Bilayers with Surfactant-induced Pores and Demixing in Micelles

Studies of Segregation in Amphiphile Systems

BY

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Abstract

The focus of this thesis has been on the effects of segregation in mixtures of amphiphilic molecules. Two different systems were investigated: fluorocarbon-hydrocarbon surfactant mixtures and lipid-surfactant mixtures.

In fluorocarbon-hydrocarbon surfactant mixtures the repulsive interactions between the chains can lead to a demixing into different types of coexisting micelles, fluorocarbon rich and hydrocarbon rich. From NMR self-diffusion measurements such a demixing was found to occur in the mixture of the partially fluorinated surfactant HFDePC and C10TAC. We furthermore suggested a demixing also within the micelles to explain 2H-NMR line width data and results from neutron scattering.

In lipid-surfactant mixtures, a segregation of the molecules may instead be caused by a difference in the preferred curvature of the lipid and the surfactant residing within the same aggregate. Using a surfactant selective electrode, binding isotherms of four different cationic surfactants (C12TAC, C14TAC, C16TAC and HFDePC) to preformed lipid (GMO) vesicles were determined. Perforated vesicles were observed by cryo-TEM in the mixture with C12TAC. To explain the results from the binding isotherms, the formation of pores in the bilayer was regarded as a cooperative process, similar to micelle formation. The surfactant accumulates at the edges of the pores, and increasing the surfactant concentration results in an increased number of pores with a constant surfactant/lipid ratio at the edges.

The lipid-surfactant mixtures were also studied at the solid/solution interface using AFM. An adsorbed mesh structure, a counterpart to the bulk perforated lamellar phase, was observed for the first time.

Keywords: fluorocarbon surfactants, amphiphile mixtures, perforated bilayers, segregation, NMR, surfactant selective electrode, cryo-TEM, SANS, AFM

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


III. Kadi, M., Hansson, P., Almgren, M., Bergström, M., Garamus, V M. Mixed Micelles of Fluorocarbon and Hydrocarbon Surfactants. A Small Angle Neutron Scattering Study. (manuscript)

IV. Kadi, M., Hansson, P., Almgren, M. Determination of Isoterm for Binding of Surfactants to Vesicles Using a Surfactant Selective Electrode. (submitted)


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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>GMO</td>
<td>Glycerol monoolein</td>
</tr>
<tr>
<td>C\textsubscript{12}TAC</td>
<td>Dodecyltrimethylammonium chloride</td>
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<tr>
<td>C\textsubscript{14}TAC</td>
<td>Tetradecyltrimethylammonium chloride</td>
</tr>
<tr>
<td>C\textsubscript{16}TAC</td>
<td>Cetyltrimethylammonium chloride</td>
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<tr>
<td>HFDePC</td>
<td>N-(1,1,2,2-tetrahydroperfluorodecanyl)pyridinium chloride</td>
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<tr>
<td>Cryo-TEM</td>
<td>Cryogenic transmission electron microscopy</td>
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<tr>
<td>SAXS</td>
<td>Small angle x-ray scattering</td>
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<tr>
<td>SANS</td>
<td>Small angle neutron scattering</td>
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<tr>
<td>TRFQ</td>
<td>Time-resolved fluorescence quenching</td>
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<td>PGSE-NMR</td>
<td>Pulse gradient spin echo NMR</td>
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<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
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1 Introduction

Amphiphilic molecules are made up of one part that readily dissolves in water and one part that dissolves in oil. Because of this, these molecules form aggregates when dissolved in water, allowing separate compartments to form and thereby shield the hydrophobic parts of the molecule from the polar media and vice versa. We find amphiphilic molecules everywhere around us, for instance in such diverse areas as washing powders and cell membranes. One usually distinguishes between polar lipids, amphiphilic molecules building up biological membranes and synthetic amphiphilic molecules, surfactants (surface active agents), so called since they prefer to reside at an interface between media of different polarity. The applications of surfactants are numerous, for instance as lubricants and wetting agents.

This thesis deals mainly with mixtures of amphiphilic molecules. The interest has been in the mixture of a polar lipid with different cationic surfactants as well as a mixture of a cationic hydrocarbon surfactant with a partially fluorinated cationic surfactant. The focus is on the effects of segregation in these mixtures.
2 Self-assembly of Amphiphilic Molecules

As stated in the introduction, due to their dual preference for solvent, amphiphilic molecules self-assemble in water solution. The driving force for this aggregation is the unfavorable interactions between the hydrophobic part of the amphiphile and the water molecules.[1] This so called hydrophobic effect is a result of the strong attractive forces between water molecules being disrupted or distorted when a solute is dissolved. The local ordering of the water molecules around the solute results in a loss of entropy that is the origin of the hydrophobic effect. The formation of aggregates minimizes the energetically unfavorable hydrocarbon-water contact area.

The optimal aggregate structure is dependent on the size and shape of the molecules and other experimental parameters such as temperature, ionic strength and surfactant concentration.

2.1 The Surfactant Parameter & Curvature

A simple theory relates the geometry of the molecule to the shapes of the aggregates that are formed. The relation between the optimal headgroup area $a_0$, the hydrophobic chain volume $v$ and the critical chain length $l_c$ defines the surfactant parameter, $S$:[2]

$$S = \frac{v}{a_0 l_c}$$ (2.1)

This parameter is used to predict the shape of the aggregates formed as illustrated in figure 1. $v$, $l_c$ and $a_0$ can often be estimated with reasonable accuracy for a given surfactant, although the headgroup area can be strongly dependent on solution conditions.
Aggregate shape

Molecular shape

Packing Parameter

\[ S \leq 1/3 \quad 1/3 < S \leq 1/2 \quad 1/2 < S \leq 1 \quad S > 1 \]

**Figure 2.1** The preferred aggregate structure as a function of the surfactant parameter and molecular shape.

Instead of looking at the individual molecules, the geometry of the aggregates can be described by the curvature of the amphiphilic layer. The principal curvatures, \( c_1 \) and \( c_2 \), define the mean (\( H = (c_1 + c_2)/2 \)) and the Gaussian (\( K = c_1 \times c_2 \)) curvature. By convention, aggregates where the interior is filled with hydrocarbon chains have a *normal* or *positive* curvature, while aggregates enclosing the polar headgroups and water have a *reversed* or *negative* curvature (inverted structures). Aggregates with a normal curvature have a surfactant parameter <1, and aggregates with a reversed curvature have a surfactant parameter >1. A simple general expression relates the packing parameter to the curvatures of the interface:[3]

\[
\frac{v}{a_0 l_c} = 1 + Hl + \frac{Kl^2}{3}
\]  
(2.2)
This expression shows that the mean and the Gaussian curvature can be varied cooperatively without varying the magnitude of the surfactant parameter, i.e. the interfacial geometry of the aggregate is not fixed by the surfactant parameter alone.

If $H_0$ represent the spontaneous curvature, i.e. the curvature of the state corresponding to the lowest bending energy, any deviation from this preferred curvature results in a bending energy cost per unit area that can be written as:[4]

$$E_{\text{bend}} = k_C(H - H_0)^2 + k_G(K)$$

(2.3)

where $k_C$ and $k_G$ denote the bending elastic modulus for cylindrical deformations and the Gaussian elastic modulus, respectively.

### 2.2 Phase Behavior

The typical sequence of different phases formed by a surfactant predicted by the packing parameter or preferred curvature is shown in figure 2.[5]

Normal micelles (L$_I$) are formed when the surfactant concentration reaches a critical value, the $cmc$ (critical micelle concentration). They are aggregates of high positive curvature whereas reversed micelles (L$_{II}$) are aggregates of high negative curvature.

At high surfactant concentrations liquid crystalline phases possessing short- and long-range positional order are formed. The lamellar phase (L$_{\alpha}$), consisting of stacked bilayer sheets separated by intervening layers of solvent, has zero mean curvature. Hexagonal phases (H$_I$, H$_{II}$) can be of positive or negative curvature and consists of cylindrical micelles assembled in a hexagonal array. The cubic phases can be of two different types, those built up by discrete aggregates (I$_I$ and I$_{II}$) and those termed bicontinuous cubic phases (Q$_I$ and Q$_{II}$).[5-7] Bicontinuous cubic phases are infinite aggregates periodically curved in three dimensions. The mid-surfaces formed by these aggregates can be described by periodic minimal surfaces.
Non-cubic phases occupying regions between lamellar and hexagonal phases are collectively termed intermediate phases.\[8\] The structure of the intermediate phases is related to the adjacent hexagonal or lamellar phases. Three different kinds have been suggested, rectangular or ribbon structures, layered mesh structures and bicontinuous structures. They are all characterized by a non-uniform interfacial curvature.

Figure 2.3 The typical phase sequence of a given surfactant as a function of surfactant parameter and curvature.

2.2.1 Vesicles

Vesicles, or liposomes, consist of bilayers self-closed into spherical aggregates enclosing a water core. The formation of vesicles from a flat bilayer minimizes the edge energy, i.e. the energy cost of exposing the hydrophobic interior of the bilayer to water, but it also involves an energy cost due to the bending of the bilayer that induces excess curvature.\[9\] By using the Helfrich expression (equation 2.3) for the elastic curvature energy, it has been shown that the excess energy per vesicle is independent of the vesicle radius.\[10\] Hence, large vesicles are energetically favored, while entropy favors smaller vesicles.

Vesicles are generally not thermodynamically stable, and are probably best described as a dispersion of a lamellar phase in excess water. The existence of equilibrium vesicles has however been discussed.\[11-14\]
3 Segregation in Amphiphile Systems

While the behavior of single amphiphile systems has been quite well characterized in the past, much less is known about mixtures of amphiphilic molecules. The focus of this thesis is on the effects of segregation in two different kinds of mixed amphiphile systems. In mixtures of fluorocarbon and hydrocarbon surfactants, the net repulsive interactions between the surfactant chains can lead to demixing into two populations of micelles coexisting in solution. On the other hand, in lipid-surfactant mixtures, segregation may be caused by a difference in the preferred curvature of the molecules residing within the same aggregate.

This chapter serves to give a general introduction to the systems of interest in this thesis.

3.1 Fluorocarbon Surfactants and Fluorocarbon-Hydrocarbon Surfactant Mixtures

Fluorinated surfactants have properties that are very different from normal hydrocarbon surfactants.[15-17] Due to the strength of the C-F bond they are chemically and thermally very stable, making them useful under conditions where normal surfactants would not survive. Furthermore, because of the larger volume of fluorine, compared to hydrogen, fluorinated chains are more hydrophobic and hence the cmc:s of fluorinated surfactants are lower than for hydrocarbon surfactants of the same chain length. Fluorinated surfactants have a tendency to form aggregates with relatively little curvature, such as bilayers and cylinders instead of spherical micelles. This is explained by the large cross sectional area and the rigidity of fluorinated chains.[16,18] Intermediate phases are more often observed for fluorocarbon surfactants, since they are more likely to occur for surfactants with long or rigid tails.[8] In the first paper in this thesis
the diffusion of a fluorinated surfactant in an intermediate phase was studied.

Because of the net repulsive interactions among fluorinated and hydrogenated chains, they do not mix well. It is known that mixtures of alkanes and fluoroalkanes with sufficiently long chains phase separate macroscopically.[19] To settle whether there is a microscopic demixing into separate populations of micelles in fluorocarbon-hydrocarbon surfactant mixtures is not easy and many attempts using a variety of experimental techniques have been made.[20-36] The question whether such a demixing occurs is still a matter of debate, and some groups have obtained results that are contradictive.[19,20] In addition to demixing into two different populations of micelles, the possibility of segregation within the micelles has been discussed by a few authors.[36,37]

Rubingh and Holland developed a pseudo-phase separation model, where the regular solution approximation was utilized for describing the non-ideal interactions in the micelles.[21] This model results in a relation between the composition of the micelles, $x_1$ and $x_2=1-x_1$, and the monomer concentrations of the surfactants at the cmc, $C_1$ and $C_2$:

$$C_i = C_i^0 f_i x_i; i = 1, 2$$  \hspace{1cm} (3.1)

where $c_i^0$ is the cmc of the pure surfactants and $f_i$ the activity factors described by:

$$\ln f_i = \alpha (1 - x_i)^2$$  \hspace{1cm} (3.2)

In an ideally mixed micelle, the interaction parameter $\alpha$ is zero and the activity factor equals unity. Repulsive interactions, that may lead to a demixing into two different types of micelles, are described by $\alpha > 0$. In Paper II and III we studied a mixture of the cationic hydrocarbon surfactant $C_{16}$TAC and the partially fluorinated surfactant HFDePC, a system in which such a demixing has been suggested to occur.[29,38]
3.2 Perforated Lamellar Phases and Lipid-Surfactant Segregation

Lamellar phases have traditionally been looked upon as stacks of intact bilayer sheets separated by intervening layers of water. In recent years lamellar phases pierced by water-filled pores, or slits, have been observed in a number of systems.[39-49] These kind of lamellar phases were, together with the related intermediate mesh phases, reviewed by Holmes.[8] According to the analysis by Hyde, mesh phases are stable for values of the packing parameter between 1/2 and 2/3,[50] and are hence found to occupy regions in the phase diagram in between lamellar and hexagonal phases. The introduction of water-filled pores introduces curvature to the otherwise flat bilayer. It releases the frustration in the bilayer connected to packing molecules normally preferring a positive curvature into an aggregate of zero mean curvature. Different defect geometries have been discussed in the literature.[42,51] A pore can be described by two principal curvatures, one that is positive and one that is negative, while a channel is described by one positive curvature and the other curvature equals zero.

A recent thesis dealt with perforated lamellar phases in lipid-surfactant mixtures.[52] The effect of surfactant chain length and added salt was investigated. The chain length of the surfactant was found to be very important for the formation of pores. Perforated bilayers were observed for C_{16}TAC with EPC (egg-phosphatidyl choline)[48] and GMO, in water and in 100 mM NaCl.[52] Using a surfactant with a shorter chain (C_{12}TAC), no signs of a perforated lamellar phase were observed.[54] A local segregation of the lipids and the surfactants was suggested to occur within the perforated bilayer. The surfactant, with a preference for a more positive curvature, would accumulate at the edges of the holes and thereby stabilize the broken lamella. Using deuterated surfactants and lipids some weak evidence for such a demixing was actually found.[54]

Although a segregation of the amphiphiles might be crucial for the formation of pores in these systems, it should be pointed out that segregation of the constituting molecules is not a prerequisite for pore formation, since perforated bilayers have been observed also in single amphiphile systems.
In paper IV perforated vesicles formed in the mixture of GMO and C₁₆TAC in 100 mM NaCl were studied. These vesicles probably originate from the perforated lamellar phase described above. Perforated vesicles were first observed by freeze fracturing microscopy,[40] and have later on been observed by cryo-TEM.[48,53,55,56] This mixture, as well as a few other amphiphilic systems, was also investigated at the solid/solution interface using AFM in paper V.
4 Experimental Techniques

4.1 NMR

4.1.1 Pulse-field-gradient spin echo NMR

Pulse-field-gradient spin echo NMR (PGSE NMR) [57,58] is a tool for studying diffusion processes in the ms-s time range. In a spin echo experiment two pulses separated by a time \( \tau \) refocuses the magnetization lost by field inhomogeneities after another time \( \tau \). The transverse relaxation time \( T_2 \) is determined by varying \( \tau \) and measuring the echo intensity. In PGSE NMR strong pulsed magnetic field gradients are applied during the delay time \( \tau \) of the spin echo. If the molecules move while a gradient is applied, there is an extra loss of magnetization, and hence a loss of intensity, not described by the \( T_2 \) processes. This attenuation of the signal is related to the inhomogeneity of the magnetic field and to self-diffusion according to:

\[
E(g) \propto e^{(-\gamma^2 \delta^2 \gamma^2 D(\Delta - \delta / 3))}
\]

(4.1)

\( D \) is the self-diffusion coefficient, \( \gamma \) the magnetogyric ratio, \( \delta \) the duration of the gradient pulse, \( g \) the applied gradient strength, and \( \Delta \) is the diffusion time. The diffusion coefficient can be determined by recording the variation of the signal intensity with increasing gradient strength, by varying the duration of the gradient pulse or the diffusion time \( \Delta \). In cases when the time-constant \( T_1 \) is rather long and the spectral lines are broad, a stimulated echo version of the PGSE NMR experiment should be used. [58] Double echo experiments have been
designed to compensate for convection artifacts caused by small temperature gradients in the sample.[59]

PGSE NMR was used in paper II for studying the fluorocarbon-hydrocarbon surfactant mixture.

In recent years, PGSE NMR methods that include dipolar decoupling have been developed.[60-63] This makes it possible to measure self-diffusion coefficients in anisotropic phases where the spectral lines are broadened by strong spin interactions such as dipole-dipole couplings. In the first paper in this thesis the lateral diffusion of a cationic fluorocarbon surfactant in an intermediate phase was measured by combining PGSE NMR with homonuclear decoupling.

4.1.2 Exchange studies by NMR

Exploiting $^{19}$F chemical shifts can reveal information about molecular environments.[34,64-71] If the molecular environments are unchanged on the time-scale of the NMR experiment the presence of different environments for the nuclei involved is revealed by spectral broadening and/or splitting. These effects are both linear functions of the magnetic field strength. If instead the exchange is fast among environments with different chemical shifts, there will be no spectral splitting. The line broadening is in this case a quadratic function of the magnetic field strength:[66,69,72,73]

\[
R_s^{ex} = (\delta \gamma B_0)^2 x(1-x) \tau_e
\]  
(4.2)

where $\delta$ represent the difference between the two chemical shifts, $x$ the relative population at one of the sites, and $\tau_e$ the exchange time. The condition for the fast exchange regime, where the above expression is valid, is:

\[
\frac{1}{\tau_e} >> \frac{x}{(1-x)\tau_e} \approx \frac{\delta \gamma B_0}{(1-x)\tau_e} \approx \delta \gamma B_0
\]  
(4.3)
19F line width measurements were performed in the studies of the mixed micelles in paper II, yielding information on the molecular environment in the micelles.

4.2 Time-Resolved Fluorescence Quenching

Time-Resolved Fluorescence Quenching (TRFQ)[74] measurements were performed to study the mixed micelles in paper II. The fluorinated surfactant, HFDePC, quenches pyrene fluorescence. Following excitation, pyrene residing in micelles containing one or more quencher molecules will only contribute to the fluorescence during the first nanoseconds. The fluorescence at longer times stems from quencher-free micelles and the decay is single exponential. By comparing the intensity at long times with that in the beginning, the fraction of quencher-free micelles is obtained and assuming a Poissonian distribution of quenchers among the micelles the average number of quenchers per micelle can be determined. This is of course an approximate value, since the non-ideal interactions in the micelles are not taken into account.

4.3 Cryogenic Transmission Electron Microscopy

Cryo-TEM is a powerful technique for studying aggregates of amphiphilic molecules in water solution with no need for staining, drying or chemical fixation of the sample.[75-77] A crucial step is the preparation of the thin sample film, which is done following the procedure first described by Bellare et al.[77] A schematic illustration of the preparation technique is shown in figure 4.1. To prevent evaporation, the preparation is done within a climate chamber with controlled temperature and high humidity. A drop of the solution under study is placed onto a copper electron microscopy grid coated with a perforated polymer film. A thin film, with a thickness between 10 and 500 nm, of the sample spanning the holes in the polymer film is obtained by a blotting procedure, where excess liquid is removed using a filter paper. Rapid vitrification of the film is achieved by plunging the grid into liquid ethane held just above the freezing point. The sample is then transferred to the electron microscope for
examination. The microscope works in transmission mode, and the higher the electron density difference between the aggregates and the water, the better is the contrast. The temperature is kept low, below –165°C, during the entire procedure to prevent sample perturbation and formation of ice crystals.

Figure 4.1 The climate chamber used for the preparation of the sample and the electron microscopy grid with the perforated polymer film. © Göran Karlsson.

Artifacts

Cryo-TEM is an extremely useful technique for studying aggregates of amphiphilic molecules, whose structure is dependent on the surrounding solvent. A rapid vitrification is necessary to ensure that the aggregates observed really are present in the sample and not an effect of the preparation of the sample. A thin sample film ensures that the vitrification is fast and thus prevents crystallization of the water. Fast vitrification also normally means that no reorganization of the amphiphilic molecules take place. However, surfactants with a strong
temperature-dependent aggregation are unsuitable for cryo-TEM. Due to the fact that the thickness of the sample film spanning the holes in the polymer film varies substantially, a “size-sorting” of the aggregates can sometimes be seen. Large aggregates are predominantly located close to the edge of the holes, where the film is thicker, and smaller objects are normally observed closer to the center where the film is thinner. Since the surface to volume ratio of the thin film is high, another possible artifact is surface adsorption, both to the air/solution interface and to the support.[78] The presence of these kinds of artifacts calls for caution when interpreting cryo-TEM micrographs.

4.4 Small Angle Neutron Scattering

The very short wavelength of neutrons allows one to probe structure at much smaller length scales than possible with light, and this method is thus a complement to light scattering when one wants to study smaller objects.[79] Neutrons interact with the nucleus of the atoms. The relation between the scattering length density and the type of nucleus has been determined experimentally for each atom. The scattering length density of a molecule can then be calculated by summing the contribution from each atom. The very large difference in scattering length densities between H and D is utilized in the contrast variation method[80] that we used for studying the fluorocarbon-hydrocarbon surfactant mixture in paper III. By varying the H₂O/D₂O ratio of the solvent either the hydrocarbon or the fluorocarbon surfactant was made invisible for the beam. This method has earlier been used for studying similar systems.[36,81]

The intensity of the scattering from a system of monodisperse interacting micelles, assuming spherical symmetry, at an angle \( \theta \) is given by:

\[
I(Q) = A(\rho_m - \rho_s)^2 V_m^2 N_m P(Q) S(Q) \tag{4.4}
\]

where \( \rho_m \) is the coherent neutron scattering length density of the micelle and \( \rho_s \) is the coherent scattering length density of the solvent.
$A$ is an instrument calibration factor. $V_m$ is the micellar volume and $N_m$ the number of micelles per cm$^3$. $P(Q)$ is the form factor and $S(Q)$ the structure factor. $Q$, the scattering vector is given by:

$$Q = \frac{4\pi}{\lambda} \sin \left( \frac{\theta}{2} \right)$$

(4.5)

where $\lambda$ is the wavelength of the radiation and $\theta$ is the scattering angle.

**Data Analysis**

One way of analyzing small angle neutron scattering is by Indirect Fourier Transformation (IFT), a method introduced by Glatter.[82] It provides the pair distance distribution function, $p(r)$, and the radius of gyration. The squared radius of gyration is given by:

$$R_g^2 = \int_0^{D_{\text{max}}} p(r) r^2 dr$$

$$2 \int_0^{D_{\text{max}}} p(r) dr$$

(4.6)

The pair distribution function is described by a linear combination of a large set of basis functions. No assumptions on the shape of the particles are made. $D_{\text{max}}$, the maximum dimension of the particle, has to be estimated and is normally chosen so as to give the most accurate fits to experimental data. A disadvantage of IFT is that correlations between the particles can not be accounted for.

A second approach is direct modeling. The scattering data is fitted to analytical or semi-analytical expressions for the form and structure factors. Some common model expressions have been summarized by Pedersen.[83]

**4.5 Surfactant Selective Electrodes**  
Free surfactant concentrations can be measured using surfactant selective electrodes. By placing a reference solution and a test solution on either side of a membrane a measurable potential difference, that is
directly proportional to the logarithm of the surfactant concentration, is obtained. By the use of calibration curves the free surfactant concentration can be determined. The experimental set-up, similar to the one described by Maeda et al.[84] is schematically illustrated in figure 4.2. We used a PVC-based membrane with bis(2-ethylhexyl) phtalate as plasticizer. In the preparation of our membranes we followed the procedure described by Hayakawa and Kwak except that the carrier complex was left out.[85] The selectivity of these kinds of electrodes for surfactant ions has been documented earlier,[84] and they give a linear response in a broad concentration range.

In paper IV we used a surfactant selective electrode to determine binding isotherms of cationic surfactants to vesicles.

This technique has earlier been used to study the binding of surfactants to polymers.[74,86] A commercially available surfactant selective electrode has been used for studying the equilibrium between the dilute monomer solution of surfactant and a mesophase for a cationic surfactant/alcohol mixture.[87]

\[ \text{Figure 4.2 A surfactant selective electrode. (A) Reference electrode (B) Agar bridge with 0.5 M NH}_4\text{Cl (C) PVC membrane (D) Reference solution (E) Test solution (F) Saturated KCl solution. This set-up was earlier described by Maeda et al.[84]} \]
4.6 Small Angle X-ray Scattering

Detailed information on the structure of liquid crystals can be obtained from small angle x-ray scattering (SAXS).\cite{88} Bragg peaks are observed at low scattering angles, since the unit cell dimensions are in the range from ten to several hundreds of Ångström. The structure of the phase is recognized from the characteristic diffraction pattern, and once the dimension of the unit cell is determined the sizes of polar and apolar regions may be calculated. In paper IV the swelling of lamellar phases was determined from how the repeat distance obtained from SAXS varied upon dilution. The bilayer thickness was determined from the repeat distance of the most concentrated sample.

4.7 Atomic Force Microscopy

AFM was used in Paper V to study amphiphilic molecules adsorbed on mica. The first successful AFM-images of the different structures formed by surfactants adsorbed to hydrophobic and hydrophilic surfaces were presented by Manne et al.\cite{89,90} AFM imaging studies of surfactant adsorbed layer structures has recently been reviewed.\cite{91} Hydrophobic graphite and hydrophilic mica are usually the preferred substrates for AFM, since flat, clean surface are easily prepared by cleaving these materials.

The basic principles of the AFM technique are illustrated in figure 4.3. Three-dimensional topographical maps of the surface are obtained by scanning a cantilever over the sample surface (or scanning the surface under the cantilever). A sharp tip, mounted at the end of the cantilever, senses the force between the sample and the tip. The deflection of the cantilever is typically measured by bouncing a laser beam off the end of the cantilever. The motion of the laser beam is detected by a multiple segment photodiode (a Position Sensitive Detector). All the images in this thesis display deflection data captured using a soft contact method.\cite{92,93} Surfactant adsorption on both the tip and the substrate allow imaging using electric double layer forces without contacting the sample.
Figure 4.3 A schematic illustration of the AMF technique.
5 Results and Discussion

5.1 Diffusion of a Fluorinated Surfactant in an Intermediate Phase

In the first paper the lateral diffusion of the partially fluorinated cationic surfactant HFDePC \text{(N-(1,1,2,2-tetrahydroperfluorodecanyl)pyridinium chloride)} in the intermediate trigonal, sometimes referred to as rhombohedral, mesh phase prepared at about 84 w\% of HFDePC in D\textsubscript{2}O was studied.\cite{94} This phase is described as bilayers pierced by water-filled pore-defects arranged in a hexagonal pattern. The arrangement of the defects in adjacent layers, with the hole in one layer positioned over the node in the next layer, lends trigonal symmetry to the phase.

The diffusion of the surfactant was measured in the temperature interval 305-340K by a recently developed method that combines PGSE NMR with homonuclear decoupling and slice selection.\cite{62} Previously, NMR studies of anisotropic phases have been limited to samples with cubic symmetry or lamellar phases that could be oriented at a particular angle, the magic angle, where line-broadening effects due to dipole-dipole coupling disappear.\cite{95}. The advantage of the PGSE NMR technique compared to other techniques for measuring diffusion, for instance FRAP or single particle tracking, is that there is no need to add a probe molecule.

The $^{19}$F homonuclear-decoupled spectra of the mixture of 84 w\% HFDePC in D\textsubscript{2}O (figure 5.1) is essentially determined by the motionally averaged chemical shift anisotropy (CSA) of the $^{19}$F nuclei within the CF\textsubscript{2} and CF\textsubscript{3} groups of the surfactant tail. The low frequency edges of the CSA powder patterns of the CF\textsubscript{2} and CF\textsubscript{3} groups correspond to lamellar domains with the surface normal perpendicular to the applied magnetic field. Since the gradient
direction was set parallel to the main magnetic field, the diffusion coefficient that was extracted from the decay of the low-frequency edges in figure 5.1 describe the movement of the surfactant along the bilayer, $D_{\text{lat}}$.

![Figure 5.1](image)

**Figure 5.1** $^{19}$F-homonuclear-decoupled spectra of the mixture of 84 w% HFDePC in D$_2$O at 310K. The dashed lines represent the areas within which the intensity decay was measured.

In figure 5.2 the lateral diffusion coefficients obtained are plotted as a function of temperature. From the linear dependence on temperature an activation of $39 \pm 4$ kJ/mol was determined assuming Arrhenius-type behavior.

It should be pointed out that the lateral diffusion of the molecules is obstructed by the water-filled pores. The extent of this obstruction depends on the volume fraction of the pores. However, the average arrangement, and hence the volume fraction of the holes, is believed to be temperature independent since the x-ray diffraction pattern recorded at different temperatures has earlier been found to be rather constant.[94] The presence of large scale defects, such as terminated bilayers, also affect the diffusion. These kinds of defects would result in deviations of the decay of the NMR signal on increasing diffusion time and gradient strength. Such deviations were not observed, and the observed temperature dependence of the lateral diffusion coefficient was seemingly determined by the inter-molecular interactions.
The obtained activation energy for the diffusion of the fluorinated surfactant is much higher than the activation energy for diffusion in fluoroalkanes.\cite{96} It was concluded that in spite of the more rigid tail, the diffusion of fluorinated surfactants is dominantly governed by their headgroup-headgroup interactions. This is similar to what has been found for hydrogenated amphiphilic molecules a long time ago.\cite{97,98}

5.2 Mixed Micelles of Fluorocarbon and Hydrocarbon Surfactants

In paper II and III we studied mixtures of a cationic partially fluorinated surfactant, HFDePC, with C\textsubscript{16}TAC, looking for effects of demixing between the fluorinated and hydrogenated components. At relatively high total surfactant concentrations or with added salt, convincing evidence of a coexistence of two different kinds of micelles, C\textsubscript{16}TAC-rich and HFDePC-rich, was found. Figure 5.3 shows the hydrodynamic radius as a function of temperature obtained from the \textsuperscript{1}H-diffusion measurements on 50:50 % mixtures of C\textsubscript{16}TAC and HFDePC at two different total surfactant concentrations and with added salt.
The hydrodynamic radius was calculated from the observed time-averaged diffusion coefficients using the Stoke-Einstein equation. The larger values of the radius observed at low temperatures and high counterion concentration for the fluorinated surfactant (figures 5.3a and c) show that it spends, on average, more of the time residing in a larger micelle than the hydrocarbon surfactant. This was explained by considering two types of micelles of different size, the larger one rich in fluorinated surfactant and the smaller one rich in hydrogenated surfactant. Earlier results on this system have suggested such a demixing to occur.[29,38] At 5 w% without added salt (figure 5.3b), there is no significant difference in the hydrodynamic radii, and no conclusions whether there is a segregation or not could be drawn from these measurements.
The mixing of the two surfactants was found to be enhanced by increasing the temperature, and above approximately 315 K (42°C) only one kind of mixed micelle seemed to be present in the solution. Increased mixing with increasing temperatures has also been found for liquid hydrocarbon-fluorocarbon surfactant mixtures[19] and for neat hydrocarbon-fluorocarbon mixtures.[99,100] Critical demixing temperatures have earlier been determined for different mixtures of anionic fluorocarbon and hydrocarbon surfactants by Asakawa et al.[31]

Figure 5.4 shows non-exponential fluorescence decay curves for pyrene in a 50-50 % mixture of C_{16}TAC and HFDePC at two different temperatures, 27 and 60°C. The fluorinated pyridinium surfactant quenches pyrene fluorescence efficiently. The upper curves represent pyrene decay in pure C_{16}TAC micelles. In (a) the lower curve has a tail with the same decay rate as in C_{16}TAC micelle (upper curve) showing that a fraction of pyrene – about 1 \times 10^{-4} – is present in micelles without quenchers. This corresponds to an average of about 10 quenchers/micelle in the hydrocarbon rich micelle population, or a mole fraction of about 0.1 assuming the aggregation number to be 100. These calculations were based on a Poissonian distribution of quenchers, and non-ideal interactions were not taken into account. For this reason the mole fraction of fluorinated surfactant is probably larger than 0.1. As will be discussed below, we have indeed found indications of a demixing also within the micelles.

At 60°C quencher-free micelles could not be observed (figure 5.4b). This is in line with the diffusion measurements that also suggested increased mixing at higher temperatures.
Figure 5.4 Non-exponential pyrene decay curves in 50-50% mixtures of HFDePC and C_{16}TAC at two different temperatures (a) 27°C and (b) 60°C. Total surfactant concentration was 5 w%. The upper curves represent pyrene decay in pure C_{16}TAC micelles.

A cryo-TEM micrograph taken on a sample with 5 w% HFDePC-C_{16}TAC (50:50) in 100 mM NaCl indicated a coexistence of small spherical micelles, probably hydrocarbon-rich, and longer probably fluorocarbon-rich cylindrical micelles (figure 5.5). The SANS experiments, to be discussed below, cast some doubt on the existence of these very long micelles in the bulk solution at 25°C and indicated that they may be an artifact from the preparation of the specimen for the cryo-TEM investigation.[78]

Figure 5.5 Cryo-TEM micrograph taken on an equimolar mixture of HFDePC and C_{16}TAC in 100 mM NaCl. Total surfactant concentration was 5 w%. Bar equals 100 nm.
SANS measurements with contrast matching were performed to further investigate the surfactant mixture (paper III). These measurements were mainly performed at lower surfactant concentrations (0.5 and 2w%) and with added salt (100 mM NaCl) to minimize effects of interactions between the micelles. The mole fraction of C\textsubscript{16}TAC to HFDePC was varied between 0 and 1. The data were analyzed by fitting to various models for ellipsoids and polydisperse rigid rods\cite{83} and also in some cases by the Indirect Fourier Transform Method (IFT)\cite{82}. Table 1 in paper III summarizes the results from the model dependent fittings. All the data were fitted to a model for oblate ellipsoids of revolution, except for pure HFDePC that was fitted to a model for prolate ellipsoids in X(D\textsubscript{2}O) = 0.05 and a model for polydisperse rods in D\textsubscript{2}O. The size of pure HFDePC micelles were hence found to be dependent on the composition of the solvent. Also results from static light scattering measurements indicated that the HFDePC micelles are larger in D\textsubscript{2}O than in X(D\textsubscript{2}O) = 0.05. D\textsubscript{2}O effects have been observed in surfactant systems earlier\cite{101,102}.

As an example, in figure 5.6 the scattering data for a sample with X(C\textsubscript{16}TAC) = 0.5 in two different solvents are shown. The curve at X(D\textsubscript{2}O) = 0.05, the calculated match point for the hydrocarbon surfactant, is very similar to the curve at X(D\textsubscript{2}O) = 0.67, the match point for the fluorinated surfactant. This can be explained either by considering a coexistence of two types of micelles, fluorocarbon-rich and hydrocarbon-rich, that are very similar in size and shape, or by the formation of mixed micelles. Since the shape of the scattering curves of pure HFDePC- and C\textsubscript{16}TAC-micelles were found to be different (figure 5.6b), indicating that pure HFDePC-micelles are elongated, the formation of mixed micelles seemed to be the most likely explanation.
Figure 5.6 The neutron scattering data, together with the results from the model dependent fittings, for samples with (a) $X(\text{C}_{16}\text{TAC}) = 0.5$ in $X(\text{D}_2\text{O}) = 0.05$ and 0.67 and (b) $X(\text{C}_{16}\text{TAC}) = 0$ and 1 in $\text{D}_2\text{O}$. Total surfactant concentration was 2 w% in 100 mM NaCl.

Further indications of formation of only one kind of mixed micelles was obtained by measuring a sample with $X(\text{C}_{16}\text{TAC}) = 0.5$ at a solvent composition of $X(\text{D}_2\text{O}) = 0.35$, the calculated match point for completely mixed micelles (Figure 2, Paper III). If we consider completely demixed micelles, the absolute intensity of the scattering at this solvent composition would be approximately two times lower than for $X(\text{D}_2\text{O}) = 0.05$ and $X(\text{D}_2\text{O}) = 0.67$ (assuming that the volumes of the two micelle-types are approximately the same). The observed intensity of the scattering at $X(\text{D}_2\text{O}) = 0.35$ is much lower.
than this, about five times, which was explained by considering one kind of mixed micelle, but with an inhomogeneous distribution of surfactant within the micelles as will be discussed below.

The results from the IFT analysis, the radius of gyration and the scattering at zero angle, are presented in table 2 in paper III. For completely mixed micelles, a plot of the square root of the scattering at zero angle vs. contrast would give straight lines with the zero crossing points moving along the x-axis as the composition of the micelles is changed (Figure 3, Paper III).[36] For two types of coexisting micelles, on the other hand, deviations from straight lines should be observed, since the scattering intensity at zero angle would not equal zero at any solvent composition. The number of data points is small, and no conclusions regarding mixing/demixing could be drawn from these plots. From the zero crossing points of the straight lines the average scattering length densities of the micelles were determined and these values were found to be in reasonable agreement with the values calculated for completely mixed micelles (table 3, Paper III).

Taken together, the results from SANS and NMR can be explained by considering a formation of mixed micelles at low surfactant concentrations and a demixing into two different kinds of micelles at higher concentrations.

In the next section, further results indicating demixing within the micelles are presented.

5.2.1 Demixing Within the Micelles

$^{19}$F-linewidth measurements were performed on 50:50% mixtures of HFDePC and C$_{16}$TAC yielding information on the molecular environment of the fluorinated surfactant.[34,64-71] The results obtained at two different resonance frequencies, 188 and 470 MHz, are shown in figure 5.7 as a function of temperature for a sample with a surfactant concentration of 20w%.
Figure 5.7 $^{19}$F line widths of the difluoromethylene (a) and the trifluoromethylene (b) groups obtained at 188 MHz (×) and 470 MHz (●). Surfactant concentration was 20 w% of an equimolar mixture of HFDePC and CTAC.

The line width of the difluoromethylene group closest to the headgroup was larger and more field dependent than the line width of the trifluoromethyl group at the end of the tail. To explain this unexpected result, an exchange between sites for which the chemical shift difference is larger for the CF$_2$ than for the CF$_3$ fluorines was required. A model for the micelles within which the surfactants are separated as illustrated in figure 5.8 was suggested. The existence of such micelles has earlier been discussed.[36,37,103] For a sample with a total surfactant concentration of 5 w% (with no added salt), this effect was not observed.

Figure 5.8 A schematic illustration of segregation within a micelle. The fluorinated surfactants, with their headgroups crowded mostly in domain A, provide a fluorocarbon environment for the CF$_2$ groups located close to the headgroups. In domain B, this environment is instead hydrogenated, providing a different $^{19}$F chemical shift for those CF$_2$ groups. On the other hand, the CF$_3$ groups at the end of the tail reside in a mixed fluorocarbon-hydrocarbon environment that changes little if the headgroup is moved from domain A to domain B. From reference [104].
Supporting evidence of intramicellar segregation of the surfactants was obtained from the SANS measurements. In figure 5.9 the square of the radius of gyration, obtained from the IFT analysis as defined in equation 4.6, is plotted as a function of the reciprocal of the contrast for three different samples with $X(C_{16}TAC) = 0.25$, $0.5$ and $0.75$. The relation between the squared radius of gyration and the contrast is described by the Stuhrmann equation.[105]

$$R_g^2 = R_{g,0}^2 + \frac{\alpha}{\Delta \rho} - \frac{\beta}{\Delta \rho^2}$$

(5.1)

where $R_{g,0}$ is the radius of gyration at infinite contrast. The parameter $\beta$ is zero for the simple core+shell model that we used to account for the inhomogeneous contrast of the mixed micelles. The parameter $\alpha$, given by the slope of the lines, was found to be positive, suggesting that the core has a higher neutron scattering density than the shell. This was explained by considering an enrichment of the fluorinated surfactant in the outer shell of the micelles.

**Figure 5.9** The squared radius of gyration plotted versus the reciprocal of the contrast for samples with $X(C_{16}TAC) = 0.25$, $X(C_{16}TAC) = 0.5$ and $X(C_{16}TAC) = 0.75$. Total surfactant concentration was 2 w% in 100 mM NaCl.
Further indications of such a radial separation of the surfactants within the micelles were obtained from plots of the pair distance distribution function for a sample with $X(C_{16}TAC) = 0.5$ in the three different solvents (figure 5 in paper III). The lowest peak value of $p(r)$ was observed for $X(D_2O) = 0.05$. The higher maxima for $X(D_2O) = 0.67$ and 1 points to a higher scattering density in the core of the particles at these contrasts, i.e. a higher density of the hydrocarbon surfactant in the core. The segregation in domains, suggested by the NMR results, is not necessarily in conflict with the radial separation from SANS. An average inhomogeneous radial contrast distribution would normally result from laterally separated domains. For example, the micelle model by Fromherz,[103] where the surfactants are aligned side by side in a block-like arrangement, may probably account for both findings, as would many other arrangements of the surfactants in distinct domains. It should also be remembered that the SANS and the NMR measurements were performed at different concentrations.

5.3 Surfactant Binding to Vesicles

In paper IV we studied the binding of four different cationic surfactants to preformed GMO/surfactant-vesicles. The surfactants studied were C$_{12}$TAC, C$_{14}$TAC, C$_{16}$TAC and HFDePC. The vesicles were prepared by sonicating mixtures of GMO and a small amount of the cationic surfactant in 100 mM NaCl. GMO itself forms a reverse bicontinuous cubic phase, which upon surfactant addition transforms into a lamellar phase.

The main interest was the perforated vesicles observed earlier in similar systems,[48,53] and the possibility of a local segregation of the lipids and the surfactants within the membrane. The free surfactant concentration was monitored using a surfactant selective electrode and the aggregate structures formed were directly visualized using cryo-TEM. The results obtained from the binding studies were compared to the phase behavior studied separately.

5.3.1 Aggregate Structure and Binding Isotemps

The solubilization of the vesicles brought about by C$_{12}$TAC, C$_{14}$TAC and HFDePC was found to follow the route described by the so called
three stage model.[106-109] At low additions of surfactants, intact vesicles were found. Increasing the surfactant concentration lead to a coexistence of vesicles and micelles, and finally only micelles were found in the solution (figure 5.10).

**Figure 5.10** A cryo-TEM micrograph from a sample at a molar ratio of C₁₂TAC to GMO of 4 showing intact vesicles (a). At a molar ratio of C₁₂TAC to GMO 6.4 mostly spherical micelles but also vesicles were observed (b). At a molar ratio of HFDePC to GMO of 4 a coexistence of vesicles and spherical micelles was observed (c), and at a molar ratio of 50 threadlike micelles were seen (d). All solutions contained 100 mM NaCl and the concentration of GMO was 1.0 mM. Bar equals 100 nm.

A large micelle-vesicle two phase region, as observed by cryo-TEM and dynamic light scattering, was found in the solubilization of the vesicles by the partially fluorinated surfactant HFDePC. The large two phase area is an effect of the repulsive hydrocarbon-fluorocarbon interactions. In the present case the micelles formed on solubilization of the vesicles contain very little of the hydrocarbon compound (GMO), whereas the bilayers seem to accept up to about 30 mol% of the fluorinated surfactant.

Only spherical micelles were found in coexistence with vesicles in the three different systems. The threadlike micelles observed in figure 5.10d is probably a consequence of the presence of HFDePC in the
micelles, reflecting the larger tendency of fluorinated surfactants to form aggregates with less curvature than hydrocarbon surfactants.[18]

If we instead turn to the solubilization of the vesicles by C_{16}TAC, a very different sequence of aggregates was found as shown in figure 5.11. Intact vesicles were observed at low surfactant concentrations and with increasing surfactant concentrations perforated vesicles and finally threadlike micelles were found. No coexistence region of vesicles and micelles could be detected.

![Cryo-TEM micrographs from samples with molar ratio of C_{16}TAC to GMO of 1.7 showing vesicles with perforated membranes (a) and with a molar ratio of 2.3 showing threadlike micelles (b). The solutions contained 100 mM NaCl. Bar equals 100 nm.](image)

The binding isotherms, constructed by subtracting the free surfactant concentration measured by the surfactant selective electrode, from the total surfactant concentration, are shown in figure 5.12.
The principal behavior of the surfactant binding to the vesicles was described by considering the chemical potential of the surfactant in the aggregates and in the aqueous pseudo-phase. At equilibrium, the chemical potential of the surfactant must be equal in the two pseudo-phases, yielding the following expression:

\[
\ln \frac{X_s}{[S]_f} = \frac{1}{RT} \left( \mu_{s,ag} - \mu_{s,b}^0 - \gamma_b A_{s,b} - \mu_{s,b}^c \right) = \ln K_b \tag{5.2}
\]

where \( b \) stands for bilayer.[110] The contributions for the mixed micelles are given by a similar equation, with index \( m \) instead of \( b \).
\( \mu^0_{s,b} \) is the standard chemical potential of the surfactant in the bilayer, \( \gamma_b \) is the bilayer-water surface tension, \( A_{s,b} \) is the area per surfactant in the bilayer, \( \mu^\ell_{s,b} \) is the contribution to the chemical potential due to the electrostatic interactions of the surfactant headgroups in the bilayer, \( X_s \) is the mole fraction of surfactant in the bilayer and \([S]_f\) the concentration of surfactant in the aqueous pseudo-phase. In the micelle case there are further contributions to the chemical potential that are neglected here. \( K_b \) is the partitioning coefficient for the surfactant between the bilayer and the aqueous solution. The coefficient, \( K_m \), for partitioning between water and mixed micelles is defined in a similar way.

Since we know that the surfactant itself forms micelles, at \( X_s = 1 \) the micellar phase is the stable phase. Bilayers at \( X_s = 1 \) would therefore give a larger chemical potential for the surfactant. This means that \( K_b \) is smaller than \( K_m \). For an ideal mixture \( K_m = 1/cmc \).

The experimental results were explained by the equation above for the variation of \( X_s \) with \( [S]_f \). At low surfactant concentrations the bilayers would be the stable phase. At a certain \([S]_f\), corresponding to the first breakpoint in the binding isotherms, mixed micelles and bilayers give the same chemical potential of the surfactant. In this two-phase region, the composition of the micelles and the bilayers, and hence the free surfactant concentration, remain constant according to the three stage model. At higher surfactant concentrations, only mixed micelles were found, with a composition that is described by equation (5.2). The free surfactant concentration varies as the composition of the micelles is changed.

This simple model predicts that the slope of the line in the bilayer region is lower than in the micelle region, determined by \( K_b \) and \( K_m \), which is also found experimentally. The reason why micelles are not formed at lower surfactant concentrations is the constraint that the radius of the micellar core can not largely exceed the length of a fully stretched surfactant chain.

The binding isotherm for C16TAC is very similar to the three other binding isotherms, although no vesicle-micelle two-phase area was observed. This indicates that the pore formation in the bilayers is a cooperative process, as described in the next section. In the simple
model described above the formation of pores was regarded in the same way as formation of micelles.

5.3.2 Pore Formation in Bilayers

The almost vertical slope of the binding isotherm implies that in the region where these perforated vesicles exist, the free surfactant concentration, and hence the chemical potential of the surfactant, is almost constant, similarly to what was observed for the two-phase regions with C_{12}TAC, C_{14}TAC and HFDePC. As mentioned above, a local segregation of the lipids and the surfactants in perforated lamellar phases has earlier been suggested to occur.[48,54] In line with these earlier results, we explained the observed constant chemical potential of the surfactant by considering such a segregation of the amphiphilic molecules. Pores, with a fixed surfactant to lipid ratio at the edges, larger than the ratio in the defect-free parts of the bilayer, would start to form at a critical surfactant concentration in the bilayer; cpc (critical pore concentration). Increasing the surfactant concentration would lead to an increased number of pores and/or a change of the diameter of the pores, but the composition at the edges would not change, similar to a micelle-vesicle phase separation. At relatively high surfactant concentrations only edges remain in the form of long entangled threadlike micelles. This process is schematically illustrated in figure 5.13.

![Figure 5.13](image-url) A schematic illustration of the formation of pores in the bilayer. Pores start to form at a certain critical surfactant concentration, cpc, in the bilayer. Adding more surfactant leads to a higher density of pores until only pore edges remain in the form of threadlike micelles.
If this kind of segregation occurs, it is driven by a difference in the preferred curvature of the lipids and surfactants. In the hydrocarbon-fluorocarbon surfactant mixture discussed above the segregation was instead caused by the intrinsic tendency of fluorinated and hydrogenated chains to demix. Perforated vesicles were not observed upon vesicle solubilization with the fluorinated surfactant. Hence, it appears that the curvature-driven separation that leads to pore formation is not facilitated by the repulsive interactions between fluorocarbon and hydrocarbon chains.

Another possible explanation for the constant chemical potential of the surfactant was considered. In the earlier determined phase diagram of GMO/C_{16}TAB/100 mM NaCl an isotropic flow birefringent fluid phase, denoted L_1^{*}, was found.[53] If we upon the vesicle solubilization pass through a L_α/L_1^{*}/L_1 three phase region, this would of course result in a constant chemical potential of the surfactant. However, such a three-phase region probably exists only in a narrow range of surfactant to lipid ratios close to the micellar phase border.

The reason for why pores are formed in the bilayers with C_{16}TAC but not with the shorter chain surfactants, C_{12}TAC and C_{14}TAC, is not clear. Probably only small changes may alter the preferred aggregate structure from perforated vesicles to instead a micelle/vesicle coexistence.

5.3.3 Phase Diagrams

The pseudo ternary phase diagram of GMO/C_{16}TAB/100 mM NaCl has been studied earlier.[53] Instead of a lamellar/hexagonal or lamellar/micellar two-phase region a lamellar phase pierced with water-filled curvature defects extending far into the solvent corner was observed. The swelling behavior of this lamellar phase was found to be highly non-ideal - at high dilution a deviation from ideal behavior of about 30% was observed from SAXS-measurements. Such a strong negative deviation has earlier been taken as an indication of pore formation,[47,48] since the introduction of water-filled pores in the bilayer would decrease the distance between the bilayers. The perforated vesicles observed by cryo-TEM probably originate from this lamellar phase with curvature inhomogenieties.
The solvent corner of the phase diagrams of GMO/C$_{12}$TAC/100 mM NaCl and GMO/C$_{14}$TAC/100 mM NaCl, determined by visual inspection supported by SAXS measurements, are shown in figure 5.14. These phase diagrams are very similar to the earlier determined phase diagram of EPC/C$_{12}$TAC/100 mM NaCl.[54]

As expected from the cryo-TEM investigation, where vesicles were found in coexistence with micelles, well-developed two-phase areas were found between the lamellar and hexagonal or lamellar and micellar phases. The swelling of the lamellar phases, obtained from SAXS-measurements, were found to be close to ideal suggesting that the bilayers are intact.

![Figure 5.14](image)

Figure 5.14 The solvent corner of the phase diagrams (a) GMO/C$_{12}$TAC/100 mM NaCl and (b) GMO/C$_{14}$TAC/100 mM NaCl. $\alpha$ denotes the lamellar phase, $L_1$, $H_1$ and $I_1$ denote the micellar, hexagonal and micellar cubic phases, while $Q_2$ denotes the reverse bicontinuous cubic phase. All the multiphase regions are not depicted in the phase diagrams.

From the binding isotersms, $X_{s}^{\text{sat}}$ and $X_{s}^{\text{sol}}$ were determined. $X_{s}^{\text{sat}}$ is commonly used to denote the molar fraction of surfactant in the bilayer at the point where the surfactant concentration in the aqueous phase reaches cmc and mixed micelles start to form. $X_{s}^{\text{sol}}$ refers to the mole fraction of surfactant in the micelles when they first appear in the solution. These values, presented in table 5.1 together with the bilayer partition coefficients obtained from the binding isotersms and the cmc:s of the surfactants, corresponded relatively well to the location of the phase borders in the phase diagrams. Moreover, the
first breakpoint in the binding isotherm of C\textsubscript{16}TAC (referred to as $X_s^{\text{sat}}$ in the table), the point where perforated vesicles were believed to form, corresponds to the surfactant to lipid ratio in the phase diagram where the perforated lamellar phase is entered.

Table 5.1. The cmc:s, the bilayer partitioning coefficients $K_b$ and $X_s^{\text{sat}}$ and $X_s^{\text{sol}}$ determined from the phase diagrams (values obtained from the binding isoterms are given in parenthesis). $X_s^{\text{sat}}$ for C\textsubscript{16}TAC refers to the surfactant to lipid ratio at which the defective lamellar phase is entered.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>cmc$^a$</th>
<th>$K_b$</th>
<th>$X_s^{\text{sat}}$</th>
<th>$X_s^{\text{sol}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{12}TAC</td>
<td>7.2</td>
<td>50</td>
<td>0.43</td>
<td>0.60</td>
</tr>
<tr>
<td>C\textsubscript{14}TAC</td>
<td>0.80</td>
<td>420</td>
<td>0.49</td>
<td>0.68</td>
</tr>
<tr>
<td>C\textsubscript{16}TAC</td>
<td>0.068</td>
<td>5400</td>
<td>0.57</td>
<td>0.71</td>
</tr>
</tbody>
</table>

$^a$The cmc:s of the surfactants in 100 mM NaCl were obtained from the calibration curves used for the surfactant selective electrode measurements.

5.4 AFM studies of Mesh Phases at the Solid/Solution Interface

In the last paper in this thesis the structure formed upon adsorption to mica was studied in different amphiphile systems using AFM. The aim was to investigate if a counterpart to the perforated bilayers also could be manifested at the solid/solution interface, as an intermediate between bilayers and cylindrical micelles.

The systems examined were:

1) Didodecyldimethylammonium bromide (DDAB) and dodecyltrimethylammonium bromide (DTAB) on mica and quartz

2) GMO and C\textsubscript{12}TAC or C\textsubscript{14}TAC in 100 mM NaCl on mica$^1$

3) a cationic gemini surfactant called 12-2-12 and CsCl on mica

$^1$ I performed the experimental work on this system.
4) C\textsubscript{14}TAC and sodium benzoate on mica

Mixtures of GMO and C\textsubscript{16}TAC were also investigated. An unstructured bilayer was found to form at all compositions examined, as was also found for pure C\textsubscript{16}TAC in 100 mM NaCl.

Figure 5.15 shows a sequence of AFM images of mixed GMO/C\textsubscript{14}TAC adsorbed layers on mica at various bulk compositions. The Fourier transforms of the images are also shown. Samples with relatively low surfactant concentrations showed no lateral structure and no periodicity was evident in the Fourier transforms (figure 15.15a). At high surfactant concentrations the characteristic stripes of adsorbed cylinders were observed (figure 5.15c). The Fourier transforms also revealed the anisotropic structure of the adsorbed aggregates. Pure C\textsubscript{14}TAC was also found to form adsorbed cylinders on mica. At intermediate GMO/C\textsubscript{14}TAC compositions an adsorbed layer with a periodic but isotropic structure in the plane was observed (figure 5.15b). The Fourier transform of these images showed faint but distinct isotropic rings. This isotropic structure was interpreted as a mesh.

![Figure 5.15](image)

**Figure 5.15** AFM deflection images, and their Fourier transforms, of mixed adsorbed layers of GMO and C\textsubscript{14}TAC on mica at the following bulk compositions (a) GMO 0.5 mM, C\textsubscript{14}TAC 2.5 mM (b) GMO 0.25 mM, C\textsubscript{14}TAC 2.75 mM and (c) GMO 0.15 mM, C\textsubscript{14}TAC 2.85 mM. All solutions contained 100 mM NaCl.

The preferred curvature of the adsorbed aggregates was varied by different mechanisms in the four systems. The transition between homogeneous bilayers and cylinders was in all these systems interrupted by the appearance of a structure of intermediate curvature - a mesh or a two-dimensional layer of highly branched micelles - similar to the mesh structure in figure 15.15b.
Earlier studies have shown that the adsorbed structures formed upon variation in surfactant structural parameters follow the same trend as expected from bulk behavior.[89,90,111-117] However, mesh structures have not, to our knowledge, been observed earlier at the solid/solution interface. It should be pointed out that these lateral structures in the AFM images were not always easily distinguished from adsorbed globular aggregates. Sometimes it was only the curvature progression with composition that made us interpret the structure as a mesh instead of adsorbed globules. We also considered the possibility of formation of hemispheres on top of a hydrophobic monolayer to explain the observed structures. We however found this less likely than the formation of mesh structures.
6 Conclusions

The diffusion of HFDePC in the intermediate trigonal mesh phase was measured as a function of temperature by using PGSE NMR combined with homonuclear decoupling. The activation energy for the lateral diffusion was determined, and it was concluded that the diffusion was dominantly governed by the headgroup-headgroup interactions, similar to what has been found for hydrocarbon surfactants.

The mixture of a partially fluorinated surfactant, HFDePC and C_{16}TAC was investigated by using NMR, fluorescence quenching, cryo-TEM and neutron scattering. Strong indications of demixing into two different types of micelles, fluorocarbon rich and hydrocarbon rich, were found at high surfactant concentrations. A demixing of the surfactants within the micelles was also suggested to occur. The segregation is an effect of the repulsive interactions between the chains.

Perforated vesicles, observed in dilute mixtures of the lipid GMO and C_{16}TAC by cryo-TEM, were investigated using a surfactant selective electrode. In this case segregation between the molecules was suggested to occur due to the difference in the preferred curvature of the lipid and the surfactant. The surfactant would accumulate at the edges of the pores and thereby enjoy a more positive curvature than in the otherwise flat bilayer.

Furthermore, adsorbed mesh structures at the solid/solution interface were visualized using AFM in a number of systems. The preferred curvature of the aggregates was varied by different mechanisms to yield morphologies between adsorbed cylinders and bilayers.
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Uppsala, September 2003
Mari
References


A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to October, 1993, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science”.)