\), and water, and are subjected to an electroneutrality constraint. Therefore, the

Figure 1  Surface tensions of SDBS, CTAT, and their mixtures at 25°C (top). The mixtures are highly nonideal, as shown by the variation of the critical aggregation concentration with CTAT fraction (bottom).
pseudoternary phase diagram for R⁺X⁻, R⁻X⁺, and water at
constant temperature represents only a portion of the phase
prism. The full prism is needed to represent compositions in
multiphase regions when the surfactant ion and associated
counterion separate into different phases, as is the case when
precipitate forms. Nonetheless, when precipitate is not present
the pseudo-ternary map is a useful guide, although the phase
alternation rule [43] need not apply.

A canonical phase diagram for anionic and cationic
mixtures is that of CTAT and SDBS (Fig. 2), where in this
case the SDBS hydrophobe is of the “soft” type and has rake-
like branching. (Hard-type SDBS has approximately a dodecane
tail bonded to the aromatic ring at a single carbon.) The phase
boundaries in Fig. 2 and all other phase diagrams of catanionic
mixtures can only be established after visual observations
remain unchanged over an extended period of time. Most
compositions equilibrate within one to two weeks, but samples
that contain vesicles or a viscous phase require longer
equilibration times. Vesicle phases are identified first by their
characteristic isotropic blue appearance and then by determin-
ing the mean diameter using quasielastic light scattering (QLS)
to verify that aggregate sizes are in the range typical of vesicles.
Cryo-transmission and/or freeze-fracture electron microscopy
(cryo-TEM) can be used to confirm the unilamellar vesicle
structure [26,32], as can small-angle neutron scattering
methods [26,30]. The long equilibration time is necessary for
the samples containing vesicles in order to distinguish between
single-phase vesicle regions and two-phase vesicle/lamellar
regions, since small amounts of lamellar structure develop
slowly compared to other phases. Visual observations and QLS
measurements over a time period of nine months or longer (in
one case over 10 years) confirm the stability of the vesicle phase
relative to a lamellar phase in one-phase regions.

At higher surfactant concentrations, Fig. 2 shows that the
vesicle phases are in equilibrium with one of two lamellar
phases: (1) CTAT-rich vesicle solutions (V⁺) are in equilibrium
with a CTAT-rich lamellar phase (L₂⁺) of lower density; and
(2) SDBS-rich vesicle solutions (V⁻) coexist with an SDBS-rich
lamellar phase (L₂⁻) which undergoes an inversion in density
at $\sim 35/65$ CTAT/SDBS. Isotropic one-phase regions border each binary surfactant–water boundary. The rod-like micelle region on the CTAT side extends into the ternary diagram and is separated from the vesicle lobe by several multiphase regions. On the other side of the diagram, SDBS forms spherical micelles in water at the water concentrations shown in the phase diagram. Within the micellar region, the viscosity is low.
and constant and there is no increase in scattered light intensity as CTAT is added. Thus it is unlikely that adding CTAT causes significant micellar growth in SDBS-rich micellar samples. A two-phase region, in which $L_a$ is in equilibrium with an isotropic solution presumably containing vesicles, separates the one-phase micellar region from the vesicle lobe. The general qualitative features of two vesicle lobes, which may differ significantly in size, an equimolar precipitation line, lamellar phases at higher concentration and micellar phases on the edges of the diagram are found in most cationic–anionic surfactant phase diagrams.

The phase diagrams evolve in a systematic way as the chain lengths of the two surfactant molecules change. Figure 3 shows the diagram for CTAT/SDBS compared to that for CTAB/SOS, which have highly asymmetric tails [31] and to DTAB/SDS, which have symmetric tails [6]. The CTAB/SOS diagram displays a very large vesicle lobe on the SOS-rich (shorter hydrophobic chain) side of the diagram, while there is only a small composition range where in CTAB-rich vesicles form. The DTAB/SDS phase diagram is dominated by a large composition range wherein precipitate forms. As the ionic head groups are the same in the two surfactant pairs, this strongly suggests an important thermodynamic role in vesicle stability for the chain packing configurations within the hydrophobic core of the bilayer.

**Figure 3** Ternary phase maps of three cationic-anionic mixtures in water at 25°C. CTAB/SOS (left) shows a large vesicle lobe on the SOS-rich side of the diagram along with other phases identified in Fig. 2 (see Fig. 4). The DTAB/SDS mixture (right) shows a wide range of precipitate formation.
The implications of the CTAB/SOS diagram are seen more clearly when plotted in rectangular coordinates (Fig. 4) [29]. When samples are initially prepared, vesicles appear to form over a wider range of compositions. However, as the samples age, the range of compositions that yields stable vesicles shrinks considerably, particularly at the lamellar phase.

**Figure 4** Phase behavior of CTAB/SOS/H₂O with no added salt plotted on a rectangular diagram. Dotted lines represent equimolar CTAB/SOS composition, at 61.4% CTAB. One-phase vesicle lobes (V) exist at dilute CTAB-rich and SOS-rich compositions. Samples here appear bluish and are isotropic. One-phase rod-like (R) and spherical (M) micelles form near the CTAB and SOS axes, respectively. Rod-like and spherical micellar phases are both clear, yet scatter more light than pure water. Rod-like micellar samples are viscous and viscoelastic. At intermediate mixing ratios, much of the phase behavior is dominated by vesicles in equilibrium with a lamellar phase (L), which appears as birefringent clouds above the vesicles. The CTAB-rich R and V phases are separated by a narrow two-phase region of rods and vesicles in equilibrium. SOS-rich micelles transform abruptly to vesicles at most concentrations, though around 3.0 wt%, an intervening region of rod-like growth occurs. Unresolved multiphase regions are at concentrations above those of the vesicle lobes. Modified from [29].
boundary. Small amounts of the lamellar phase form in two-phase samples close to the vesicle phase, and become visible only after aging for days or weeks. Boundaries are assigned only when the sample appearance does not change with time. As expected, micellar phases exist on the binary surfactant–water axes of the diagram. Vesicles form in the water-rich corner of the phase diagram, and are found in both CTAB-rich and SOS-rich samples as confirmed by QLS and cryo-TEM. The CTAB-rich vesicle lobe is small and narrow in extent, while the SOS-rich vesicle lobe is considerably larger. Note that the lobe extends to nearly the CMC value of SOS, so that compositions where vesicles form all have compositions such that the SOS concentration is below its pure component CMC. As SOS is added to CTAB-rich micellar solutions, there is strong rod-like micellar growth as indicated by increased viscosity and viscoelasticity. Samples become increasingly more viscoelastic at high surfactant concentrations. SOS-rich micelles are spherical at low amounts of added CTAB, while at higher ratios of CTAB to SOS and higher concentrations, rod-like micelles form.

The micelle-to-vesicle phase transition is of considerable interest. For CTAB-rich samples and SOS-rich samples at higher surfactant concentration, there is an intervening two-phase region of rod-like micelles and vesicles. Samples separate into two phases: one phase scatters more light than a micellar solution and is viscous, while the other phase is not viscous. Depending on the composition, the appearance of the second phase ranges from clear and colorless to bluish and somewhat turbid. At higher surfactant concentrations, samples contain more than two phases and may contain vesicles, rod-like micelles, and liquid crystalline microstructures. SOS-rich samples exhibit different behavior for low surfactant concentrations (see Fig. 4). In these samples there is limited micellar growth with added CTAB or increased dilution, and the colorless micellar solutions progressively scatter more light than micellar solutions of pure SOS as the micellar phase boundary is approached. Samples become noticeably turbid over a very narrow increment of concentration, and the phase boundary between micelles and vesicles is set at the point at
which samples appeared turbid. In no case is an intermediate
two-phase region observed, so this is not a first-order phase
transition.

A crystalline precipitate, presumably the equimolar salt
\(\text{CTA}^+ : \text{OS}^-\), forms in equimolar mixtures as well as in dilute
(<0.1–0.2 wt%) solutions at all mixing ratios. Dilute samples in
the vesicle phase close to the precipitate phase boundary often
contain turbid wisps that are so easily dispersed by mixing that
attempts to characterize them with optical microscopy are
unsuccessful. Because of the proximity of the precipitate phase
boundary, the wisps may be a fine precipitate, or a dilute
dispersion of multilamellar vesicles (MLVs).

Electrostatic attractions between the cationic and anionic
surfactants are responsible for this interesting phase behavior,
so it is no surprise that addition of excess electrolyte markedly
alters the phase behavior of catanionic solutions [29]. This has
been examined in some detail for mixtures of CTAB/SOS
with added NaBr (Fig. 5). The most conspicuous effect is the
destabilization of the large one-phase vesicle lobe with increas-
ing amounts of added NaBr. The vesicle region shrinks in all
dimensions. Vesicles still form in the water-rich corner of the
lobe, but small turbid clouds form over the vesicle phase after
the addition of salt. These clouds are either lamellar or
multilamellar vesicle (MLV) phases, or a fine dispersion of
precipitate. Adding NaBr also drives the two-phase lamellar/
vesicle region on the CTAB-rich side of the vesicle lobe to more
SOS-rich compositions. The lamellar phase becomes increas-
ingly favorable as salt is added, as observed visually by the
increasing predominance of a turbid white birefringent phase
over the vesicle phase. This is consistent with a decreased
electrostatic interaction between the vesicle bilayers leading to
a more attractive interaction [44]. The vesicle/micelle phase
boundary, however, shifts to compositions richer in CTAB with
increasing NaBr. Thus, overall, the most SOS-rich section of
the larger vesicle lobe becomes unstable with respect to the
neighboring micelle phase, while the most CTAB-rich section of
the lobe becomes unstable with respect to the lamellar phase. It
is striking that the addition of salt in some cases drives the
vesicles to form small micelles rather than larger MLVs or
lamellar phases, as would be the prediction based on either colloidal stability arguments (aggregation) or by appeal to analogy to charged phospholipids, in which salt addition drives fusion.

The differences in behavior of mixtures of homologous surfactants with identical headgroups but different hydrocarbon tails further points to the important role of the packing of surfactant tails in the bilayer. This can be explored more directly by examining mixtures of a hydrogenated cationic surfactant with a fluorinated anionic surfactant [25,26]. The antipathy of the tails, and even their potential demixing, is balanced by the attractions between the head groups. Figure 6 shows the phase map for the mixture of CTAB/FC₅/water in the water-rich corner (<8 wt%) at 25°C. The CMC of each
Figure 6  Phase maps for mixtures of CTAB/FC₅ (a) and CTAB/FC₇ (b) measured at 25°C. The gray lines in each graph correspond to constant weight mixing ratios (δ) as indicated. The CMC for each surfactant is shown by the arrows on the binary axis. At intermediate mixing ratios in each system, the phase behavior is dominated by a two phase region consisting of vesicles in equilibrium with a denser lamellar phase (V + Lα). Rodlike micelles (R) exist on the CTAB–water binary axis for both FC₅ and FC₇ mixtures. The most important feature is the existence of a single vesicle phase (V) on the fluorinated surfactant rich side of the phase map.
surfactant is indicated by an arrow on the appropriate binary axis and the gray lines represent constant values of the surfactant mixing ratio, \( \delta(=\text{wt. FC}_5/(\text{wt. FC}_5 + \text{wt. CTAB})) \). The majority of the phase space at intermediate values of \( \delta \), including equimolar mixtures, is composed of a two-phase region denoted by \((V + L_a)\) containing a vesicle phase in equilibrium with a denser, birefringent lamellar phase. When CTAB is present in large excess, a viscoelastic solution of long rodlike micelles is formed. The phase behavior on the FC5-rich side on the phase map is more interesting. Along the FC5–water binary axis (\( \delta = 1 \)), at concentrations below the CMC, the surfactant presumably exists as monomers. Over the majority of this range, the addition of small amounts of CTAB leads to the formation of larger aggregate including vesicles between 2 and 4 wt%. The vesicle region extends to about \( \delta = 0.8 \). Vesicles do not form in FC5–water binary mixtures, but quasielastic light scattering measurements and cryo-TEM images indicate that large structures form with the addition of only a small amount of CTAB (\( \delta \sim 0.995 \)). Thus on this scale, the vesicle lobe appears to almost touch the binary axis. For \( \delta \) below about 0.80, the samples also contain a more dense birefringent phase, with the phase boundary between the vesicle lobe (V) and the two-phase region \((V + L_a)\) set by the formation of this dense phase. Mixtures of CTAB and FC7 show a similar phase diagram, and neither of these mixtures shows a substantial change in phase behavior with temperature, suggesting that there is no demixing of the tails.

C. Microstructure Characterization

Once phase behavior has been established the microstructure can be explored with a variety of techniques. Especially useful methods are quasielastic light scattering, which provides a rapid size estimate and can be used to track the evolution of structure with time; small angle neutron scattering, which can provide information about aggregate shape, structure and, under ideal conditions, size distribution; and cryo-TEM, which can provide unambiguous size, shape, and size distribution information.
As an example of the evolution of apparent vesicle radius determined by QLS as a function of time, consider the properties of CTAB/FC₅ and FC₇ mixtures. Figure 7(a) shows the measured hydrodynamic radius plotted as a function of the sample age for CTAB/FC₅ concentrations 3 wt% and δ = 0.80, 0.90, and 0.99. At the two higher δ values, vesicle growth is observed over the first 50 days with a leveling off to an equilibrium size seen at longer times. For the lowest δ, there is a more dramatic change in size initially and the measured size continues to increase even after 110 days. In these samples a lamellar phase is eventually observed visually. The situation in the FC₇ case is different, with vesicles at 2 wt% concentration reaching a constant size more quickly at δ values of 0.8 and 0.85.

Cryo-TEM provides the simplest and most model-free measure of microstructure, although artifacts due to sample preparation are possible [45]. Figure 8 is a cryo-micrograph of a 2 wt% CTAB/SOS mixture (7 : 3 wt : wt). This sample shows unilamellar vesicles with radii varying from about 20 to >100 nm. Note that the bilayers appear flexible and the vesicles are not all spherical. Such shapes may be the equilibrium conformations of vesicles either because of the low bending rigidity of mixed bilayers or may reflect deformations due to shear during sample preparation. The radii of the vesicles imaged are in good agreement with those measured with QLS, although the cryo-TEM average radius is typically less than the QLS average. It is extremely important to note that QLS sizes measure a higher moment of the size distribution than the number average measured by cryo-TEM, so the QLS size is very sensitive to the presence of negligible (below 1 part in 1000) numbers of larger aggregates [32], and this may explain some size growth reported by others [46]. Such large aggregates can result from even a slight degree of chemical or physical degradation of the surfactants.

Small-angle neutron scattering (SANS) is also a useful method for determining average vesicle dimensions and, to some extent, size distributions [47] and is very complementary to cryo-TEM. SANS scattering spectra (intensity I as a function of the scattering vector magnitude q) can be analyzed either
Figure 7 Quasielastic light scattering results for CTAB/FCₘ vesicles. (a) CTAB/FC₅ vesicles at a surfactant concentration of 3 wt% and δ values of 0.99, 0.90, and 0.80. (b) CTAB/FC₇ vesicles measured at a surfactant concentration of 2 wt% and δ values of 0.85 and 0.80. The lines are shown to guide the eye. All measurements were performed at 25°C.
by fitting a model function describing the presumed structure to the data, or by using an indirect Fourier transform (IFT) method to extract model-free estimates of microstructure dimensions. Both methods have been used to analyze the spectra from samples known (by cryo-TEM) to contain vesicles. Generally spectra are fitted using a polydisperse core-shell model [48] where the vesicles are assumed to have a polydisperse core with constant shell thickness ($t$). For a polydisperse system of unilamellar non-interacting vesicles, the scattered intensity as a function of the scattering vector is given by

$$I(q) = \frac{d}{dq} = n \int_0^{\infty} G(r_c)P^2(qr_c) \, dr_c$$

where $n$ is the number density of vesicles, $P(qr_c)$ is the form factor of a single particle (e.g., vesicles) consisting of a core and an outer shell, and $G(r_c)$ is the normalized probability of finding a particle with a core radius between $r_c$ and $r_c + dr_c$. $G(r_c)$ is modeled as a Schulz distribution, so

$$G(r_c) = \frac{r_c^Z}{\Gamma(Z+1)} \left( \frac{Z+1}{r_c} \right)^{Z+1} \exp \left( \frac{-r_c}{r_c} (Z + 1) \right)$$  (4)
where $\bar{r}_c$ is the mean core radius and $Z$ is related to the polydispersity of the particles ($p$) and the variance ($\sigma^2$) of the core radius by

$$p^2 = \frac{1}{Z + 1} = \frac{\sigma^2}{\bar{r}_c^2}$$

(5)

The form factor is

$$P(x) = \frac{4\pi}{q^3} (\rho_b - \rho_c) \left\{ J_1 \left( x + \frac{t}{\bar{r}_c} x \right) - J_1(x) \right\}$$

(6)

where $x$ is the dimensionless variable $qr_c$, $J_1(x) = \sin(x) - (x)\cos(x)$, and $\rho_b$ and $\rho_c$ are the scattering length densities (SLDs) of the bilayer and the core (taken as the solvent), respectively. The SLDs were calculated by adding the scattering amplitudes of each group or atom in a molecule and dividing the total by the corresponding molecular volume. The SLD of the bilayers is calculated assuming the bilayer is made of an equimolar composition of the oppositely charged components. This assumption has little influence on the results. Interestingly, use of one deuterated surfactant allows the SLD of the bilayer to be changed, and combining this information with mass balances allows the composition of the vesicle to be determined [49]. It is not, of course, generally equal to the bulk composition.

Typical scattering spectra for polydisperse vesicles are relatively featureless and show only a broad $q^{-2}$ decay of intensity, which is consistent with the presence of a two-dimensional bilayer (Fig. 9a). More monodisperse populations (Fig. 9b) yield spectra with undulations that reflect the oscillations of the form factor. In this case, the vesicles are about 20–22 nm in number average radius with ca. 20% polydispersity. Further analysis shows the bilayers to be rather thin (2–3 nm), and IFT methods allow the cross-sectional SLD profile to be determined [34].
Figure 9 SANS results for CTAB/FC\textsubscript{m} vesicles plotted as the scattering intensity in absolute units versus the scattering vector \( q \).

(a) CTAB/FC\textsubscript{5} vesicles measured at a surfactant concentration of 3 wt\% and \( \delta \) values ranging from 0.85 to 0.99. The top three curves have been offset by the indicated scale factors for clarity. Each scattering curve shows the characteristic \( q^{-2} \) decays as indicated by the line in the graph. (b) CTAB/FC\textsubscript{7} vesicles measured at a surfactant concentration of 2 wt\% and \( \delta \) values of 0.80 and 0.85. The top curve is offset by a factor of 10 for clarity. The \( q^{-2} \) region is observed at higher \( q \) values and minima from the form factor are evident around \( q \approx 0.01-0.02 \).
III. THEORETICAL APPROACHES

Much work has focused on development of theoretical models that can account for the behavior of surfactant solutions. These models range from the simple but useful geometric packing model of Tanford [50] and Israelachvili [44] to more quantitative molecular thermodynamic models. The level of detail required from a model varies widely with the circumstance of its application. A simple conceptual model is useful when attempting to organize results or guide experimentation, and both the geometric packing and the curvature elasticity models fulfill this need. These models require the input of parameters that characterize the surfactant properties, and given these parameters, both models can be used to rationalize observations and to suggest an experimental plan.

Often, solution properties such as micellar composition or monomer concentrations for mixtures of surfactants are required for a given surfactant application. In this case, a model based on classical thermodynamics in which aggregates are treated as a single phase in equilibrium with an aqueous monomer phase may be suitable. In this approach, deviations from ideal mixing in the aggregate phase are accounted for by activity coefficients for each surfactant in the mixed aggregate [51,52]. Activity coefficients can be calculated using a suitable theory for nonideal mixing (such as regular solution theory) with interaction parameters determined from the experimental values of the CMC of each surfactant and the cac measured for at least one mixing ratio. Finally, there are powerful predictive models based on a detailed statistical thermodynamic analysis of aggregate formation [53–58]. These molecular thermodynamic models are capable of predicting the equilibrium state of aggregation, aggregate sizes and composition, and equilibria between multiple phases, given data about the pure surfactants that is readily available in the literature.

The surfactant inventory in monomeric and aggregated (micellar or vesicular) form is needed in many applications of surfactant mixtures. The inventory may be calculated in
several ways, with each method having a different degree of complexity and accuracy. In a first approach, micellar size distributions can be computed by considering each aggregate to be a distinct chemical species. From thermodynamics, the chemical potential of a surfactant molecule has the same value regardless of the size of the aggregate it is in. Combining the condition of chemical equilibrium with a mass balance on surfactant yields the aggregate size distribution. This approach is the basis of the mass action model, which has been widely applied in the analysis of surfactant properties to predict the micellar size distribution, monomer concentration, electrical conductivity of micellar solutions, and degree of counterion binding to micellar aggregates (see, e.g., Kamrath and Franses [59]).

In a second model, the effect of finite aggregate size on solution properties is neglected. Instead, the collection of aggregates is viewed as a separate phase with uniform properties. This approach, known as the pseudo-phase separation model, can account for many properties, such as the micellar composition and monomer concentration in mixtures of surfactants [60]. Since this model ignores the finite size of aggregates, the monomer concentration above the CMC is predicted to be constant, which is at odds with experimental results and the predictions of more realistic theories, both of which show a moderate decrease in monomer concentration above the CMC. This decrease is a consequence of the mass action effects for a collection of micelles. Also, the pseudo-phase separation approach yields no information about the optimal aggregate size or distribution of sizes.

A third class of models is based on a molecular-level thermodynamic analysis, and provides monomer and micellar compositions as well as detailed information on aggregate structure and stability. This last approach requires significantly more computational effort than the other two, but yields more information, ideally without requiring data for mixtures of the two surfactants.

Some theoretical work has been directed at modeling the important properties of mixtures of anionic and cationic surfactants [1,51,61,62]. Regular solution theory, combined
with the pseudo-phase separation model, has been used to predict the monomer concentrations, micellar compositions, and critical aggregation concentrations (cac) for anionic and cationic surfactant mixtures [51]. In the pseudo-phase separation approach, the contribution to the solution entropy by individual aggregates is assumed small and the aggregate phase is treated as a bulk phase. Although realistic predictions are made for the dependence of the cac on the solution composition, the theory is strictly applicable only to molecules of similar size and functionality [63], which is clearly not the case for mixtures of oppositely-charged surfactants (also see Hoffmann and Pössnecker [52]). Further, some of the reported interaction parameters actually characterize precipitate/monomer equilibria rather than micelle/monomer equilibria [61].

The phase equilibria between monomer, micelles, and precipitate in mixtures of SDS and dodecylpyridinium chloride has been reported and interpreted in terms of a model that combines regular solution theory to describe the micellar phase and a solubility product to characterize the precipitate phase [61]. The thermodynamic machinery needed to combine an expression for Gibbs free energy for micellar aggregates with the mass action law has been developed for the calculation of micellar properties, size distributions, and phase separation [58]. This model was used to make qualitative predictions of trends for micellar solutions of anionic and cationic surfactants. Application of Puvvada and Blankschtein’s model [62] to mixtures requires the input of interaction parameters for each of the binary components. Finally, a statistical thermodynamic cell model has been used to predict the phase equilibrium between various phases for mixtures of amphiphilic molecules [64]. Of these approaches, the cell model structure provides the most careful way to account for the main contributions to the aggregate free energy. In the cell model, the nonidealities due to electrostatic interactions are calculated using the nonlinearized Poisson–Boltzmann equation and the effect of excluded volume is accounted for by the dependence of cell volume on surfactant concentration and aggregate size.
A. Thermodynamics of Aggregation

The surfactant solution consists of monomeric surfactant, surfactant ions contained in aggregates of a given geometry with aggregation number $N$, water, and counterions. Each aggregate is treated as a distinct species with chemical potential $\mu_N$, and at equilibrium, the Gibbs free energy of the system is minimized. For a mixture of two surfactants, A and B, dispersed in water, the Gibbs free energy is

$$G = n_w \mu_w^0 + n_{A,\text{mon}} \mu_{A,\text{mon}}^0 + n_{B,\text{mon}} \mu_{B,\text{mon}}^0 + \sum_{N>1,X_A} n_N X_A \mu_N^0$$

$$+ k_B T \left\{ n_w \ln x_w + n_{A,\text{mon}} \ln x_{A,\text{mon}} + n_{B,\text{mon}} \ln x_{B,\text{mon}}$$

$$+ \sum_{N>1,X_A} n_N X_A \ln x_N, x_A \right\}$$

$$+ N X_A \lambda_A + N X_B \lambda_B$$  \hspace{1cm} (7)$$

where $X_A$ is the fraction of component A in the mixed aggregate, $n_i$ is the number of molecules of species $i$ in the aqueous solution: $n_{i,\text{mon}}$ is the number of surfactant ions $i$ ($i = A$ or $B$) in monomeric form, $n_w$ is the number of water molecules, and $n_N, X_A$ is the number of surfactant aggregates with aggregation number $N$ (an “$N$-mer”) with composition $X_A$. $\mu_i^0$ is the standard chemical potential of species $i$ ($i$ refers to water, monomeric surfactant A or B, or $N$-mer of composition $X_A$), $k_B$ is the Boltzmann constant, $T$ is the temperature, and the mole fraction of species $i$, $x_i$, is defined as

$$x_i = \frac{n_i}{n_w + n_{A,\text{mon}} + n_{B,\text{mon}} + \sum_{N>1,X_A} n_N, X_A}$$  \hspace{1cm} (8)$$

The summation $(N > 1, X_A)$ includes all possible combinations of aggregates of aggregation number $N$ and composition $X_A$, excluding monomeric surfactant since this is already accounted...
for by the terms \( n_{A,\text{mon}} \) and \( n_{B,\text{mon}} \). The Lagrangian multipliers, \( \lambda_A \) and \( \lambda_B \), are chosen to satisfy the mass balance constraints for components A and B

\[
\sum_{N,X_A} N X_A n_{N,X_A} \lambda_A - n_{A,\text{total}} = 0
\]

\[
\sum_{N,X_A} N (1 - X_A) n_{N,X_A} \lambda_B - n_{B,\text{total}} = 0
\]  

(9)

In this equation, \( n_{i,\text{total}} \) is the total number of molecules of species \( i \) in the sample. Minimization of Eq. (7) relative to \( n_{N,X_A} \) yields the size distribution for mixed aggregates [65]

\[
x_{N,X_A} = x_{A,\text{mon}}^N x_{B,\text{mon}}^N \times \exp \left[-\left(\mu_{N,X_A}^0 - N_A \mu_{A,\text{mon}}^0 - N_B \mu_{B,\text{mon}}^0\right)/k_B T\right]
\]  

(10)

where \( N_A \) and \( N_B \) are the number of surfactant ions of type A or B contained within the mixed aggregate having aggregation number \( N \) and composition \( X_A \). Note that for monomers, \( N = 1 \).

The aggregate size distribution is calculated by simultaneously solving Eqs (7)–(9) for the monomer mole fractions, \( x_{A,\text{mon}} \) and \( x_{B,\text{mon}} \). Typically, the mole fraction of monomeric surfactant is very small and the exponent of the difference in chemical potentials is large, so that solution of these equations may present numerical problems [66]. A good initial guess for solution of the equations is the mole fraction of the pure surfactant at the CMC, which is often available in the literature.

With the help of the size distribution defined in Eq. (10), various useful quantities can be calculated, including the average aggregation number (neglecting the contribution from monomeric surfactant)

\[
\langle N \rangle = \sum_{N>1,X_A} N x_{N,X_A} \left/ \sum_{N>1,X_A} x_{N,X_A} \right.
\]  

(11)
the mixing ratio, $Y = n_{A,\text{total}}/(n_{A,\text{total}}+n_{B,\text{total}})$, in the bulk solution

$$Y = \frac{x_{A,\text{mon}} + \sum_{N>1} N x_{A} x_{N} x_{A}}{x_{A,\text{mon}} + x_{B,\text{mon}} + \sum_{N>1} N x_{N} x_{A}}$$  \hspace{1cm} (12)

the average mole fraction of component A in aggregate form

$$\langle X_{A} \rangle = \sum_{N,X_{A}} N_{A} x_{N} x_{A} / \sum_{N,X_{A}} N x_{N} x_{A}$$  \hspace{1cm} (13)

and the monomer mixing ratio

$$Y_{\text{mon}} = x_{A,\text{mon}} / (x_{A,\text{mon}} + x_{B,\text{mon}})$$  \hspace{1cm} (14)

The equations developed in this section are very general and may be used to predict size distributions of aggregates of various geometries, given a suitable function for the variation of the chemical potential of the aggregate as a function of size and composition. Thus, size distributions of micelles or vesicles may be calculated. In addition, this approach can be used to predict the distribution of surfactant between coexisting microstructures such as small micelles and large vesicles.

B. Curvature Elastic Energy

1. Overview

Models based on the concept of the curvature elastic energy of a surfactant film are well suited to describe the properties of aggregates composed of monolayers (microemulsions) or bilayers (lamellar bilayers, vesicles, sponge phases) [20,23, 67–70]. In this approach, the bilayer is modeled as a two-dimensional elastic film. Trends in morphology are then predicted as a function of the elastic constants and the spontaneous curvature of the film. This model is of limited use in the treatment of very small aggregates, such as micelles, because the quadratic curvature expansion breaks down at high curvature. To apply this model quantitatively, the
variation of the elastic constants and spontaneous curvature with composition or surfactant properties is required.

Helfrich wrote the curvature elastic energy of a bilayer per unit area, $E/A$, in a quadratic approximation in curvatures as given in Eq. (1). For spherical deformations, $c_1 = c_2 = 1/R$, where $R$ is the radius at the surface of inextension or “neutral” surface. For a symmetric bilayer, the area per molecule is invariant with curvature at the midplane, and so the neutral surface is the midplane. The energy to bend a bilayer away from the spontaneous curvature is proportional to $\kappa$ and the cost of making a saddle splay deformation is proportional to $\bar{\kappa}$.

Application of the Gauss–Bonnet theorem shows that the integral of the Gaussian curvature, $c_1c_2$, over the bilayer surface is independent of the shape and size of a closed surface and depends only on the number of handles, $n_h$, or components, $n_c$, of the surface

$$\int c_1c_2d^2S = 4\pi(n_c - n_h)$$ (15)

Handles are defined as pores or passages from one side of the surface to the other and each closed surface is one component. A vesicle has no handles and one component per vesicle. Hence, shape variations of simple closed vesicles produce no change in the Gaussian curvature energy. However, for spherical vesicles, the saddle splay (“Gaussian”) contribution to the bending energy is $4\pi \bar{\kappa}$ per vesicle, while for infinite lamellar bilayers it is zero [71]. Hence, the Gaussian curvature can play a significant role in determining vesicle size distributions [26,71].

At a molecular level, the tendency of a surfactant molecule to bend towards or away from the aqueous region is determined by the chemical make-up of each surfactant [22,71]. For example, a monolayer composed of single-tailed ionic surfactants with large head groups and small tail volumes will tend to bend away from the water, while a monolayer of a double-tailed zwitterionic surfactant such as lecithin will tend to curve more towards the aqueous region. By convention, positive curvature is away from the aqueous region. If a bilayer is made up of two chemically and physically identical monolayers, the bilayer has
no net spontaneous curvature, $c_0 = 0$ in Eq. (1). As the bilayer is bent, the molecules in one monolayer may be approaching their preferred curvature, but the molecules in the other monolayer are far from theirs. The bending energy is given by Eq. (1) with $c_0 = 0$. It is important to note that, in addition to allowing for a nonzero spontaneous curvature, a multicomponent bilayer consisting of monolayers with different compositions may have bending constants, $\kappa$ and $\bar{\kappa}$, considerably lower than for a uniform bilayer [71–73].

2. Implications of Curvature Elastic Energy for Vesicle Formation

Vesicles form spontaneously typically only in mixtures of two or more surfactants in water. Considering curvature elasticity alone, energy is required to bend a symmetric bilayer away from the planar configuration [Eq. (1)]. Thus, for single component (i.e., symmetric) bilayers with large bending rigidity, vesicles are formed only with the input of chemical or mechanical energy. However, this need not be the case for bilayers containing two or more components. Two factors are at work here. First, the elastic constants are strongly affected by bilayer composition, as observed experimentally [24,74] and as supported by theoretical calculations [72,73]. In mixtures, the bending rigidity can be reduced an order of magnitude, thus reducing the bending penalty that opposes the formation of an ensemble of vesicles. This mechanism gives rise to vesicles stabilized by the entropy of mixing. Alternately, a bilayer composed of two surfactants could have an asymmetric composition, thus producing a bilayer with a spontaneous curvature. This could occur if the two components in the bilayer have different spontaneous curvatures, so that when mixed the surfactants assemble into monolayers of equal and opposite curvature, resulting in an effective bilayer spontaneous curvature. This situation corresponds can lead to an enthalpic stabilization.

Consider first the case of a vesicle with a small bending constant ($\kappa, \bar{\kappa} \leq k_B T$) in the absence of a spontaneous curvature. Unilamellar vesicles can be stabilized if the bending
energy per vesicle relative to the infinite lamellar phase $[8\pi\kappa + 4\pi\tilde{\kappa}, \text{Eq. (1)}]$ is offset by the increased translational entropy of the larger number of independent vesicles. If there is a nonzero spontaneous curvature, the effective bending energy is zero for a vesicle population with a mean radius of $\sim 1/c_0$ and entropy dictates that vesicles are the stable phase.

A small value of $\kappa$ also promotes unilamellar vesicles as the repulsive undulation force (Eq. 2) reduces attraction between bilayers, leading to stable unilamellar vesicles, especially when combined with electrostatic repulsion in charged systems [24]. Theory and experiment have shown that surfactant mixing can lead to sufficiently low values of $\kappa$ for entropic stabilization to be effective [24, 72, 73].

Undulations of the bilayer may also decrease the effective bending constant at long length scales. Helfrich and others have developed an expression relating the effective bending rigidity of a membrane to the length scale of observation, $\eta (= R$ for vesicles) and a molecular distance, $\delta$ ($\sim$ bilayer thickness) [68, 69, 75].

$$\kappa = \kappa_0 \left[ 1 - \alpha \frac{k_B T}{4\pi\kappa_0} \ln(\eta/\delta') \right]$$ (16)

The numerical value of $\alpha$ is probably between 1 and 3 [68, 75]. Similarly, the effective value of the Gaussian modulus is

$$\tilde{\kappa} = \tilde{\kappa}_0 \left[ 1 + \tilde{\alpha} \frac{k_B T}{4\pi\tilde{\kappa}_0} \ln(\eta/\delta') \right]$$ (17)

where the prefactor $\tilde{\alpha}$ is estimated to be zero [69] or 10/3 [75]. The form of the bending constants, renormalized for thermal fluctuations, represents a logarithmic decay in bilayer rigidity with distance. The effect of these thermal fluctuations is to increase the apparent membrane rigidity at short distance (high curvature or small vesicles) and to decrease the effective rigidity at long distances (large vesicles or free bilayers). When both entropy and undulations are accounted for, there are various predictions of how the vesicle size distribution should
vary with surfactant concentration [24,76–78], but these have not been verified experimentally except for a single system [24]. Safran and coworkers have studied in detail enthalpic stabilization that results if the bilayer has a spontaneous curvature and if the bending rigidity is of the appropriate magnitude [22,79]. They recast Eq. (1) in terms of the interior and exterior spontaneous curvatures, \(c_I\) and \(c_E\)

\[
E/A = \frac{1}{2} K [(c + c_E)^2 + (c - c_I)^2]
\]

(18)

again with the convention that the curvature of the outer monolayer is positive. For symmetric bilayers, \(c_I = c_E\) and the free energy is minimized for \(c = 0\) (flat bilayers). However, for surfactant mixtures, nonideal mixing of surfactant molecules in the bilayer could allow the interior and exterior monolayers to have equal and opposite curvatures: \(c_I = -c_E = c\). For this to happen, the effective head group size of the mixed surfactants on the inside monolayer must differ from that of molecules in the outside monolayer. This can be achieved either with a mixture of amphiphiles with widely different areas per head group, or in a mixture in which surfactant complexes form such that the complex has a small area per head group. By placing more of the smaller head group component (or complex) in the inside monolayer (and more of the larger head group or uncomplexed component in the exterior monolayer) of the vesicle, the spontaneous curvatures could be adjusted to suit a particular composition of the vesicle [22,79]. Vesicles with such a curvature would then be stable with respect to flat, symmetric bilayers, especially in the limit that the bending modulus of the bilayer is large compared to \(k_B T\) [22,79]. The net result is a composition-dependent spontaneous curvature for the bilayer that determines the radius and size distribution of the vesicle population. In the event that vesicle formation is promoted by a spontaneous bilayer curvature, the size distribution is predicted to be a Gaussian peaked at a size near \(1/c_0\) with a relative standard deviation that is inversely proportional to the sum of the Helfrich bending constants, \(K = \kappa + (\kappa/2)\). The model does not treat bilayer interactions that undoubtedly become important at higher surfactant
concentrations, but it does account for several features of the experimental phase diagram [79].

In the spontaneous curvature model, interactions between surfactants are critical in stabilization of a phase of vesicles. In particular, attractive interactions are necessary to alter the balance between the area required for head groups relative to that needed for the tail groups. Applied to mixtures of oppositely charged surfactants, the interaction between the oppositely charged head groups can produce a surfactant pair that occupies a smaller area per molecule at the interface than the two individual surfactants when separated. Within this model, spontaneous vesicle formation with a well-defined size distribution is predicted for mixtures of surfactants with equal length tails as well as in mixtures with asymmetric tail groups.

IV. EXPERIMENTAL MEASUREMENTS OF BENDING CONSTANTS

A. Size Distributions

To distinguish between these two mechanisms of vesicle stability, the bending elasticity and bilayer spontaneous curvature has been measured for a number of different systems of spontaneous, unilamellar vesicles [26,27,32] by analysis of the vesicle size distribution using cryo-microscopy and freeze-fracture replication. The size distribution is calculated as follows.

The equilibrium curvature, calculated from the minimization of the curvature energy given by Eq. (1), is

$$c_{eq} = c_0 \frac{2\kappa}{2\kappa + \tilde{\kappa}}$$  (19)

The stability criterion for the bilayer bending energy is $2\kappa + \tilde{\kappa} > 0$. When $\kappa > -\tilde{\kappa}/2$, this theory predicts that the stable state of the bilayer, even for the case of $c_0 = 0$, is a spherical deformation, with higher order terms in the free energy expansion required to limit the vesicle size from becoming
vanishingly small. For \( \kappa > 0 \), infinite films are unstable towards formation of a surface with many handles and in this case cubic or sponge-like phases are predicted to be the stable bilayer configuration. For sufficiently dilute systems with finite bilayer fragments, vesicles are still possible [27] as systems with only negative Gaussian curvature cannot close on themselves, which results in bilayers with energetically unfavorable edges in contact with water.

The equilibrium size distribution of a population of vesicles is determined by a subtle competition between the entropy of mixing and the curvature elasticity of the bilayers. For the case of spherical vesicles, \( R_1 = R_2 = R \), and Eq. (1) can be simplified [22,79] to

\[
E/A = 2K \left( \frac{1}{R} - \frac{1}{R_0} \right)^2 2K = 2\kappa + \tilde{\kappa} R_0 = \frac{2\kappa + \tilde{\kappa}}{2\kappa} r_0
\]

(20)

\( R_0 \) is the radius of the minimum energy vesicle (\( = 1/c_{eq} \)), and \( K \) is an effective bending constant [22,79].

The distribution of surfactant between vesicles of aggregation number \( M \), corresponding to the minimum energy radius, \( R_0 (M = 8\pi R_0^2/A_0, \text{in which} A_0 \text{is the mean molecular area}) \), relative to vesicles of aggregation number \( N \) and radius \( R \), is dictated by a balance between the entropy of vesicle mixing and the curvature energy [80], and can be written in terms of the law of mass action [cf. Eq. (10)]

\[
\frac{X_N}{N} = \frac{X_M}{M} \exp \left[ \frac{M(\mu_M^0 - \mu_N^0)}{k_B T} \right]^{N/M}
\]

(21)

\( X_M, \mu_M^0 \) and \( X_N, \mu_N^0 \) are the mole fraction of surfactant and the standard chemical potential per molecule in vesicles of aggregation numbers \( M \) and \( N \), respectively. Equation (21) assumes ideal mixing of the vesicles (not the molecules within the bilayers, which will be assumed to be nonideal allowing for a spontaneous curvature) and is valid for dilute vesicle dispersions in which the Debye length is small in comparison to the inter-vesicle distance. In practice, this is always the case for catanionic vesicles as the surfactant co-ions result in at least
10–100 millimolar electrolyte concentrations. The chemical potential difference is due to the change in curvature energy per molecule for surfactant distributed between a vesicle of radius $R$ and aggregation number $N$ and the minimum energy vesicle of radius $R_0$ and aggregation number $M$

$$\left( \mu_N^0 - \mu_M^0 \right) = \frac{4\pi R^2 (E/A)}{N} = \frac{8\pi K [1 - (R/R_0)^2]}{N}$$  \hspace{1cm} (22)

Inserting Eq. (22) into Eq. (21) and substituting $M = 8\pi R_0^2 / A_0$ and $N = 8\pi R^2 / A_0$, in which $A_0$ is the area per surfactant molecule, gives a two parameter vesicle size distribution as a function of $R_0$ and $K$ \cite{80,81}

$$C_N = \left\{ C_M \exp \left[ -\frac{8\pi K}{k_B T} \left( 1 - \frac{R_0}{R} \right)^2 \right] \right\}^{R^2 / R_0^2}$$  \hspace{1cm} (23)

$C_M (= X_M / M)$, and $C_N$ are the molar or number fractions of vesicles of size $M$ and $N$, respectively. A consequence of Eq. (23) is that vesicles stabilized by thermal fluctuations ($K \sim k_B T$) have a much broader size distribution than vesicles stabilized by the spontaneous curvature ($K \gg k_B T$). This is the opposite of vesicle size distribution models that do not include a spontaneous curvature \cite{24,44}; larger bending constants predict more polydisperse vesicles of larger size.

B. Experimental Results

Cryo-TEM images were used to determine the radius of vesicles \cite{32}. Histograms of the vesicle size distributions were built up by measuring the size of $\sim 3000$ spherical vesicles per concentration ratio taken from many different samples over several weeks. The measured distribution was fit to Eq. (23) (solid line) to determine $R_0$ and $K$. Figure 10(a) shows a typical image of cetyltrimethylammonium bromide (CTAB)/sodium octyl sulfate (SOS)/water (0.3/0.7/99\% by wt) vesicles, while Fig. 10(b) shows the measured size distribution of vesicles and excellent agreement with the equilibrium distribution. The best fit to Eq. (23) gives $K = 0.7 \pm 0.2 k_B T$ and $R_0 = 37$ nm,
indicating these vesicles have a low bending constant and thus are entropically stabilized. Even though the $K \sim k_B T$, single parameter size distributions with no spontaneous curvature could not fit the experimental size distribution [24,82].

Figure 11(a) shows that the vesicles in a 2 wt% 2/8 CTAB/perfluoroctanoate (FC7) dispersion are smaller, and much more monodisperse (Fig. 11b) than the CTAB/SOS dispersion (Fig. 10b). Fitting this distribution to Eq. (23) gives $R_0 = 23$ nm.
and $K = 6 \pm 2k_B T$, indicating that the hydrocarbon–fluorocarbon (CTAB/FC$_7$) bilayers are much stiffer than the hydrocarbon–hydrocarbon bilayers (CTAB/SOS). Replacing the FC$_7$ fluorocarbon surfactant with the shorter chain sodium perfluorohexanoate (FC$_5$) lowers $K$ to $0.5k_B T$ while increasing $R_0$ to 56 nm [26,27]. For the CTAB/SOS and CTAB/perfluorohexanoate (FC$_5$) systems, thermal undulations due to the small value of $K$ stabilize the hydrogenated vesicles against formation of multilamellar liposomes, even in the absence of electrostatics. CTAB/SOS vesicles are stable even with 1.4 wt% added salt, at which point a phase transition to micelles occurs. The large bending constant and narrow size distribution suggest that CTAB/FC$_7$ vesicles are stabilized by the energy costs of deviations from the spontaneous curvature.

C. Theoretical Estimation of Elastic Constants

Factors such as the chemical identity of the surfactants, the addition of cosurfactants, and the value of the ionic strength strongly influence the elastic properties of surfactant films. Theoretical treatments of both electrostatic [83–87] and chain contributions [73,88] to the overall bending moduli are available. To summarize the results of theoretical predictions, the contribution to the bending moduli from the chain region is found to be the dominant factor, with bending moduli ranging from $>10k_B T$ for pure surfactant bilayers to several $k_B T$ in mixed bilayers. The electrostatic contribution to the bending modulus is found to be of order $k_B T$, and is predicted to exceed $k_B T$ only for highly charged membranes with low salt concentrations [83–86,89,90]. However, in mixtures of short- and long-chained surfactants, and in microemulsions, the chain contribution to the bending moduli is reduced considerably, and in these cases, the electrostatic contribution can be of similar magnitude and should not be neglected.

Szleifer and coworkers have calculated the elastic moduli as a function of surfactant chain length, area per molecule, and composition for mixed surfactant bilayers [73,88]. The model is based on a mean field treatment of the organization of the surfactant chains within an aggregate of a given geometry.
The model contains no adjustable parameters and the only assumption made is that the chains pack at uniform density within the aggregate, as is seen experimentally [91,92]. Calculations based on chain conformation free energy show that the bending constants increase strongly with increasing chain length or decreasing area per molecule. The bending modulus $k$ scales with chain length as $n^{3/2}$ and with area per molecule as $a^{-7.5}$ [73,88]. The bending constants are predicted to decrease sharply in mixtures of short- and long-chain surfactants. For example, in mixtures of chains with 16 (C16) and 8 (C8) carbons, the bending constant is reduced from $\sim 40k_B T$ for pure C16 bilayers to as low as $\sim k_B T$ in mixtures [72,88].

D. Close-packed Vesicle Dispersions and Vesicle “Nesting”

The hollow bilayer shells of vesicles exclude a substantial volume, and so vesicles can overpack at relatively low surfactant concentrations. Overpacking occurs roughly when the volume fraction of solution enclosed by vesicles, $\Phi$

$$\Phi = \frac{4\pi}{3V_{\text{total}}} \sum_{N, X_A} R_N^3 n_N$$

is of order 60%. Clearly, when the distribution shifts towards large vesicles, the surfactant mole fraction at which vesicles become close packed, $x^*$, will drop considerably. This concentration is relevant experimentally, and will occur at surfactant concentrations of a few percent for larger vesicles. It probably marks the high concentration end of the vesicle “lobes” in the phase diagram, as shown in Fig. 2.

At surfactant concentrations above $x^*$, the added surfactant can be accommodated either by shifting the vesicle size distribution to lower radii, by forming multilamellar vesicles, or by forming a lamellar phase. Simons and Cates give a thermodynamic analysis of the stability of the various phases in the region of the overlap concentration $c^*$ [76]. Their model combines curvature elasticity with entropy of mixing and
focuses on symmetric bilayers with zero spontaneous curvature. They find that as the overlap concentration is approached, the large vesicle sizes are eliminated from the size distribution. This is accompanied by a decrease in polydispersity and an accompanying decrease in the entropic contribution. At the same time, there is an energetic penalty for forcing the surfactant to assemble into more highly curved aggregates. Consequently, the entropic term that favors formation of unilamellar vesicles decreases, and the lamellar phase, which has a lower bending energy than an ensemble of vesicles, becomes more favorable. This corresponds to a first order phase transition from vesicles to a lamellar liquid crystalline phase [76]. Another possibility is that above the overlap concentration, the vesicles can “nest” within each other, forming multilamellar vesicles. In this case, surfactant can more efficiently pack into the available volume, and the favorable entropic contribution arising from a polydisperse distribution of vesicles continues to stabilize the vesicle phase relative to the lamellar phase. Simons and Cates found that MLVs can be stable at concentrations intermediate to the unilamellar vesicle phase and the lamellar phase [76].

There is an additional interesting possibility for vesicle nesting that arises for dispersions stabilized by spontaneous curvature. In this case it is possible for vesicles to form with a discrete number of bilayers, depending on the magnitude and sign of the bilayer interactions. This was observed experimentally when 1 wt% NaBr was added to screen any residual short-range electrostatic interactions between the bilayers, the result was the spontaneous formation of a population of primarily two-layered vesicles (Fig. 12). In Fig. 12, two layer vesicles are distinguished from one layer vesicles by the darker rim on the inside edge of the apparent vesicle membrane (arrows). This dark rim is due to the greater projection of the electron beam through both the interior and exterior vesicle bilayers. From examining many images, about 90% of the vesicles with added salt have two bilayers, while the rest have one bilayer. There were essentially no vesicles with three layers or more. The vesicles in 1% NaBr sample also had a greater tendency to adhere to each other and the carbon coated electron microscope.
grid and flatten, consistent with the enhanced attraction between the vesicle bilayers [93].

The distribution between one layer and two-layer vesicles can be derived using the mass action model for vesicles with a spontaneous bilayer curvature. The analysis shows that vesicles stabilized by spontaneous curvature can have a narrow distribution of the number of bilayers when the attractive interactions just balance the curvature energy. In the absence of a spontaneous bilayer curvature, each additional layer added to a vesicle has a decreasing curvature energy, but the attractive interaction energy grows with the net bilayer area in contact, and a polydisperse population of multilamellar liposomes result. Hence, typical phospholipid vesicles, with \(1/R_0 = 0\), are unstable relative to multilamellar liposomes. The combination of a narrow size distribution, a large bending
elastic constant, and the formation of two-bilayer vesicles shows that the CTAB/FC$_7$ vesicles are stabilized by spontaneous curvature. Interesting other shapes (rods and disks) can also be analyzed quantitatively [27].

V. EQUILIBRIUM?

Unilamellar vesicles form in a wide variety of mixtures of cationic and anionic surfactants, and do so spontaneously; that is, without substantial input of energy. Whether or not the vesicles are the equilibrium state of aggregation has been explored by several authors [46,94,95], all of whom conclude for various reasons that the vesicles are unstable with respect to a lamellar phase. A corollary argument is that the vesicles that are observed are the consequence of shear forces, which might be vanishingly small, that disrupt the stacked bilayers of a lamellar phase. Experimental conformation is hampered by the extremely slow evolution of these structures with time, which in turn must reflect a combination of small thermodynamic driving forces and slow mass transfer processes.

The question is clearer from a theoretical point of view. Consider first a patch of bilayer with zero spontaneous curvature in water in the limit of low concentrations. The patch of bilayer has hydrophobic edges exposed to water. This “edge energy” is recovered by closing the bilayer into a vesicle, with the energy penalty given by Eq. (1). For large enough values of edge energy and small enough values of the bending constant, the vesicle is stable. The situation is more interesting when the bilayer has a preferred curvature, as can happen for a mixed bilayer. Several theoretical approaches all yield the result that the vesicle is the thermodynamically preferred structure.

Thus, at least at low concentrations, bilayers with either a low bending constant or with a non-zero spontaneous curvature form vesicles are absolutely the preferred thermodynamic state compared to a stacked bilayer for the reasons laid out above. The experimental data show that vesicles are stabilized by one of two distinct mechanisms depending on the value of
the bending constant. Helfrich undulations ensure that the
interbilayer potential is always repulsive when the bending
constant, $K$, is of order $k_B T$. When $K \gg k_B T$, unilamellar
vesicles are stabilized by the spontaneous curvature that picks
out a particular vesicle radius; other radii are energetically
disfavored. Measurements of the bilayer elastic constant and
the spontaneous curvature, $R_0$, for three different systems of-vesicles by an analysis of the vesicle size distribution
determined by cryo-transmission electron microscopy show
cases for both entropically and enthalpically stabilized vesicles.

An absolute resolution of this question is probably
impossible, but there are several relevant observations at
hand. In particular, Hoffmann and co-workers have produced
both lamellar phases and vesicles by chemical synthesis to
produce one of the surfactant species in situ, thereby avoiding
shear. They see the formation of lamellar phases that are stable
in time but that form vesicles upon a single inversion of the
sample tube. These vesicles are also stable with time, so the
experiment simply does not allow resolution of the question of
which is the stable phase. Further, the lamellar phases are
observed by freeze-fracture electron microscopy, which requires
exposing a thin layer of solution to a solid surface, and solid
surfaces are well known to nucleate lamellar sheets from
vesicles [96]. Thus the question of the metastability of the
lamellar phase is open. For the reasons given above, creating a
lamellar phase by specific chemical or physical treatments that
is unstable to small perturbations does not show that the
lamellar phase is the preferred state of organization.

Almgren [46] recently summarized many observations
around swollen lamellar phases, and notes that they can be
easily dispersed to form vesicles. This observation was also
offered by Laughlin as the explanation for the observed vesicles
[94]. Almgren also reports QLS measurements of mixtures of
CTAB and SOS at a given mixing ratio that show an increase in
mean radius with time, which agrees with our results at that
mixing ratio [31]. Other ratios yield vesicles whose average size
does not change with time. Careful, long term studies of
catonic mixtures at certain well-defined compositions have
shown uniform phases of unilamellar vesicles for well over a
decade [28]. The size distributions of these vesicles remain constant over time and are well described by equilibrium theories of self-assembly [26,27,32]. Of course, not every mixture of anionic and cationic surfactants will form equilibrium vesicles and care must be taken to understand the phase diagrams and carefully delimit the concentration ranges that do form such vesicles. That various experimental observations are often at odds highlight the experimental challenges in resolving the issue of equilibrium vesicles.

REFERENCES

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