Structural Studies of Surfactants at Interfaces
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Structural Studies of Surfactants at Interfaces

by

Mario Campana

For the degree of Doctor of Philosophy

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School of biological and Chemical Sciences

Queen Mary, University of London
Declaration

“I confirm that this thesis and the research presented in this thesis are the product of my own work, any ideas or quotations from the work of other people, published or otherwise, are fully acknowledged in accordance to standard reference practices of the discipline.”

______________

Mario Campana

02 July 2012
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“E quindi uscimmo a riveder le stelle.”

Dante, Inferno, Canto XXXIV, 139
Abstract

This thesis consists of a collection of studies on surfactant adsorption at different interfaces. Particularly, this dissertation focuses on adsorption processes occurring at buried interfaces (solid-liquid and liquid-liquid). Because of complexity in the sample environment, the study of buried interfaces is experimentally challenging.

Neutron reflectivity enables the study of adsorption processes at interfaces at atomic length scale. Furthermore, neutrons can be transmitted through solid substrates, permitting the analysis of buried interfaces. The technique was used to describe adsorption processes both qualitatively and quantitatively, delivering information regarding structure of adsorbed layers and adsorbed amount.

Different investigations were carried out during the PhD and the results are grouped into two main sections. Investigations at the solid-liquid interface are presented in the first section. Chapter 3 provides an example of structural study of complex multi-layers at the silicon-water interface; a surfactant adsorption study at the technologically relevant metal-oil interface is presented in Chapter 4.

The second section discusses a series of neutron reflectivity experiments at the important oil-water interface. The structural study of a series of non-ionic dodecanol ethoxylate surfactants is discussed in Chapter 6. The structure of a lipid monolayer as model for a biological membrane is reported in Chapter 7. Chapter 8 is a comparative study of fatty acid-alkylated azacrown ether co-adsorption at the air-water and oil-water interface. These mixtures are used for metal ion extraction processes. This was the first analysis of a surfactant mixture at the oil-water interface using neutron reflectivity.

Some of the studies reported here are the first of their kind and the advances affect different technologically and biologically relevant areas. As a result of this PhD project a number of follow-up studies have been planned and several neutron reflectivity experiments will be performed in the future to further explore these interesting areas of science.
The following publications were produced as part of this PhD project:


- Structural Conformation of Lipids at the Oil-Water Interface. Mario Campana, John R P Webster, Jayne M Lawrence, Ali Zarbakhsh; *Soft Matter*, article in press.
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Surfactants and interfaces: an introduction

1.1 Interfaces

The boundary surface between two different phases is commonly referred to as an interface. An interface could separate a liquid from a gas, two immiscible liquids or a solid from a liquid. As in any other system, an interfacial region reaches equilibrium by minimising its free energy. The interfacial free energy is a fundamental property of any interface and can be easily determined by measuring the interfacial tension.

The existence of condensed phases is caused by the attractive forces between molecules being sufficiently large to overcome Brownian motion. Cohesive forces between molecules in a bulk liquid are responsible for the surface tension. If we consider a water molecule in the bulk, it establishes attractive interactions in every direction with neighbouring water molecules. Because of these attraction forces, the energy of a molecule surrounded by others is lower than in conditions of isolation. The reduction of neighbouring molecules would lead to a decrease in the number of attractive interactions and an increase in energy, and will be opposed by the system. This is what happens at the surface: a water molecule at the surface has a reduced number of neighbouring molecules and, therefore, has higher energy. The attractive forces are unbalanced and consequently molecules at the interface are pulled towards the bulk (see Figure 1.1). As a result, the liquid surface contracts and tends to assume the minimal area.
Figure 1.1. Attractive forces for a molecule at the surface (left) and in the bulk. For molecules at the surface the inter-molecular forces are not balanced, therefore interfacial molecules present a higher energy compared to molecules in the bulk.

This phenomenon is known as surface tension and is a measure of the strength of the cohesive forces in the bulk. It is expressed in energy per unit area, or more generally force per unit length (mN m$^{-1}$).

The surface tension, or more generally the interfacial tension $\gamma$ is defined as:

$$\gamma = \frac{\partial G}{\partial A}$$  

Equation 1.1

$G$ is the interfacial free energy and $A$ is the interfacial area.

The situation is slightly more complicated in the case of two immiscible liquids such as oil and water. A water molecule at the oil-water interface may have the same number of neighbours as one in the bulk, but the attractive interactions between water molecules will be more favourable than water-oil interactions. Therefore the force pulling water molecules at the interface toward the bulk will be lessened. This results in an interfacial tension at the oil-water interface lower than the surface tension.
1.1.1 Interfacial tension measurements

Over the years a number of methods have been developed to measure the surface tension of a system. The most commonly used are discussed briefly below.

1.1.1.1 Capillary rise method

Attractive interactions between glass and most molecules are often highly favourable and most liquids display a concave meniscus when placed in a glass tube. If the section of the tube is sufficiently small, the liquid will be subject to capillary action and will be drawn up.

When the liquid is in contact with the capillary tube, it wets the walls, thus increasing the surface area (Figure 1.2 (a)). The surface tension opposes this increase, pulling the liquid up the column (Figure 1.2 (b)). Wetting continues, causing further rise of the liquid, which is again opposed by the surface tension (Figure 1.2 (c)). The capillary rise stops when the weight of the risen liquid is exactly balanced by the surface tension, resulting in a constant height (Figure 1.2 (d)).

![Figure 1.2](image-url)

**Figure 1.2.** Surface tension acts in a thin tube causing capillary rise (a → d). The rise equilibrates when the hydrostatic pressure of the raised liquid balances the surface tension.

The capillary rise can be used to calculate $\gamma$ using the following equation:
In Equation 1.2, \( h \) is the capillary rise, \( \rho \) the density of the liquid, \( g \) the acceleration of gravity, \( r \) the radius of the tube and \( \theta \) is the contact angle. Note that if the contact angle is greater than 90° the capillary rise will be negative, meaning that the cohesive forces within the bulk phase are stronger than the attractive forces with the glass surface. This is, for example, the case with liquid mercury, which presents an upwardly convex meniscus. Equation 1.2 also shows that the capillary rise is inversely proportional to the tube radius, explaining why the phenomenon can be quantitatively observed only for a small cross-section of the tube (Figure 1.3).

\[ \gamma = \frac{h \rho g r}{2 \cos \theta} \]

Equation 1.2

**Figure 1.3.** The increase in capillary rise with decreasing diameter can be visualized by using colored solutions.

### 1.1.1.2 Drop volume

In this methodology, the weight (or volume) of a drop falling from a capillary with radius \( r \) is measured. A series of drops are formed at the end of the tube, allowing them to fall into a container until enough have been collected so that the weight per drop can be accurately measured.
Figure 1.4. The size of a drop falling from a capillary tube is a function of the surface tension (a). As the drop falls off the tube, a portion is inevitably left behind (b). The volume left in contact with the tube is accounted for by the correction factor in Equation 1.4.

The weight of the drop falling off the capillary \( W \) is linearly related to the surface tension of the liquid:

\[
W = 2\pi r \gamma
\]

Equation 1.3

When the drop has reached the point of instability and it falls off the capillary, a portion of the drop normally remains attached to the tip. This is schematically shown in Figure 1.4. Generally a correction factor \( f \) is applied so that the measured weight of the drop \( (W') \) is:

\[
W' = 2\pi r \gamma f
\]

Equation 1.4

The correction factor \( f \) is expressed as:

\[
f = \frac{r}{V^{1/3}}
\]

Equation 1.5

\( V \) is the drop volume.
1.1.1.3 Plate method

The plate method is also called the Wilhelmy method after its creator. It requires the use of a tensiometer from which a plate of known geometry is suspended. The lower edge of the plate is initially brought in contact with the interface. Attractive forces take place between the plate and the liquid so that a meniscus is formed. The raised liquid pulls the plate into the bulk phase. By measuring the weight of the meniscus one can accurately determine the surface tension:

\[ W_{\text{meas}} = \gamma p \]  

Equation 1.6

In Equation 1.6, \( W_{\text{meas}} \) is the force measured by rising the plate back to the level of the surface and \( p \) is the perimeter of the plate. A typical tensiometer and a schematic representation of the plate method are shown in Figure 1.5.

![Tensiometer and schematic representation of plate method](image)

**Figure 1.5.** A tensiometer used for the plate method is shown on the left. A schematic representation of the forces acting upon the plate is shown on the right. The thickness of the plate is generally negligible compared to its length. The same tensiometer can be also used for the Du Nouy ring method, discussed in the following section.

One significant advantage offered by the Wilhelmy method is that the measurement is static. This means that it is possible to continuously measure the surface tension of the
system without the need of the constant formation of a new surface. This makes the plate method ideal for studying the evolution of the surface tension in a system over long periods of time.

1.1.1.4 Du Nouy ring method

The Du Nouy ring method takes its name from its creator. The method is similar to the plate method discussed above, but in addition to surface tension its use can be easily extended to the investigation of interfacial tension. Instead of a plate, a circular platinum wire of known cross section is used for the measurement. The force measured is the maximum force required to pull the ring through the interface. As opposed to the plate method, the ring method is a dynamic measurement.

The investigated interface is flat at the beginning of the measurement and the ring is fully immersed in the lower medium, Figure 1.6 (a). As the ring goes through the interface, a meniscus is formed (Figure 1.6 (b)), meaning that new surface is being formed and added to the initial surface. Given the ring method is a dynamic measurement, it is not suitable for studying the changes of interfacial tension over time. The pull increases up to a maximum when the meniscus forms a 180° angle with the ring (Figure 1.6 (c)). Because of the geometry of the ring, the meniscus may not detach from it right after it has reached a maximum, in which case a decrease in pull on the ring is observed before the detachment (Figure 1.6 (d)).

Similar to the plate method, the interfacial method is a function of the wetted length, hence the inner and outer circumference of the ring. The cross-section of the wire is small, so that the difference between the two circumferences can be considered negligible.

\[ W_{\text{meas}} = 4\pi R\gamma F \]  
Equation 1.7

In Equation 1.7, \( R \) is the radius of the ring and \( F \) is an empirical correction factor. The correction factor is required as some bulk liquid underneath the ring is also raised during the measurement, affecting the measured surface (or interfacial) tension. \( F \) has been experimentally determined [1] and a correlation equation has been developed [2]:
Figure 1.6. Schematic representation of the Du Nouy ring method. We consider the meniscus raised by the wire supporting the ring negligible; when the ring is immersed in the lower bulk phase (a) no force is measured. As the ring is brought in contact with the interface, a meniscus is formed and the balance starts registering an increasing pull on the ring (b). The pull increases up to a maximum when the meniscus forms a 180° angle with the ring (c). The meniscus may not detach from the ring right after it has reached a maximum and a decrease in pull on the ring can be observed before the detachment.

\[ F = 0.725 + \frac{0.01452 \gamma^*}{\sqrt{0.25 l^2 \Delta \rho}} + 0.04534 - \frac{1.679}{R/r} \]  

Equation 1.8
In Equation 1.8, $\gamma^*$ is the un-corrected interfacial tension, $l_b$ is wetted length ($l_b = 4R\pi$), $\Delta\rho$ is the difference in density between the two bulk phases, $R$ is the radius of the ring and $r$ the radius of the wire cross section. Equation 1.8 is verified for $\gamma^* < 25 \text{ mN m}^{-1}$ and $\Delta\rho > 0.1 \text{ g cm}^{-3}$, which was the case for all interfacial tension measurements carried out in this thesis. However, the equation is often used above the range of validity.

1.1.2 Interfacial tension in the presence of solutes

The presence of very small amounts of solutes in a liquid may not significantly alter its bulk properties but major changes may be observed at interfaces. A solution can reduce its surface tension by increasing or decreasing the concentration of solutes at the interface depending on the relative magnitude of solvent-solvent and solvent-solute interactions. For an aqueous saline solution, ion-water interactions are generally stronger than water-water interactions and the concentration of ions at the surface becomes depleted. The presence of ions decreases the free energy of the solution; therefore the interfacial depletion of ions leads to a small increase in the surface tension compared with pure water. On the other hand, if the water-solute interactions are less favourable than water-water interactions, the solute concentration at the interface will increase. This phenomenon is known as adsorption and it occurs alongside a decrease in surface tension. Surfactants are known readily to adsorb at interfaces and are capable of lowering the surface tension.

1.2 Surfactants

The word surfactant is an abbreviation of SURFace ACTive AgeNT. Surfactants in solution spontaneously and promptly adsorb onto the water surface forming a monolayer. Because of their ability to lower the surface tension of a liquid surfactants
are used in a broad range of applications including detergents, food, cosmetics, paints, drug-delivery and many others.

Surfactants possess both a hydrophilic and a hydrophobic part; therefore they are also called amphiphiles. The hydrophobic part, commonly referred to as the tail group, is generally a linear or branched hydrocarbon group. Other common hydrophobic tail groups include fluorinated carbon chains which are broadly applied as stain repellents and paint additives [3]. The tail group region is generally oil soluble and its presence in bulk water is not energetically favoured as it tends to disrupt the water molecule network.

![Figure 1.7](image-url)  
Figure 1.7. General representation of the three surfactant categories: ionic, non-ionic and zwitterionic.

The hydrophilic part, or head group, consists of a polar group. Different degrees of hydration can be observed depending on the nature of the head group, but this part of the surfactant is generally water soluble (although some degree of solubility in oil could be observed, particularly for non ionic surfactants). The size of the head group can vary
from a few atoms to very complex structures and its nature extensively influences the surfactant’s behaviour. In fact, surfactants are generally classified depending on the nature of the head group, i.e. presence or absence of charges. There are three main surfactant categories: they are schematically shown in Figure 1.7 and briefly presented here. Each class can be further divided in sub-categories.

### 1.2.1 Ionic surfactants

Ionic surfactants carry a net charge on the head group. The charge can be negative (anionic) or positive (cationic surfactants). Sulphates, sulphonates and phosphates are among the most common anionic surfactants. All these compounds carry a permanent charge on the head group. Carboxylates are also very common anionic surfactants, but it must be stressed that the presence of the charge in the head group depends on the pH of the solution. Anionic surfactants are extensively used as cleaning products because of their marked detergency properties.

Cationic surfactants mostly consist of amines. These compounds can also carry a permanent or a pH dependent charge. Quaternary ammonium compounds, for example, carry a permanent charge, while other amines can be charged or neutral depending on the pH. These compounds are known to be excellent conditioners. The surface of most fabric and hair contains sites which are negatively charged: the positive head group of cationic surfactant is attracted to these sites and the long hydrophobic tail will tend to lie along the fabric or hair surface, providing a smooth coating.

### 1.2.2 Non-ionic surfactants

These surfactants do not carry any charges in the head group. Their chemical structure is relatively simple and the majority of them are only composed of hydrogen, carbon and oxygen atoms. Fatty alcohols and alkyl ethoxylates are amongst the most used non-ionic surfactants, but the structure of both the head and tail group can vary significantly.
These are used mainly in detergents, as emulsifying agents in paints, agrochemicals, drugs and cosmetics and also as dispersing agents.

1.2.3 Zwitterionic surfactants

Zwitterionic surfactants present similar behaviour to non-ionic surfactants [4]. They are interesting compounds because they can carry opposite charges in the head group. However, these charges could be permanent or pH dependent, so that some of these compounds can be cationic or anionic in certain conditions. The most common zwitterionic surfactants are lecithins and betaines which are particularly important as cleansing agents and emulsifiers.

1.2.4 Surfactant solutions in water

Surfactants are generally soluble in water because of the hydrophilic nature of the head group. Once in solution, the water molecules in the vicinity of the hydrophobic tail groups order themselves in a cage around them, resulting in an overall increase in the free energy of the system. By concentrating the surfactants at the surface and orienting the tails out of water, hence away from the solvent, the free energy of the system can be efficiently reduced.

The adsorption of surfactant molecules at the interface reduces the surface tension of the solution, the decrease being a function of the surfactant concentration. A typical variation of surface tension with increasing concentration is plotted in Figure 1.8. At very low concentration (Figure 1.8 (a)) little adsorption occurs and the surface tension varies little. As the concentration increases (Figure 1.8 (b)), more adsorption occurs and the surface tension decreases rapidly and steadily. As the concentration increases further (Figure 1.8 (c)), surfactants saturate the interface and the surface tension becomes constant: the solution has now reached the critical micelle concentration (CMC).
Figure 1.8. Surface tension isotherm of a generic surfactant. The surface tension gradually drops with increasing concentration. When the CMC is reached, no variations are observed with further increase of concentration.

At the CMC, it is no longer energetically favourable for more surfactant molecules to be in solution as monomers. Upon addition of further surfactant molecules, monomers start forming aggregates, or micelles, in order to minimise the free energy of the system.

Figure 1.9. Section of a typical spherical micelle.
This process, called micellisation, is a mechanism that surfactants adopt to mask their hydrophobic parts from the bulk aqueous phase and maximise the interaction between the hydrophilic head groups and water. A simplified representation of a spherical micelle is shown in Figure 1.9. The surfactant tails form a hydrophobic core, which is surrounded by a disordered corona composed of surfactant head groups, water, some alkyl groups and (if the surfactant presents charges) counterions [5].

When the concentration is not much higher than the CMC, inter-micellar forces are limited. The shape of the micelle is then mainly determined by intra-micellar forces which are highly dependent on the surfactant structure. The area per surfactant molecule (APM) also seems to play a key role in the shape of micelles. It has been observed that for a spherical micelle with radius $r$, the volume $V$ is directly related to the APM [6]:

$$V = \frac{APM \times r}{3}$$  \hspace{1cm} \text{Equation 1.9}

The radius of the micelle cannot extend further than the fully extended alkyl chain, and assuming that the volume of the micelle $V$ is fixed, there seems to be a lower limit for APM in a spherical micelle. This lower value for APM is inversely related to the alkyl chain length. For surfactants with a dodecyl chain, this lower value is 70 Å$^2$ [7]. This implies that for surfactants with a dodecyl chain, only those presenting APM $> 70$ Å$^2$ are allowed spherical geometry. With decreasing APM ($70 < \text{APM} < 47$ Å$^2$) spherical micelles cannot form and only rod or disc micelles are allowed by geometry. Upon further decrease in APM, generally only bilayer lamellae or reversed phases are observed. This principle is known as the “packing constraint”.

As approaching the CMC, surface tension is not the sole property of the solution changing. Also properties such as conductivity, conductance and osmotic pressure, for example, undergo drastic changes at the CMC (Figure 1.10).
Figure 1.10. Changes in physic-chemical properties of a generic surfactant solution as a function of concentration.

1.2.5 Surface excess and the Gibbs equation

For the purpose of a convenient representation, often interfaces are treated as neat planes (Figure 1.11, left). This is clearly an oversimplification of the real interface. When adsorption processes are discussed quantitatively the interface must be described as an interfacial region whose characteristics are significantly different from the bulk phases. In need of defining a reference point in the interfacial region, Gibbs introduced the concept of the dividing surface. The dividing surface is defined as a geometrical plane passing through an arbitrary point in the interfacial region and all the other points that are similarly located along the interfacial region. The surface excess, $\Gamma$, is then defined as the difference between the amount of a species that is actually present in the interfacial region and that which would have been present had the bulk phase extended to the dividing surface without changing its composition [8]. Depending on the arbitrary location of the dividing surface, $\Gamma$ can be either positive or negative. The surface excess for the species A is shown in Figure 1.11, right, by the shaded areas. Given that the
position of the dividing surface is arbitrary, it is convenient to choose a position such that the surface excess of one of the components is zero. The dividing surface in Figure 1.11 is placed at $z_0$ position, such that the two shaded areas are equal, therefore the surface excess of the component A is 0.

![Figure 1.11. Left: schematic representation of the interface between two bulk phases A and B. The interface is represented as a neat plane, indicated by $z_0$. Right: a more realistic representation of the interfacial region between the two bulk phases. The composition is gradually changing from “pure” A to “pure” B. The dividing surface $z_0$ represents the ideal interface and is chosen such that the surface excess of A is zero.](image)

Let us consider the situation of a surfactant adsorbed at the air-water interface. The surface tension of the system, $\gamma$, is determined by the surface excess of the two components, surfactant ($s$) and water ($w$).

$$d\gamma = - \sum_i \Gamma_i d\mu_i = -\Gamma_s d\mu_s - \Gamma_w d\mu_w$$

Equation 1.10

d$\mu_i$ is the chemical potential of the species $i$. By positioning the dividing surface at $z_0$, such that the surface excess for water is zero, Equation 1.10 can be written as:

$$d\gamma = -\Gamma_s d\mu_s$$

Equation 1.11
According to the Equation 1.11, for a system where a surfactant is adsorbed at the air-water interface, an increase in $\Gamma$ will cause a decrease in surface tension.

The chemical potential, $d\mu$, can be defined as:

$$d\mu_s = RTd\ln a_s$$

Equation 1.12

$a_i$ is the activity of the species $i$ in the bulk. By substituting Equation 1.12 in Equation 1.11, we have:

$$dy = -RT\Gamma d\ln a_s$$

Equation 1.13

For dilute concentrations it is convenient to use the molar concentration of the surfactant instead of the activity, therefore:

$$dy = -RT\Gamma d\ln C$$

Equation 1.14

At constant temperature one could rearrange the equation to obtain $\Gamma$:

$$\Gamma = -\frac{1}{RT} \frac{dy}{d\ln C}$$

Equation 1.15

$\frac{dy}{d\ln C}$ represents the variation in interfacial tension as a function of logarithm of concentration and can be easily determined experimentally as it corresponds to the slope of the linear regression in Figure 1.8 (b). It is noteworthy that when the adsorbed species is an electrolyte, electrical neutrality must be maintained at the interface and a counterion must also adsorb at the interface. Should this be the case, it must be taken into account by adding a factor $1/2$ on the right hand side of Equation 1.15.

Knowing $\Gamma$, one can calculate APM:

$$APM = \frac{1}{N_A\Gamma}$$

Equation 1.16

where $N_A$ is Avogadro constant. The APM is not only important for the assessment of the packing constraint in micelles, as discussed earlier, but it could also give some indications with regard to the orientation of adsorbed molecules.

As regards the liquid-liquid interface it may not be possible to choose a dividing surface such that the surface excess of both liquids is exactly zero. Our group is currently analysing neutron reflectivity data from the bare hexadecane-water interface in terms of
the “partial structure factors” [9,10]. Preliminary results suggest that there is very little separation between the inflection points of the \( \tanh \) functions used to describe the composition profiles for the two bulk liquids. In other words, at the dividing surface where \( \Gamma_{\text{water}} = 0, \Gamma_{\text{oil}} \sim 0 \). Therefore, in presence of strongly adsorbing solutes the surface excess of the two bulk liquids will be comparably much smaller than that of the solute, and Equation 1.15 can still be applied.

### 1.3 Langmuir monolayers

The adsorption processes discussed in the previous section refer to water soluble surfactants that adsorb at interfaces from the bulk solution. Some surfactants present a particularly marked hydrophobic character and are, therefore, practically insoluble in water. However, these surfactants can still form ordered monolayers when trapped between two immiscible phases; such layers are called Langmuir monolayers. Although the formation of Langmuir monolayers at the oil-water interface has been reported [11], these films are particularly exploited at the air-water interface. In laboratories Langmuir monolayers are generally formed onto a PTFE trough containing the water sub-phase, where the monolayer conditions can be accurately controlled. Such a trough is called a “Langmuir trough” and an example is shown in Figure 1.12.

Langmuir monolayers are formed by spreading insoluble material onto the water surface from a volatile solvent. The solvent rapidly evaporates and the insoluble surfactant is trapped at the interface. By compressing the monolayer with suitable barriers, the surfactant molecules are forced to get closer together and form a compressed and ordered monolayer. These films are easy to prepare, offer surprising reproducibility and their general behaviour is well understood.
The state of the monolayer can be monitored by means of the Wilhelmy plate. This device simply consists of a strip of chromatographic paper hanging from a microbalance and the underlying physics is similar to that involved in the plate method described earlier. When in contact with the surface, the strip is pulled towards the water phase by the surface tension. As for the plate method, the surface tension is given by the weight of the meniscus formed:

$$W_{\text{meas}} = \gamma p$$  \hspace{1cm} \text{Equation 1.6}$$

The Wilhelmy plate is generally zeroed at the air-water interface before spreading, so that any change in surface pressure ($\Delta \gamma$) would affect the force measured by the Wilhelmy plate.

$$\Delta W_{\text{meas}} = (\gamma^* - \gamma)p = \Delta \gamma p$$  \hspace{1cm} \text{Equation 1.17}$$
\( \gamma \) is the surface tension of pure water (72.8 mN m\(^{-1}\)). \( \Delta \gamma \) indicates the deviation in surface tension from that of pure water and is caused by the presence of surface active material at the interface. \( \Delta \gamma \) is called surface pressure and is generally indicated by the Greek letter \( \Pi \). Equation 1.17 then becomes:

\[
\Delta W_{\text{meas}} = \Pi p
\]

Equation 1.18

The study of monolayers at the air-water interface is generally carried out as a function of surface pressure \( \Pi \). Upon compression of the monolayer, a reduction in surface tension can be observed, which in turn leads to an increase in surface pressure. Because of the monolayer being insoluble in water, the compression of the film goes along with a reduction in surfactant area per molecule (APM). The variation of surfactant APM as a function of \( \Pi \) at constant temperature is characteristic of the molecule adsorbed at the interface and is known as “pressure-area isotherm”. More information regarding the different phases typically observed upon compression in a pressure-area isotherm is given in Chapter 7.

### 1.3.1 Langmuir-Blodgett technique

Besides being an excellent methodology for the study of monolayers at the air-water interface, one of the most interesting applications of Langmuir monolayers can undoubtedly be found in the Langmuir-Blodgett technique. This technique allows the deposition of monolayers from the air-water interface onto solid substrates. The typical experimental set-up is shown in Figure 1.13 and a close-up of the interfacial phenomena occurring during deposition is shown in Figure 1.14 (a) - (d). It was observed by Katherine Blodgett [12] that when a hydrophobic (or artificially hydrophobed) substrate is slowly dipped through the water surface where a Langmuir film is present, the monolayer can be transferred onto the surface of the solid (Figure 1.14 (a) and (b)). For the deposition to take place, the water surface meniscus must dip below the surface and wet the substrate at an angle of \( \sim 180^\circ \). These conditions are generally met for hydrophobic surfaces.
Figure 1.13. Experimental details for Langmuir-Blodgett deposition.

Figure 1.14. Schematic representation of the Langmuir-Blodgett deposition. An ordered Langmuir monolayer is present on the water surface (a) and a hydrophobic substrate is slowly dipped through the interface (b). This leads to the deposition of a monolayer. The substrate surface now presents a hydrophilic nature (c) and when it is slowly removed from the water phase, the deposition of a second monolayer occurs (d). For the deposition to take place, a meniscus must form between the water phase and the substrate in the direction of the moving substrate. Such meniscus is not shown in figure.
Once the first monolayer has been deposited, the solid substrate is found completely immersed in the water sub-phase. The substrate surface is now coated with the deposited surfactant monolayer which is exposing the head group region to the bulk aqueous phase. The substrate surface has, therefore, become hydrophilic (Figure 1.14 (c)). When the substrate is slowly removed from the aqueous phase, head-head interactions take place between the Langmuir monolayer and the deposited layer (Figure 1.14 (d)). This leads to the deposition of a second monolayer onto the solid substrate. Multilayers can also be deposited by sequentially dipping and removing the solid substrate from the water sub-phase. In order to have homogeneous deposition, it is essential that the monolayer is kept at a fixed surface pressure throughout the whole deposition process. This is possible by adjusting the area of the trough with the moveable barriers.
1.4 References for Chapter 1

Chapter 2

Neutron reflectivity

2.1 Introduction

The leading thread of this dissertation is the application of neutron reflectivity to very different systems. The physics behind neutron scattering and neutron reflectivity is extremely complicated and a full discussion goes beyond the scope of this dissertation. However, in order to fully understand and interpret the results discussed in the following chapters, the key aspects of the technique must be discussed.

In a neutron reflectivity experiment a highly collimated neutron beam is reflected from a macroscopically flat interface. The intensity of the specular reflection is then measured as a function of momentum transfer perpendicular to the interface, $Q$, which is defined by the beam incident angle $\theta$ and the neutron wavelength $\lambda$:

$$ Q = \frac{4\pi \sin \theta}{\lambda} $$

Equation 2.1

The reflected intensity is related to the refractive index profile normal to the interface; therefore the technique gives information regarding the composition and density profiles of surfaces and interfaces.

In the first part of the chapter, the optics involved in reflection techniques is discussed. This is followed by a more detailed discussion about neutron reflectivity. The procedure that is generally adopted for the data analysis is also briefly discussed in this section.

The second part of the chapter focuses on the instrumentation required for a neutron reflectivity experiment. How neutron beams are being produced and detected is
discussed, along with the general features of a neutron reflectometer. Details are also given about the reflectometers used during the PhD.

2.2 Principles of reflection

The properties of reflection and refraction of neutrons are exactly analogous to light incident on a surface. The reflected and transmitted parts of the incident beam vary accordingly with the nature of the surface, specifically with the neutron refractive index of the media that constitute the interface. By analysing the intensity of the reflected beam, one can gain information regarding the neutron refractive index of the sample perpendicular to the interface, which in turn provides structural information regarding the interface itself, such as composition, thickness and roughness of layers on the surface.

2.2.1 Reflection from a single interface

When radiation hits the surface between two continuous media it is split into a reflected and a transmitted beam [1]. Let be \( \phi_i \) the incident angle, \( \phi_t \) the transmitted angle, \( n_1 \) and \( n_2 \) the refractive indices of the first and second medium respectively. A schematic representation is shown in Figure 2.1.
Figure 2.1. Reflection and transmission from a single interface.

The reflected \( r \) and transmitted \( t \) intensities can be described by the Fresnel reflection coefficients (Equation 2.2 – 2.5).

\[
\begin{align*}
  r_p &= \frac{n_1 \cos \varphi_t - n_2 \cos \varphi_i}{n_1 \cos \varphi_t + n_2 \cos \varphi_i}, & \text{Equation 2.2} \\
  t_p &= \frac{2n_1 \cos \varphi_i}{n_1 \cos \varphi_t + n_2 \cos \varphi_i}, & \text{Equation 2.3} \\
  r_s &= \frac{n_1 \cos \varphi_i - n_2 \cos \varphi_t}{n_1 \cos \varphi_i + n_2 \cos \varphi_t}, & \text{Equation 2.4} \\
  t_s &= \frac{2n_1 \cos \varphi_i}{n_1 \cos \varphi_i + n_2 \cos \varphi_t}, & \text{Equation 2.5}
\end{align*}
\]

The subscripts \( p \) and \( s \) refer to the polarisation of the incident wave: \( p \) polarisation refers to polarisation in the plane of incidence, whereas polarisation perpendicular to the plane
of incidence is referred to as s polarisation. The reflectivity is then expressed as the square of the reflection coefficients:

\[ R_p = |r_p|^2 \quad \text{Equation 2.6} \]

\[ R_s = |r_s|^2 \quad \text{Equation 2.7} \]

Applying Snell’s law, Equation 2.8, the Fresnel coefficients for reflectivity \((r_P\) and \(r_s\)) can be written as in the form shown in Equation 2.9 and Equation 2.10.

\[ \frac{\sin \phi_t}{\sin \phi_t} = \frac{n_2}{n_1} \quad \text{Equation 2.8} \]

\[ r_p = \frac{\tan(\phi_t - \phi_i)}{\tan(\phi_t + \phi_i)} \quad \text{Equation 2.9} \]

\[ r_s = \frac{\sin(\phi_t - \phi_i)}{\sin(\phi_t + \phi_i)} \quad \text{Equation 2.10} \]

Although Equation 2.9 and Equation 2.10 are of little help in multi-layer problems, it is interesting to consider them in the case of neutron reflectivity. Because of the neutron refractive indices being close to 1 for most materials, generally neutron reflectivity is performed at grazing angle of incidence. In these conditions, \(\phi_t - \phi_i \sim 0\) and \(\phi_t + \phi_i \sim \pi\), therefore \(R_s \sim R_p\). As a result, from now on only the reflectivity for the s-polarised wave \(R_s\) will be used.

The reflection from a surface where \(n_1 = 1\) and \(n_2 = 1.5\) is shown in Figure 2.2, for \(n_1 = 1.5\) and \(n_2 = 1\) in Figure 2.3.
Figure 2.2. Reflectivity from a single interface between two media with refractive index $n_1 = 1$ and $n_2 = 1.5$ as a function of incident angle for both $s$-polarised (red line) and $p$-polarised light (blue line). The dashed line indicates the Brewster’s angle.

Figure 2.3. Reflectivity from a single interface between two media with refractive index $n_1 = 1.5$ and $n_2 = 1$ as a function of incident angle for both $s$-polarised (red line) and $p$-polarised light (blue line). The dashed line indicates the critical angle above which total external reflection occurs.
2.2.1.1 Special features: Brewster’s angle

The dashed line in Figure 2.2 shows the Brewster’s angle which corresponds to the angle of incidence at which \( p \)-polarised light is totally transmitted and no reflection occurs. The schematic representation of Brewster’s angle geometry is shown in Figure 2.4. For this to happen, the refracted beam must be perpendicular to the direction in which the light is predicted to give specular reflection. The Brewster’s angle (\( \varphi_B \)) can then be calculated using Snell’s law (Equation 2.8):

\[
\varphi_B = \arctan\left(\frac{n_2}{n_1}\right)
\]

Equation 2.11

It is noteworthy that when unpolarised light approaches a reflecting surface at Brewster’s angle, the reflected radiation is entirely \( s \)-polarised (Figure 2.4). This principle is broadly used in devices such as polarisers.

![Brewster’s angle geometry](image)

**Figure 2.4.** Brewster’s angle geometry. \( p \)-polarised light is entirely transmitted and only the \( s \)-polarised portion of the beam is reflected.
2.2.1.2 Special features: total external reflection

When radiation travels from a medium with a higher refractive index to one with a lower refractive index \( (n_2 < n_1, \text{ Figure 2.3}) \) the phenomenon known as total external reflection occurs if the incident angle \( \phi_i \) is bigger than the critical angle \( \phi_c \). The critical angle is, by definition, the angle of incidence above which total reflection occurs and is shown by the dashed line in Figure 2.3. It corresponds to the angle of incidence that gives a transmitted angle \( \phi_t = 90^\circ \) and it can be calculated from Snell’s law:

\[
\phi_c = \arcsin \left( \frac{n_2}{n_1} \right) \tag{Equation 2.12}
\]

This phenomenon can be observed for example when approaching the glass-air interface from the glass side or the water-air interface from water (but of course not the other way around).

2.2.2 Reflection from a single film

In Figure 2.5 is shown a beam of light travelling through a medium with a refractive index \( n_0 \). The beam hits a homogeneous film, with thickness \( d \) and refractive index \( n_1 \), deposited onto a substrate with a refractive index \( n_2 \), at an incident angle \( \phi_i \). As observed for the reflection from a single surface, the incident beam is split into a reflected and transmitted beam. This occurs every time the reflected beam strikes an interface: as a result, the total reflected (or transmitted) beam is obtained by summing the multiple reflections (or transmissions). The reflection from a single film is conveniently discussed in terms of Fresnel coefficients, so that the \( R_e \) and \( R_p \) can be obtained by substituting the appropriate coefficients.

The Fresnel coefficients for reflection and transmission for the beam moving from \( n_0 \) to \( n_1 \) are indicated as \( r_i \) and \( t_i \) respectively. The corresponding coefficients for propagation between \( n_1 \) and \( n_0 \) are denoted with \( r_i' \) and \( t_i' \). Similar notation is adopted for the beam propagation between \( n_1 \) and \( n_2 \), and vice-versa \( (r_2, t_2, r_2' \text{ and } t_2' \text{ can be distinguished}).
The total reflected amplitude is the sum of all the beams’ reflected intensities from the film into the medium $n_0$. The change in phase of the beam upon crossing the film is given by $e^{i\delta}$, where:

$$\delta = \frac{2\pi}{\lambda} n_1 d \cos \phi$$

Equation 2.13

is the optical path length in the film. The reflected amplitude can, therefore, be expressed as:

$$R = r_1 + t_1 t'_1 r_2 e^{-2i\delta} - t_1 t'_1 r_1 r_2 e^{-4i\delta} + t_1 t'_1 t_2 r_3 e^{-6i\delta} + \cdots$$

Equation 2.14

From the Fresnel reflection coefficients (Equation 2.2 – 2.5), one can see that $r_j'$ is equal to $-r_j$. Likewise, it is possible to demonstrate that $t_1 t'_1 = 1 - r_1^2$. Therefore, Equation 2.14 can be written as:
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\[ R = \frac{r_1 + r_2 e^{-2i\delta_1}}{1 + r_1 r_2 e^{-2i\delta_1}} \]  
Equation 2.15

The transmitted amplitude can be calculated following the same procedure as for the reflected amplitude and is given by:

\[ T = \frac{t_1 t_2 e^{-i\delta_1}}{1 + r_1 r_2 e^{-2i\delta_1}} \]  
Equation 2.16

The reflected amplitude \( R \) and the transmitted amplitude \( T \) can be used as Fresnel coefficients for the single film and used to calculate the reflectivity as described for the single interface. The reflectivity from a single layer is then the squared amplitude:

\[ R = |R|^2 = \frac{r_1^2 + r_2^2 + 2r_1 r_2 \cos(2\delta_1)}{1 + r_1^2 r_2^2 + 2r_1 r_2 \cos(2\delta_1)} \]  
Equation 2.17

### 2.2.3 Reflectivity of a multilayer: the optical matrix method

The above approach can be easily applied to systems with two or three layers. This is done by building a single reflection coefficient by applying the single film formula in sequence to all the layers at the interface. With increasing number of layers, however, the calculation becomes tedious and a more general solution is desirable. Consider an interface consisting of a multilayer system represented by \( n \) layers. It is convenient to represent each layer by a matrix containing only the Fresnel coefficients relevant to the specific layer. The matrix associated with the layer at the interface between \( m-1 \) and \( m \) is shown in Equation 2.18:

\[
M_m = \begin{bmatrix}
e^{i\delta_{m-1}} & r_m e^{i\delta_{m-1}} \\
r_m e^{-i\delta_{m-1}} & e^{-i\delta_{m-1}}
\end{bmatrix}
\]  
Equation 2.18

This derivation was developed by Abeles [2] for electric vectors in successive layers. It was shown by Parratt [3] that such an approach can be used to represent the relation between successive reflection coefficients. For \( n \) layers at the interface, the matrix elements \( M_1, M_2 \) of the resultant matrix \( M_R = [M_1][M_2]...[M_n] \) give the total reflectivity:
The above calculation can be applied to a virtually infinite number of layers, each of them characterised by a certain thickness \( d \) and refractive index \( n \). This formalism is, however, valid only for smooth interfaces. Roughness between layers must in some cases be considered and this subject will be addressed in the following section.

### 2.3 Neutron scattering and neutron reflectivity

The reflected intensity of a neutron beam can be adequately described by standard optical theory discussed in the first section. One key aspect of neutron refractive index is that it can be directly calculated from the atomic composition of a material. Neutrons interact with matter in two different ways: through direct interaction with nuclei (so-called nuclear scattering) or through interactions of unpaired electrons with the magnetic moment of the neutron (magnetic scattering). The discussion below will only focus on the nuclear scattering; magnetic scattering will be briefly addressed later in the section regarding polarised neutron reflectivity.

It has been demonstrated that the neutron refractive index can be written as [4]:

\[
 n = 1 - \frac{N b}{2\pi} \lambda^2 + i \frac{N \sigma_a}{4\pi} \lambda
\]

\( N \) is the atomic number density, \( b \) the coherent scattering length and \( \sigma_a \) is the absorption cross-section. The imaginary component can be generally considered negligible unless in the presence of strong neutron absorbers such as cadmium or boron.

The neutron refractive index is very close to unity and reflectivity must be performed at grazing incidence. The grazing angle \( \theta \) is defined as the complementary angle to the angle of incidence \( \theta = \frac{\pi}{2} - \vartheta \). Another consequence of the refractive index being close to unity is that reflectivity is more conveniently discussed in terms of scattering length density \( Nb \). The \( Nb \) of a medium can be directly calculated from its composition:
The importance of neutron reflectivity resides in the fact that the scattering length $b$ varies in a random fashion within the different elements in the periodic table; also $b$ varies within different isotopes. Particularly, $b$ is substantially different between hydrogen ($b_H = -3.74$ fm) and deuterium ($b_D = 6.67$ fm).

The $Nb$ for most materials is positive. According to Equation 2.20, a positive $Nb$ gives a refractive index $n < 1$; therefore when a neutron beam approaches the surface of most materials from air ($n = 1$) there will be total reflection below a certain critical angle $\theta_c$. Measurements of the reflectivity below $\theta_c$ are generally performed as a means to determine the unit reflectivity and to ensure that an adequate scale factor is applied to all the measured reflectivity profiles. The reflectivity below $\theta_c$ is unity and no structural features can be observed in this region.

When the incident angle is larger than the critical angle, the Fresnel coefficient for the $s$ wave (Equation 2.4) can be conveniently written as:

$$r_{ij} = \frac{(q_i - q_j)}{(q_i + q_j)}$$

Where $q_i$ and $q_j$ are the wave vector of the radiation normal to the interface in layer $i$ and $j$ respectively are defined as:

$$q_i = n_i \sin \theta_i$$

$$q_j = n_j \sin \theta_j$$

It can be demonstrated that the change in wave vector from layer $i$ to layer $j$ is [5]:

$$q_i^2 - q_j^2 = 4\pi(Nb_i - Nb_j)$$

We have already shown in Equation 2.1 that the incident angle $\theta_c$ and the wavelength $\lambda$ are related by the momentum transfer $Q$:

$$Q = \frac{4\pi \sin \theta}{\lambda} = 2q_0$$
We can now define the momentum transfer at which total reflection between layers $i$ and $i+1$ occurs by using $q_j = 0$:

$$Q_{ci}^2 = 4q_{ci}^2 = 16\pi(Nb_{i+1} - Nb_i)$$  \hspace{1cm} \text{Equation 2.27}$$

Combining Equation 2.22 and Equation 2.25, we can calculate the reflectivity from a smooth surface:

$$R = |\eta_{12}|^2 = \left[\frac{\sqrt{Q^2 - Q_c^2} - Q}{\sqrt{Q^2 - Q_c^2} + Q}\right]^2 \approx \frac{Q_c^4}{[\sqrt{Q^2 - Q_c^2} + Q]^4}$$  \hspace{1cm} \text{Equation 2.28}$$

When the reflectivity is measured at $Q >> Q_c$, Equation 2.27 can be used to calculate the total reflectivity from Equation 2.28:

$$R \approx \frac{16\pi^2 \Delta Nb^2}{Q^4}$$  \hspace{1cm} \text{Equation 2.29}$$

Similarly, the reflectivity from a single layer of thickness $d$ and scattering length density $Nb_1$ can be calculated:

$$R \approx (Nb_1 - Nb_0)^2 + (Nb_2 - Nb_1)^2 + 2(Nb_1 - Nb_0)(Nb_2 - Nb_1)\cos(Qd)$$  \hspace{1cm} \text{Equation 2.30}$$

2.3.1 Contrast variation

The different scattering length $b$ within isotopes is at the basis of the methodology called contrast variation. Equation 2.21 shows that the $Nb$ of a molecule is the sum of the contributions of all its components. By changing the isotopic ratio, the $Nb$ of the molecule can be varied to match that of other molecules in the system. Substitution between different isotopes is particularly exploited with hydrogen and deuterium as they possess very different scattering length. Moreover, deuterated materials are nowadays easily accessible and custom deuteration has reached a point where even specific parts of molecules can be deuterated. The potentiality of contrast variation is shown in Figure 2.6.
Figure 2.6. Schematic representation of a layer adsorbed at the air-water interface. By changing the isotopic ratio, mainly H / D, different contrasts can be obtained.

Figure 2.6 (a): using H\textsubscript{2}O as aqueous sub-phase and a hydrogenous surfactant, little sensitivity to the adsorbed layer is achieved. Figure 2.6 (b): when the aqueous phase is contrast matched to air (NRW) and the adsorbed surfactant is deuterated, the reflectivity arises exclusively from the adsorbed layer. This contrast is generally adopted to determine the adsorbed amount of surfactants at the air-water interface. Figure 2.6 (c): to achieve maximum contrast between the aqueous sub-phase and the adsorbed surfactant, D\textsubscript{2}O can be chosen in combination with the hydrogenous surfactant.

In Figure 2.6 (a) a monolayer of fully hydrogenous surfactant is adsorbed at the interface between H\textsubscript{2}O and air. The scattering length density of H\textsubscript{2}O is close to that of air (\(N_b\text{water} = -0.56 \times 10^{-6} \, \text{Å}^{-2}\), \(N_b\text{air} = 0\)); therefore little reflection occurs from a bare air-water interface. The \(N_b\) of the adsorbed layer would change depending on the structure of the surfactant, but most hydrogenous surfactants possess an \(N_b\) not much higher than 0. As a result this contrast would prove to be rather poor for the adsorption study of such a molecule at the air-water interface.

In Figure 2.6 (b) the surfactant monolayer is now deuterated and its \(N_b\) is much higher than air. The aqueous phase consists of a mixture of H\textsubscript{2}O and D\textsubscript{2}O the Nb of which is contrast-matched to air (CMair water or null reflecting water, NRW, \(N_b = 0\)). In the absence of adsorbed species, no reflection would occur from such an interface, whereas in the presence of adsorbed layers, the reflectivity is proportional to the adsorbed amount at the interface. This contrast is generally adopted to obtain the adsorbed amount \(\Gamma\) at the air-water interface; details on this matter are given in Chapter 6.
The aqueous sub-phase in Figure 2.6 (c) consists of D$_2$O and has a high Nb, whereas the adsorbed layer is fully hydrogenous. This contrast has proved to be particularly sensitive to variations in the structure of the adsorbed layer; therefore it is often used to determine the conformation of adsorbed surfactants.

### 2.3.2 Roughness

We mentioned earlier in this chapter that the calculations shown are only valid for smooth surfaces and roughness is not taken into consideration. However, most surfaces show some degree of roughness and one must be able to take this into account when calculating the reflectivity. There are two types of interfacial roughness. Classical roughness consists of undulations originating from lateral inhomogeneities at the interface. If such undulations are of long range order they can be considered locally flat thus not affecting the above calculations [6]. Diffuse roughness arises from intermixing between adjacent layers. Molecules in neighbouring layers tend to have some degree of interpenetration; this localised roughness is of the same order of magnitude as the neutron wavelength. The effect of the two types of roughness on a reflectivity experiment is indistinguishable; namely both effects cause a reduction in reflected intensity compared to a smooth interface. The effect of roughness on the reflectivity can be calculated by applying a Gaussian factor to the Fresnel coefficients for each interface [7]:

$$r_{ij} = \left( \frac{q_i - q_j}{q_i + q_j} \right) e^{-0.5(q_i q_j)/(\sigma)^2}$$  \hspace{1cm} \text{Equation 2.31}

Where $\langle \sigma \rangle$ represents the root mean square roughness.

The reflectivity is then calculated as discussed in Equation 2.28 – 2.30. It must be stressed that by applying the above roughness treatment one does not calculate the real roughness, but simulates a Gaussian roughness. It would be more appropriate to represent the rough interface by using a large number of thin layers which mimic a
Gaussian distribution. The latter approach is preferable as it leads to the exact calculation of the reflectivity, but its application is rather cumbersome [8].

2.3.3 Polarised neutron reflectivity

Polarised neutron reflectivity has proved to be a powerful technique to probe surface magnetisation profiles. It has been developed as a tool to study magnetic films and superconductors and it has been applied to the investigation of the nature of magnetism in multilayers [9]. The technique has its foundation in the fact that the scattering of neutrons occurs, as previously discussed, either through interaction with nuclei (nuclear scattering), or through interactions of unpaired electrons with the magnetic moment of the neutron (magnetic scattering). In the presence of a magnetic field, neutrons can be polarised either parallel (+) or anti-parallel (-) to it. For magnetic materials which are magnetised in the plane of the surface, the neutron refractive index $n$ is dependent on the spin of the neutron. The measured reflectivity, $R_\pm$, is spin-dependent and the refractive index must be written as a combination of the nuclear ($n_{\text{nuclear}}$) and magnetic ($n_{\text{magnetic}}$) contributions [8]:

$$n_\pm = n_{\text{nuclear}} \pm n_{\text{magnetic}} = 1 - \left( \frac{N\lambda^2}{2\pi} \right) (b \pm C\mu)$$  

Equation 2.32

$C$ is a constant ($0.265 \times 10^{-12} \mu_B \text{cm}^{-1}$) and $\mu$ is the moment per atom.

Although the technique has been traditionally used to study magnetic features of thin films and heterostructures [10], in Chapter 4 is reported an example of how polarisation can be used to enhance the sensitivity of neutron reflectivity by providing additional contrasts for the study of surfactant adsorption at the metal-oil interface.
2.3.4 Data analysis: from the reflectivity profile to the scattering length density profile

In the previous sections we have shown how to calculate a reflectivity profile when the structure of the interface is known. In a neutron reflectivity experiment the opposite problem is encountered: a set of reflectivity profiles is experimentally measured and the structural parameters must be extracted from such profiles. Two methods generally can be used to analyse reflectivity profiles and deduce the structural parameters: iterative fitting and the analysis of the partial structure factors. All the experimental data discussed in this thesis have been analysed using the iterative fitting method as it requires less extensive isotopic labelling. However, other methods of data analysis have been reported, such as direct inversion and the analysis of partial structure factors. A detailed description can be found elsewhere [5].

2.3.4.1 Iterative model fitting

Model fitting is the most commonly used method to analyse reflectivity data. The interfacial region is divided into a finite number of layers, each characterised by a certain thickness, scattering length density and roughness, from which the reflectivity is calculated using the optical matrix method. A flat background is generally added to each point and instrumental resolution may also be taken into consideration in the calculations. The calculated reflectivity is then compared to the experimental data and the goodness of the fit is evaluated in terms of $\chi^2$:

$$
\chi^2 = \sum_N \left( \frac{(R_f - R_m)^2}{\sigma^2} \right)
$$

Equation 2.33

$N$ is the number of data points, $R_f$ and $R_m$ are the fitted and measured reflectivity respectively and $\sigma$ is the standard error associated with the measured reflectivity. The value for $\chi^2$ is minimised by the least squares routine. Each parameter (number of layers, thickness, scattering length density and roughness of each layer) can be kept
fixed or varied and the reflectivity is calculated from the model until the value for $\chi^2$ has reached a minimum. The optimum fitted parameters combine to give the scattering length density profile of the sample which represents the variation in composition perpendicular to the interface.

One complication arises from the fact that because of the loss of the phase information, more than one scattering length density profile can correspond to the same reflectivity profile i.e. there is more than one solution to a reflectivity profile. The most efficient way to reduce the possible solutions is to use the minimum number of fitted parameters, hence the minimum number of layers, to model the reflectivity. Isotopic substitution is also extensively used to provide additional contrasts; a satisfactory solution is generally achieved when the same model can fit the reflectivity profiles for all the different contrasts measured.

### 2.4 Neutron reflectometers

A neutron reflectometer is a relatively simple instrument. Although the design may significantly vary from instrument to instrument, all neutron reflectometers necessarily have some features in common. A highly collimated neutron beam is focused on the sample position. After the interaction with the sample, the reflected beam reaches the detector area where the reflected intensity is measured. The generation and the detection of a neutron beam are not trivial tasks and are worth discussing. Some details regarding the instruments used during the PhD are given and an accurate description for each of them can be found in the referenced literature.
2.4.1 Neutron sources

Several methods are available for the production of a neutron beam [11], however only two mechanisms are currently adopted for the production of neutron beams in present-day facilities.

2.4.1.1 Reactor sources

Reactor sources provide a very large quantity of neutrons. Neutrons are produced from fission: when a fissile nucleus, generally \(^{235}\text{U}\), captures a neutron, it is split into two smaller nuclei. The process is extremely rapid (in the order of femtoseconds) and as a result an average of 2.5 neutrons are produced [12]. Two reactor source facilities were used during the PhD.

- ILL, in Grenoble (France), currently hosts the brightest neutron source in the world. The beam produced is a polychromatic beam with a wavelength range between 2 and 30 Å.
- HMI, in Berlin (Germany), produces a monochromatic neutron beam.

2.4.1.2 Spallation sources

The spallation process involves the interaction of high energy particles (in our case protons) with heavy atoms. Negatively charged hydrogen ions are produced by an ion source and accelerated by means of a linear accelerator. The ions, which already possess a very high energy, collide with an aluminium oxide foil losing the two electrons and are, therefore, converted to protons. The proton beam is further accelerated in a synchrotron. When the high energy proton beam strikes a heavy metal target, such as tantalum, tungsten, uranium or mercury, a number of high energy neutrons are released. Particles released from the collision may strike other nuclei much like balls in a pool. As a consequence of the process, each proton produces an average of 20 neutrons.
2.4.2 Neutron detection

After the beam has interacted with the sample, the intensity of the reflected beam must be accurately measured. Neutrons do not possess a charge and they do not interact with electrons; the detection of the reflected neutron beam, therefore, is not a trivial task. Neutrons can interact with different nuclei, causing the release of charged subatomic particles. Such charged particles give rise to an electrical signal that can be easily processed in the detector.

Although a variety of neutron detectors are available [13], the most common are gas-filled detectors. Helium-3 is universally used in neutron detection as it readily interacts with neutrons to give a nuclear reaction:

\[ ^3\text{He} + n \rightarrow ^3\text{H} + ^1\text{H} + 765\text{ keV} \]

Such reaction is exothermic, therefore the released charged particles are highly energetic. Such particles create a charge cloud in the surrounding gas that can be easily detected.

Currently, with the shortage of Helium-3, the need for alternative neutron detection systems is emerging. For example, the helium-3 inside the chamber can be replaced by other gasses such as $^{10}\text{B}$ enriched BF$_3$. The reaction then becomes:

\[ ^{10}\text{B} + n \rightarrow ^7\text{Li}^* + ^4\text{He} + 2310\text{ keV} \]

Over 94% of the time the lithium is left in an excited state and faces a subsequent decay:

\[ ^7\text{Li}^* \rightarrow ^7\text{Li} + 480\text{ keV} \]

The energy deposited in the detector is, therefore, higher than in $^3\text{He}$ detector. However, BF$_3$ is highly toxic and corrosive. More recently $^{10}\text{B}$-lined detectors have been developed where $^{10}\text{B}$ is directly deposited onto the detector wall [14]. The efficiency is lower than BF$_3$ detectors but there is no risk associated with the presence of BF$_3$ gas.
2.4.3 Reflectometers used during the PhD

Four different reflectometers in three different research centres were used during the project. A brief description is given for each of them in this section. A more detailed description can be found for each instrument in the referenced literature.

2.4.3.1 The SURF reflectometer

SURF is a time-of-flight reflectometer at ISIS, Rutherford Appleton Laboratory, in Didcot, UK [15]. Neutrons are produced by spallation process and are slowed to the required energies for scattering experiments by means of a hydrogen moderator kept at a temperature of 20 K. A chopper operates at 50 Hz, selecting a wavelength range between 0.5 and 7.0 Å. Two sets of slits before the sample position select the beam footpath. The incoming beam comes at an incident angle $\theta = 1.5^\circ$ but it can be varied by means of a goniometer in the sample position. For liquid interfaces, where the sample cannot be tilted, the beam incident angle can be varied by means of a supermirror which is located between the two sets of slits before the sample position.

2.4.3.2 The INTER reflectometer

INTER is a time-of-flight reflectometer at ISIS, located in the newly developed second target station [16]. A hydrogen moderator is used to slow neutrons and a new solid methane moderator is currently being developed. The chopper operates at 10 Hz, enabling a much broader wavelength range compared to the SURF reflectometer (wavelength between 1.5 and approximately 20 Å). The incoming incident beam comes at an angle $\theta = 2.3^\circ$ and, similarly to SURF, it can be varied by means of either a goniometer in the sample region or by use of a supermirror.
2.4.3.3 The FIGARO reflectometer

FIGARO is a recently built time-of-flight reflectometer at ILL in Grenoble, France [17]. The chopper speed can be adjusted to have different wavelength ranges, from 2-16 Å to 2-30 Å, the latter being the standard operating setting. The incoming beam comes at an angle $\theta = 0.62^\circ$ and can be deflected by means of two supermirrors in sequence. The choice of two supermirrors instead of a single one is to minimise the loss of short wavelength neutrons upon deflection of the beam. This way, the beam incident angle can be varied to $\theta = 4.0^\circ$. Higher incident angles can be achieved by means of a goniometer in the sample position. The detector is an area detector with a vertical resolution of 2 mm. This type of detector allows the observation of off-specular peaks and significantly facilitates the alignment procedure.

2.4.3.4 The V6 reflectometer

V6 is a reflectometer at HMI, Berlin, Germany [18]. The beam has a fixed-wavelength $\lambda = 4.66$ Å and the beam incident angle is varied by tilting the sample position. Recently the instrument has been provided with an area detector providing a vertical resolution of 3 mm, although the old 1D detector was used for the experiment discussed in Chapter 4. The instrument is equipped with a polariser and a flipper before the sample position and a second flipper between the sample position and the detector to perform polarised neutron reflectivity.
2.5 References for Chapter 2

Chapter 3

Structural studies of phthalocyanine-lipid multilayers

3.1 Introduction

The availability of fresh water is of upmost importance in the global context and water pollution is amongst the leading causes of death and diseases [1]. The presence of pollutants in water does not only affect developing countries, but it is also a major concern for industrialised countries. We refer to water as polluted when it is not suitable for human use (such as drinking water) or when its ability to support life is markedly reduced.

The main causes of water pollution can be classified into two big categories:

1. Chemical contaminants. This group includes both organic (for example detergents, insecticides, pesticides, fuels, lubricants) and inorganic compounds (such as acids or alkalis, nitrates, phosphates, heavy metal ions).

2. Pathogens.

Contaminants can also be present in water as a result of a deliberate act: threats to attack or contaminate drinking water systems are common, numbering in the hundreds annually all over the world. Public water supplies are, therefore, included in the list of potential targets for malevolent acts. Small amounts of toxic chemicals, even if not directly harmful, can cause panic and great economic disruption. It is also crucial to keep in mind that biological agents, and especially toxins, can be harmful at very low levels.
3.1.1 Monitoring of the water quality

Analytical methods that are available in laboratories to evaluate water quality are time-consuming and hugely expensive. It is important that robust, low cost and real time monitoring techniques with an appropriate sensitivity are available to complement the currently used spot-sampling and conventional laboratory techniques.

There is an urgent need for low cost, broad spectrum and highly sensitive biosensors to efficiently detect the presence of different contaminants in water. For this purpose, the Queen Mary University of London is currently collaborating with the University of East Anglia to design and engineer an integrated optical biosensor. This device detects a minor change in refractive index of a sensing medium encapsulated between a bio-mimic membrane and the solid support by means of a planar optical waveguide. The membrane works as the sensing biological element: contaminants in the water would damage the membrane, ultimately changing the response of the sensor. An illustration of the device is schematically shown in Figure 3.1.

![Figure 3.1. Schematic representation of the currently studied device. Damages to the membrane, caused by contaminants, would cause a drop in the intensity of the signal detected by the waveguide.](image)

These devices meet the requirements for an efficient sensor for water quality; however, because of the optical waveguide aspect of the device, it is cumbersome and lacks portability. Hence its application is limited and it is bound to be a laboratory based technique.
3.1.2 Development of a new portable optical biosensor

A novel way to make such devices portable and hence more practical would be to eliminate the need for the optical waveguide part. This can be achieved by replacing the optical waveguide of the sensor by a multilayer of an aliphatic substituted phthalocyanine (Figure 3.2), which can incorporate a metal centre.

![Chemical structure of the substituted phthalocyanine ligand](image)

**Figure 3.2. Chemical structure of the substituted phthalocyanine ligand. The R groups are C_{10}H_{21}.*

Phthalocyanine complexes with copper are known to exhibit a colour change when in contact with nitrous oxide (NO$_2$) [2]. They are colourless when they form a complex with NO$_2$, whereas they are dark green when the gas escapes. The presence or absence of NO$_2$ would determine the colour of the phthalocyanine multilayer, leading to a colour change that can be easily visually detected. This provides a route for a possible design of a portable and relatively simple device. In this chapter, a possible design and the structure characterisation of a portable sensor device based on an aliphatic substituted phthalocyanine will be discussed.

The structure of the proposed biosensor, Figure 3.3, is relatively simple compared to the existing devices. A multilayer of aliphatic substituted phthalocyanine (only a bilayer is shown in the figure for clarity) is deposited on a solid substrate which has been rendered hydrophobic by means of silane coupling [3]. The structure is then saturated with NO$_2$ and capped with a lipid monolayer that works as the sensitive biological element. The NO$_2$ would remain confined between the solid substrate and the lipid monolayer, keeping the phthalocyanine colourless. When the biosensor is in...
contact with uncontaminated water, the integrity of the lipid monolayer is maintained and there is no colour change in the sensor (Figure 3.3 (a)). When contaminants are present in the water (Figure 3.3 (b)), the lipid monolayer is compromised (red circles in the figure) and NO₂ is free to leave the phthalocyanine region of the sensor. This would produce a colour change which can be easily detected visually.

![Figure 3.3. Schematic structure of the phthalocyanine sensor device.](image)

For the lipid monolayer to work efficiently as a sensing layer, it must meet some requirements:

1. The layer needs to be homogeneously deposited
2. The layer must form an efficient partition for NO₂ from the external environment, i.e. it needs to be able to trap the NO₂ within the phthalocyanine multilayer region.

Clearly, the biosensor cannot be used immediately after manufacturing: it needs to be transported to the location where it would be utilised and it most likely will have to be stored for some time. Even with excellent lipid coverage, some gas would leave the phthalocyanine inner structure over long periods of time. This problem would be overcome by sealing the biosensor under NO₂.

Non-peripherally 1,4,8,11,15,22,25-octa-substituted phthalocyanines were synthesised over 20 years ago [4]. They possess high solubility in organic solvents which, along with suitable balance between hydrophilic and hydrophobic parts,
renders these compounds amongst the most suitable in the phthalocyanine derivates family for the production of highly ordered Langmuir films [5]. These films can be transferred onto solid substrate by Langmuir-Blodgett depositions. It has been observed that the molecular stacking tends to remain unaltered during the film transfer from the air-water interface to the solid substrate. Nabok et al., reported an in-plane structure within the Langmuir-Blodgett film consisting of two dimensional domains having herringbone molecular arrangement [6]. They also observed, using IR optical spectroscopy, that the phthalocyanine molecules are oriented almost vertically to the substrate plane. This observation was also reported by Palacin [7].

3.2 Neutron reflectivity experiment

The composition of the biosensor is relatively complex and it is of upmost importance to understand fully the structure of the interfaces. Particularly, it is essential to establish the suitability of the hydrophobed silicon block as the anchoring substrate (hence preventing the detachment of the multilayer structure from the solid support) and the effectiveness of the lipid monolayer to separate the sensing region of the device from the bulk water. Neutron reflectivity has proved to be amongst the most suitable techniques to probe the structure of multilayers. Because of the small size of the biosensor (preferably not more than 1 cm$^2$) and the complexity of its structure (ideally several phthalocyanine bilayers are stacked upon each other), the structural analysis of an actual biosensor would be extremely complicated. Therefore, it was chosen to produce an idealised version of the device, substituting the phthalocyanine multilayer structure with a single phthalocyanine bilayer and capping this bilayer with the sensing lipid monolayer (as shown in Figure 3.3).

3.2.1 Materials

The lipids constituting the sensing layer, d-1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and functionalized poly(ethylene glycol) distearoyl-
phosphatidyl-ethanolamine (DSPE-PEG(2000)), were purchased from Avanti (Avanti, Polar Lipids, Inc.). The aliphatic substituted phthalocyanine (C\textsubscript{10}) was synthesised by our collaborators in the University of East Anglia and details of the synthetic procedure are reported elsewhere [8]. The silicon blocks used as solid substrates were circular in section with a diameter of 100 mm and a thickness of 10 mm. The silicon surface was initially cleaned using the RCA method [9]. The substrates were then chemically dried prior to treatment with a silane coupling agent (octadecyltrichlorosilane, C-18) using established methods [3]. This has been shown to provide a chemically bound hydrophobic layer on the silica surface [10].

3.2.2 Sample preparation

The experiment was performed at the ISIS spallation neutron source using the reflectometer SURF (see section 2.4.3.1). Two silicon substrates, S1 and S2, were used in this experiment. In sequence, 2, 4 and 6 layers of substituted phthalocyanine were deposited onto the first hydrophobed silicon substrate, S1, using Langmuir−Blodgett dipping technique. The deposition was performed using a standard Langmuir trough with a pool sufficiently big to allow the complete immersion of the silicon substrates. The trough was filled with ultrapure water and the interface was carefully cleaned to avoid possible contaminations before spreading. A solution of phthalocyanine in chloroform was prepared and then carefully spread at the air-water interface. An ordered Langmuir film was then obtained by moving the barriers until a surface pressure of 25 mN m\textsuperscript{-1} was reached. The same surface pressure was maintained constant during the dipping of the substrate. The sample holder allowed dipping and removing the block from the aqueous phase at a constant speed of 5 mm min\textsuperscript{-1}.

First, one phthalocyanine bilayer was deposited. The block with the bilayer was then characterized using neutron reflectometry with D\textsubscript{2}O forming the aqueous sub-phase. After the first measurement, the block was carefully removed from the cell and a second phthalocyanine bilayer was deposited on top of the pre-existing one using the same procedure described above. Now those two bilayers of phthalocyanine were
again characterized using neutron reflectometry. The same procedure was followed for the deposition of the third bilayer.

In a second experiment, a mixture of DPPC and DPPE carrying a PEG (2000) group (3% w/w) monolayer was transferred on top of the second hydrophobed silicon substrate, S2, to characterise the structure of the lipid monolayer in absence of the underlying phthalocyanine structure. The presence of the PEG group is known to enhance the stability of a lipid monolayer [11], for example large PEG groups are desirable in products for skin care as they possess mildness enhancing properties. The polymer works as a steric barrier that enhances the stability of the device. The Langmuir-Blodgett deposition of the lipid mixture was also performed at a constant surface pressure of 25 mN m$^{-1}$ and a dipping speed of 5 mm min$^{-1}$. As only a monolayer had to be deposited, the block was immersed in the aqueous phase, and then the air–water interface was carefully cleaned by vacuum suction. Only then was the block slowly removed from the trough. The structure of the lipid monolayer was then determined. After the reflectivity profile had been measured the monolayer was washed away and a bilayer of phthalocyanine followed by a lipid monolayer was deposited. In actual devices the NO$_2$ would be entrapped between the lipid monolayer and the silicon substrate. This deposited layer sequence represents a simplified ideal biosensor. A series of neutron reflectivity profiles were then obtained to characterize these layers. All measurements were carried out at room temperature.

The C-18 hydrophobic layer chemically bound to the silicon blocks has already been fully characterized by our group using neutron reflectivity [10].
Figure 3.4. Ellipsometry data obtained at the Si-air interface for (a) Si/C-18: oxide layer of 16 ± 2 Å and a C-18 layer of thickness 17 ± 4 Å and (b) Si/C-18/1 × bilayer of phthalocyanine with a layer thickness of 41 ± 2 Å. Ellipsometry measures the reflectance ratio $\rho$, which is a function of the change of polarisation of light upon reflection from an interface $\left(\rho = \frac{r_p}{r_s} = \tan(\psi) e^{i\Delta}\right)$. $\Delta$ represents the phase shift and $\tan(\psi)$ the amplitude ratio upon reflection. Empty circles are the $\Delta$ component and the filled circles are the $\psi$ component (solid lines are fit to the data).

The silicon oxide layer and the C-18 hydrophobic layer were initially characterized using ellipsometry at the air-silicon interface (Figure 3.4 (a)). In addition, the solid
substrate with a phthalocyanine bilayer was also characterised with ellipsometry (Figure 3.4 (b)). The layer thickness of the oxide layer and C-18 layer were then used as a starting point in the subsequent fitting procedure.

### 3.2.3 Results and discussion

The measured reflectivity data for the first hydrophobed silicon block S1 with 2, 4 and 6 layers of phthalocyanine with D$_2$O forming the aqueous sub-phase are shown in Figure 3.5. The fits to the data are also shown in the figure by the solid lines. The scattering length density profiles are given in Figure 3.6.

<table>
<thead>
<tr>
<th>Si (S1) substrate</th>
<th>Layer</th>
<th>Layer thickness / Å</th>
<th>Nb $\times$ 10$^{-6}$ / Å$^{-2}$</th>
<th>Roughness / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>silicon oxide</td>
<td>13</td>
<td>3.6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2 layers of phthalocyanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-18</td>
<td>30 ± 2</td>
<td>0.84</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>phthalocyanine</td>
<td>15 ± 2</td>
<td>4.50</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>phthalocyanine</td>
<td>28 ± 2</td>
<td>5.60</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>4 layers of phthalocyanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-18</td>
<td>30 ± 2</td>
<td>-0.43</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>phthalocyanine</td>
<td>15 ± 2</td>
<td>2.83</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>phthalocyanine</td>
<td>30 ± 2</td>
<td>4.50</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>phthalocyanine</td>
<td>30 ± 2</td>
<td>5.60</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>6 layers of phthalocyanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-18</td>
<td>30 ± 2</td>
<td>-0.43</td>
<td>2</td>
</tr>
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<td>1.46</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>phthalocyanine</td>
<td>30 ± 2</td>
<td>2.83</td>
<td>2</td>
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<tr>
<td></td>
<td>phthalocyanine</td>
<td>30 ± 2</td>
<td>4.50</td>
<td>2</td>
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<tr>
<td></td>
<td>phthalocyanine</td>
<td>30 ± 2</td>
<td>5.60</td>
<td>7</td>
</tr>
</tbody>
</table>

In the modelling of the data, the thickness and scattering length density of the oxide layer was found to be $d = 13 \pm 2$ Å, $Nb = 3.6 \times 10^{-6}$ Å$^{-2}$. The thickness of the oxide layer is slightly thicker than that observed in other experiments in this thesis ($d = 8 \pm 2$ Å). The difference is, however, not substantial and could have been
caused by an increase in temperature during the RCA cleaning procedure. The fitted structural parameters obtained from these fits are given in Table 3.1.

![Graph showing reflectivity profiles for 2 layers (O), 4 layers (Δ) and 6 layers (□) of phthalocyanine at the silicon-D$_2$O interface. The solid lines show the fits to the data. The profiles are shifted by a factor of ×10 for the purpose of clarity.](image)

The thickness of the C-18 layer was found to be 30 ± 2 Å, which is thicker than the expected value of 24 Å reported previously [10] and a value of 17 ± 4 Å for a dry C-18 layer coupled silicon sample deduced from the ellipsometry data. This indicates an entanglement of the C-18 chain with the aliphatic tails of the phthalocyanine layer, hence the good anchoring characteristic of the C-18 Layer. Because of the similar values of scattering length density, neutron reflectometry cannot distinguish between the C-18 chain and the aliphatic tail of the phthalocyanine because of the lack of contrast between the protonated C-18 and the aliphatic part of the phthalocyanine which is also protonated. It is noteworthy that as the number of phthalocyanine layers increases, the water (D$_2$O) content of the underlying structures decreases.
Figure 3.6. Scattering length density profiles for 2 layers (black line), 4 layers (red line) and 6 layers (blue line) of phthalocyanine at the silicon-D$_2$O interface.

This decrease in water content can be visualised in the scattering length density profiles (Figure 3.6): a decrease in scattering length density in the phthalocyanine regions indicates a reduction in the volume fraction $\Phi$ of D$_2$O. The total layer thickness for the aliphatic substituted phthalocyanine for the sample with 2 layers was found to be 43 Å, for 4 layers 75 Å, and for 6 layers 105 Å, in good agreement with atomic force microscopy results at the glass-air interface reported in the literature [12]. The deposition of 2, 4 and 6 layers of phthalocyanine at the glass-air interface showed that each bilayer has the same thickness [12], whereas a slight reduction in overall thickness is observed in our case in presence of water. Roughly a 13% and an 18% reduction in the overall layer thickness for 4 and 6 layer samples was observed, which could result from the D$_2$O expulsion from the inner layers as more layers are deposited.

In the second experiment, a mixture of DPPC and DPPE carrying a PEG group (3% w/w) was initially deposited as a monolayer on the second hydrophobed silicon surface (S2). The reflectivity profile is shown in Figure 3.7.
The fit to the data indicates a mixing of the deuterated tail of DPPC and the protonated C-18 chain over a 19 Å region. This confirms the interpenetration between the C-18 hydrophobic region and the phthalocyanine aliphatic chains and can now be seen because of better contrast. A three layer model was required to fit the profile: a 19 Å layer consisting of a mixture of C-18 and lipid tail, a 16 Å layer representing the lipid tail region, followed by a 60 Å layer representing the large PEG and lipid head group region. The fitted parameters are given in Table 3.2. The lipid monolayer was then washed from the silicon substrate and a bilayer of phthalocyanine was deposited followed by a lipid monolayer. The reflectivity data obtained are shown in Figure 3.8.

This deposited layer sequence represents an ideal device structure. The scattering length density profile for the fit is shown in Figure 3.9 by the red line along with the scattering length density profile of 1 bilayer of phthalocyanine (measured with S1, black line). The fitted structural parameters are given in Table 3.2. The black rectangle in Figure 3.9 highlights how the D$_2$O penetration into the phthalocyanine
bilayer in the presence of the capping lipid monolayer was found to be much less than that in the case of the uncapped phthalocyanine bilayer.

Figure 3.8. Reflectivity profiles for 2 layers of phthalocyanine capped by DPPC-DPPE+PEG at the silicon-D$_2$O interface. The fit is shown by the solid line.

Figure 3.9: Scattering length density profiles for 2 layers of phthalocyanine (black line) and 2 layers of phthalocyanine capped by the DPPC-DPPE+PEG layer (red line) at the silicon-D$_2$O interface. The box represents the phthalocyanine layer contribution to the data.
Table 3.2: Fitted structural parameters obtained from the fits to the reflectivity profiles shown in Figure 3.7 and Figure 3.8.

<table>
<thead>
<tr>
<th>Si (S2) substrate</th>
<th>Layer</th>
<th>Layer thickness / Å</th>
<th>Nb × 10^-6 / Å^-2</th>
<th>Roughness / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>si (silicon oxide)</td>
<td>13 ± 2</td>
<td>3.6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>C-18 + lipid monolayer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-18 + d-lipid</td>
<td>19 ± 2</td>
<td>0.55</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>lipid chain</td>
<td>16 ± 2</td>
<td>3.30</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>lipid head-PEG</td>
<td>60 ± 5</td>
<td>5.83</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>C-18 + 2 layers of phthalocyanine + lipid monolayer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-18</td>
<td>30 ± 2</td>
<td>-0.12</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>phthalocyanine</td>
<td>15 ± 1</td>
<td>1.22</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>phthalocyanine</td>
<td>30 ± 1</td>
<td>2.32</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>lipid chain</td>
<td>16 ± 1</td>
<td>3.48</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>lipid head-PEG</td>
<td>60 ± 5</td>
<td>5.83</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

There are two possible explanations for the decrease in scattering length density in this region:

1. Knowing the scattering length density of a phthalocyanine molecule (Nb ~ 1.0 × 10^-6 Å^-2), one can estimate the total volume fraction of water (Φ_w) in the phthalocyanine bilayer. When the phthalocyanine bilayer is in contact with D_2O, we calculated Φ_w1 ~ 0.65 in the inner layer and Φ_w2 ~ 0.85 in the second layer. For the phthalocyanine bilayer capped by the lipid monolayer instead, we calculated Φ_w1 ~ 0.05 in the inner layer and Φ_w2 ~ 0.25 in the second layer. This is a very simple estimation and does not take into account the interdigitation between the phthalocyanine alkyl chains and the lipid monolayer or the C-18 hydrophobic layer. The reduction in Φ_w when the phthalocyanine bilayer is capped by the lipid monolayer is significant and it could suggest water exclusion from the inner part of the structure. However, a decrease in Φ_w must go along with an increase in phthalocyanine volume fraction Φ_p. As the experimental conditions were the same for the deposition of the phthalocyanine bilayer, this argument does not seem probable.

2. All the layers were deposited using Langmuir-Blodgett technique filling the Teflon trough with ultra-pure H_2O. Small amounts of H_2O would remain associated with the deposited layers and would rapidly exchange with bulk...
D$_2$O once the cell is assembled. This is extremely likely to happen during the analysis of phthalocyanine multilayers. Possibly when the phthalocyanine bilayer is capped by the lipid monolayer the rate of H$_2$O / D$_2$O exchange is much reduced and the observed decrease in scattering length density is because of the presence of residual H$_2$O in the layers.

The second explanation seems far more likely. However, for the sake of argument the contrasts used in this experiment do not permit the exclusion of either of the two reasons discussed above. Some possibilities to resolve this ambiguity would involve using deuterated phthalocyanine or filling the Teflon trough with D$_2$O for the Langmuir-Blodgett deposition. Even if we do not know which of the two events is occurring, in both cases the efficiency of the lipid monolayer to form a barrier against water penetration, and thus partitioning the deposited phthalocyanine layers from the bulk water, is proved. It is essential to stress that the low permeability of the lipid monolayer is of utmost importance as this is a requirement for these types of devices.

### 3.3 Conclusions and future work

This work provided a better understanding of the structure of these biosensors. It has been shown that as the number of phthalocyanine bilayers increases, water is progressively excluded from the inner regions of the sensor, leading to a relatively compact and uniform structure. This is a key aspect as in the real biosensor several phthalocyanine layers would be used. In this chapter, the effectiveness of the lipid layer in partitioning (sealing) the deposited phthalocyanine layers from the bulk water has also been shown. This is crucial for the viability of these systems as the biosensors’ operation relies on the entrapment and subsequent release of nitrous oxide gas depending on the integrity of the lipid membrane.

The next possible step in the development of this class of biosensors is to test the efficiency and the response time to any damage caused to the lipid monolayer by contaminants. A follow-up experiment involving neutron reflectivity has been
planned for the future. The experiment is aimed to evaluate the biosensor’s response to damage in the lipid capping monolayer. When the lipid monolayer is damaged we expect the nitrous oxide to escape from the inner structure, causing changes in the biosensor’s composition and ultimately a visual colour change. Anti-microbial surfactants possess the ability to alter the structure of lipid membranes and they are effective within 10-20 minutes of contact [13]. Therefore it is intended to characterise the structural changes in the biosensor by studying the interaction of anti-microbial surfactants with the lipid monolayer. This experiment would demonstrate the real viability of these new biosensors. Other possible future developments of these sensors would be the use of functionalised lipids enabling sensing selectivity for these devices.
3.4 References for Chapter 3

Chapter 4

Adsorption of palmitic acid at the oil-metal interface: a polarised neutron reflectivity study

4.1 Introduction

Lubricants have extensively been used throughout the ages to reduce friction but it is only quite recently that the study of lubrication as a science has begun. The advent of the steam engine and the automobile, in the early 20th century, drastically increased the demand for far more complex lubricant systems. Nevertheless, the development over at least the first half of the past century has been mostly empirical and the detailed mechanism of the chemistry involved in lubrication was not completely understood.

During the second half of the 20th century there was a substantial increase in number of analytical techniques which offered a unique opportunity to resolve the complex chemistry involved in lubrication.

The advances made were so fast and significant that the scientific community was impelled to give a new name to this exponentially expanding branch of engineering. In 1966 the term tribology was coined. The word tribology originates from ancient Greek word *tribos*, which means rubbing. Tribology today refers to the interdisciplinary science of interacting solid surfaces in relative motion. The areas covered by this science include lubrication, friction and wear. It is an interdisciplinary research area between chemistry, physics, solid and fluid mechanics, material science and lubricant rheology.
At the beginning of the 20th century, Striebeck proposed for the first time the relationship between friction coefficient $\mu$ and applied load $P$ in an important original paper [1]. According to his classification, there are three broad regimes of lubrication [2] depending on the applied load: (a) the boundary regime where the high load pushes most oil out, leaving a molecularly thin film between the surfaces; (b) an intermediate “mixed” regime and (c) the hydrodynamic regime, where a reasonable layer of oil can survive between the moving surfaces. This classification is shown in Figure 4.1, the so called Striebeck curve.

![Figure 4.1. Characterisation of the friction regimes according to the Striebeck curve. In the plot, $\mu$ is the friction coefficient, $\eta$ is the fluid viscosity, $N$ is the relative speed of the surfaces and $P$ the applied load.]

Lubrication is obtained by maximising both the physical and chemical properties of the lubricating fluid. Physical properties, which include viscosity, density, heat capacity and thermal conductivity, are essential for the lubricant to operate under hydrodynamic lubrication. Chemical properties such as detergency, solvency, anti-wear, frictional and anti-corrosion properties are vital for successful boundary lubrication. Clearly, with so many requirements to be met, base stock oil alone would fail to be an efficient lubricant; hence chemical additives are usually added to enhance the lubricant performances.
4.1.1 Base stock oils

Base stock oil is normally derived from mineral oil or petroleum. It must possess the ability to dissolve chemical additives, to be inert toward all the parts of the engine and have some ability to reduce wear and friction by itself. In addition, low volatility is a requirement, along with long term stability to high temperature and oxidative stresses. Viscosity is also a key aspect: the oil must be fluid to avoid localised heat, but viscous enough to maintain a lubricant film under operating conditions. Petroleum derived base stocks mainly contain molecules with 18 to over 40 carbon atoms which can be classified in three main hydrocarbon categories: paraffins, naphthalenes and aromatics. Nonetheless, most of the molecules contain at least two of these basic structures. In addition, these base stock oils contain a small fraction of heteroaromatic compounds (containing mainly oxygen, sulphur or nitrogen). Although these compounds range from 0.5 to 4 wt% [3], they have a major influence on lubrication properties and stability. Some of these heteroaromatic complex molecules perform well as antioxidant and anti-wear compounds (although some of them could be quite corrosive) and they tend to interact both chemically and physically with additives.

4.1.2 Chemical additives

There are five important components which are required when formulating a lubricant [4,5]:

I. Viscosity index improvers: these compounds reduce the viscosity-temperature dependence;
II. Detergent inhibitors: they keep the engine clean from deposits;
III. Dispersants: they disperse sludge in solution;
IV. Antioxidants;
V. Anti-wear.

• Some additional components are also desirable:
  o Anti-rust;
  o Anti-foam;
  o Friction modifiers;
4.1.2.1 Viscosity index improvers

Ideally the lubricant viscosity should be constant over a large range of temperature. This is not usually obtainable as inevitably viscosity significantly reduces with increasing temperature. Viscosity index improvers are used in formulations to enhance lubricant performances over a broad range of temperature of an engine. They are hydrophobic polymers with the tendency to have a folded conformation at low temperature and unfold at high temperature, therefore partly counteracting the viscosity decrease over temperature.

4.1.2.2 Detergent inhibitors

The main functions of detergent inhibitors are to prevent deposition of material on the metal components of the engine surface and to work as a buffer. They are micro-dispersions of alkaline or alkaline earth metal carbonates in oil using surfactants. Their particle size is generally less than 30 nm, thus they are optically transparent.

4.1.2.3 Dispersants

The amount of solid waste produced in an engine is such that it would rapidly and inevitably clog oil pipes and filters. This waste must be efficiently and rapidly dispersed so that it is kept in suspension in the oil and can be efficiently removed through the filters. Dispersants are typically produced by combining low molecular weight (~1000) polyisobutenes with anhydrides or carboxylic acids and then reacting these with polymers such as polyamines and polyalcohols. Their mechanism is similar to detergent inhibitors but as bigger particles are involved, longer chain hydrocarbons are employed to provide sufficient steric stabilisation.
4.1.2.4 Anti-wear additives

In an engine, metal to metal contact often takes place, especially under the boundary regime. It must be stressed that an engine has to face rather extreme conditions and significant pressure, in the order of GPa, and temperatures can rapidly increase to hundreds of degrees. Under these circumstances, partial surface melting could occur and, if the oxide layer is sheared away because of friction, metal to metal welding could take place, leading to irreversible engine failure. Anti-wear agents protect the metal surfaces by adsorbing onto the oxide surfaces, where they form an easily removable sacrificial layer that gets sheared instead of the metal oxide [6]. For such layers to be effective, the film needs to instantaneously adsorb from the bulk oil after its removal from the metal oxide surface by shear. The most common anti-wear agents are zinc dialkyl dithiophosphates (ZDDP). The chemical structure of ZDDP is shown in Figure 4.2.

![ZDDP molecule, R group ranges from C₃ to C₁₈.](image)

It is generally recognised that the anti-wear properties of ZDDP are attributed to its ability to form a reaction film on the metal surface. It seems that this film inhibits fluid film formation on the bare metal surface: because of the smoothness of ZDDP reaction films, the liquid lubricant may slip against them [7].

4.2 Fatty acids as lubricant additives

Fatty acids cannot be classified under one of the specific categories above as their function is very broad. In fact, they are known efficiently to inhibit corrosion on metal surfaces [8] and to reduce the friction coefficient [9]. There is also evidence that they may have anti-wear properties [10]. Therefore their versatility makes this class of surfactants amongst the most widely used lubricant additives.
Despite their broad use as lubricant additives, their mechanism is not yet fully understood. The adsorption processes that take place at the oil-metal interface are of utmost importance to elucidate the role of fatty acids in lubrication. A better understanding of the adsorption profiles will enable the development of more efficient and effective lubricants, with both economic and environmental advantages [11].

The tribological properties of fatty acids as lubricant additives have been studied by Kajdas et al. [12]. They studied the relative change in wear for hexadecane and 1-methylnaphthalene containing palmitic acid at a concentration varying from 50 to 1000 ppm. The main technique used was the pin-on-disc contact method [13] performed at 25°C. The relative ball wear % as a function of palmitic acid concentration is shown in Figure 4.3.

![Figure 4.3. Relative ball wear in systems lubricated with 100-1000 ppm solutions of palmitic acid in hexadecane and 1-methylnaphthalene (pin-on-disc apparatus).](image)

The wear results indicate that adsorption processes onto the metal surface are taking place and the palmitic acid film seems significantly to reduce wear. Interestingly,
although the reduction in wear is observed at all concentrations, there seems to be a
decrease in anti-wear performances when the concentration of palmitic acid in
hexadecane is 750 ppm. Anti-wear properties are, however, almost fully recovered
when the concentration is raised to 1000 ppm. The reason for this unexpected
behaviour is still unclear.

The study presented in this chapter was the first part of a larger investigation aimed
at understanding the role of surfactants and the nature of the oil in lubrication
processes. Neutron reflectivity has the appropriate sensitivity to resolve the
adsorption processes at the oil-metal interface, which are believed to play a key role
in the lubrication properties. In this study, the neutron reflectivity technique was
used for the first time to study the adsorption of palmitic acid from bulk hexadecane
onto the metal-oil interface.

4.3 Neutron reflectivity experiment

4.3.1 Materials and solid substrate functionalisation

Hexadecane-d$_{34}$ was obtained from Cambridge Isotope Laboratories, UK (> 98%
deuterated) and palmitic acid was purchased from Sigma-Aldrich UK. An iron block
would not be a suitable substrate for neutron reflectivity. Instead, a metal layer was
deposited onto a silicon substrate (diameter 55 mm) using the sputtering technique
[14].

The iron depositions were carried out at Helmholtz Zentrum Berlin using a UHV DC
magnetron sputtering system with the argon pressure and sputter power of $1.3 \times 10^{-3}$
mbar and 0.65 kW respectively (the base pressure was $6.5 \times 10^{-6}$ mbar). An iron
layer was also deposited on a float glass (3 mm thick) substrate for the purpose of
calibration of the sputtering machine. The reference layer deposited on the float glass
was analysed with X-ray reflectivity. The reflectivity profile is shown in Figure 4.4
along with the fit; the parameters used for the data fitting are shown in Table 4.1. A
thin tin layer was deposited onto the float glass prior to the deposition of iron as it
was necessary to ensure adhesion. This layer, however, was not required for the deposition of iron onto silicon.

Table 4.1. Structural parameters of the iron coated float glass as determined from X-ray reflectivity.

<table>
<thead>
<tr>
<th>Material</th>
<th>Thickness / Å (± 1)</th>
<th>Roughness / Å</th>
<th>Nb × 10^6 / Å^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron oxide</td>
<td>21.0</td>
<td>5.0</td>
<td>3.93</td>
</tr>
<tr>
<td>Fe</td>
<td>259.0</td>
<td>4.0</td>
<td>5.94</td>
</tr>
<tr>
<td>Sn</td>
<td>10.0</td>
<td>4.0</td>
<td>2.70</td>
</tr>
<tr>
<td>Float glass</td>
<td>-</td>
<td>4.5</td>
<td>1.89</td>
</tr>
</tbody>
</table>

Figure 4.4. X-ray reflectivity for the calibration sample sputtered on a float glass substrate. The solid line is the fit to the data. The fitted parameters are given in Table 4.1.

It was shown many decades ago that very little adsorption of fatty acids occurs onto oxide-free metal surfaces; whereas adsorption is much higher and faster at metal oxide surfaces [15,16]. For this reason, an iron oxide layer was allowed to form on
the iron surface by brief exposition to air. However, the substrate was stored in an inert atmosphere prior to the neutron experiment to avoid excessive thickness and roughness of the oxide layer.

4.3.2 The need for polarised neutron reflectivity

With the deployed experimental setting, the neutron beam would approach the interface travelling through the silicon block. This implies that before reaching the iron-oil interface, where the adsorption process takes place, the neutron beam would be subject to reflection from the silicon-iron interface. The difference in scattering length density between the silicon ($Nb = 2.07 \times 10^{-6}$ Å$^{-2}$) and the iron layer ($Nb \approx 8 \times 10^{-6}$ Å$^{-2}$) is so large that the total reflectivity would be strongly dominated by the silicon-iron interface contribution. This would lead to a significant reduction in sensitivity to the adsorption of palmitic acid at the iron-oil interface. Because of the presence of the iron layer, the use of polarised neutron reflectivity, instead of standard neutron reflectivity, allows for different contrasts from the same sample to be obtained. For this reason, the choice of deploying polarised neutron reflectivity was purely to enhance the sensitivity of the technique to the structure of the adsorbed surfactant layer at the metal-oil interface.

The neutron refractive index for a magnetic sample which is magnetised in the plane of the surface can be written as a sum of the nuclear ($n_{\text{nuclear}}$) and the magnetic ($n_{\text{magnetic}}$) component of the sample.

$$n_\pm = n_{\text{nuclear}} \pm n_{\text{magnetic}}$$  \hspace{1cm} \text{Equation 4.1}

The neutron spin-dependent refractive indices result in a neutron spin-dependent reflectivity ($R^+$ and $R^-$), more details are given in section 2.3.3. The idealised scattering length density profiles for both the magnetic and the nuclear contributions of silicon, iron and iron oxide layers with hexadecane forming the bulk oil are shown in Figure 4.5.
Figure 4.5. Idealised neutron scattering length density of the iron coated silicon substrate in contact with d-hexadecane: spin up neutrons (blue line), spin down neutrons (red line) and non-polarised neutrons (black line). The contrast using spin down neutrons minimises the reflection from the silicon-iron interface, allowing the adsorption of palmitic acid onto the iron oxide-oil interface to be dominant.

These scattering length density profiles clearly show that for the spin up (\(\delta^\uparrow\)) neutrons the reflectivity would be even more strongly dominated by the Si-iron interface contribution, hence a reduction in sensitivity to any adsorbed palmitic acid at the iron oxide-bulk oil interface. The spin down (\(\delta^\downarrow\)) reflectivity profile, however, enables minimisation of the reflection from the silicon-iron interface; hence it is the spin down (\(\delta^\downarrow\)) reflectivity that will provide the optimum contrast scheme for resolving the structure of the palmitic acid at the iron oxide-bulk oil interface. However, both the spin up (\(\delta^\uparrow\)) and spin down (\(\delta^\downarrow\)) reflectivities (R+ and R-) were measured for all concentrations. The schematic diagram of the reflection geometry used is shown in Figure 4.6.
Figure 4.6. Schematic representation of the experimental geometry.

The polarised neutron reflectivity experiment was undertaken using the neutron reflectometer V6 at HMI in Berlin (section 2.4.3.4). The V6 instrument deploys a cold neutron wavelength of 4.66 Å, and the beam incident angle was varied by tilting the sample to obtain a Q range up to about 0.15 Å⁻¹. The polarisation ratio is ~98.5% and this was taken into account during all the fitting procedures. A magnetic field of 300 Gauss was applied in the plane of the sample perpendicular to the direction of the incident neutron beam.

4.3.3 Results

Before analysing the adsorption of palmitic acid at the oil-metal interface, the iron layer was first characterised with polarised neutron reflectivity. The substrate was placed in the sample holder facing upwards (in sequence, the neutron beam went through air, iron oxide, iron and silicon) and both the spin up (δ↑) and spin down (δ↓) reflectivities were measured. The reflectivity profiles and the fits to the data are shown in Figure 4.7, whereas the fitted parameters are given in Table 4.2.

The magnetic moment $\mu$ of the oxide layer is intermediate between Fe₂O₃ (non-magnetic, $\mu = 0.0 \mu_B$) and Fe₃O₄ (magnetic, $\mu = 4.1 \mu_B$), suggesting that the layer consists of a mixture of the two oxides. Examples of mixtures of such oxides have been reported for the sputtered iron layers in literature [17].
Figure 4.7. Reflectivity profiles for spin up (●) and spin down (○) neutrons at the air-metal interface. The solid lines correspond to the simultaneous fit to the data.

Table 4.2. Structural parameters of the iron coated silicon substrate as determined from polarised neutron reflectivity.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Thickness / Å (± 2)</th>
<th>Roughness / Å</th>
<th>Magnetic moment $\mu / \mu_B$</th>
<th>$N_b \times 10^{-6} / \text{Å}^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Oxide</td>
<td>28</td>
<td>1</td>
<td>2.0</td>
<td>6.19</td>
</tr>
<tr>
<td>Fe</td>
<td>300</td>
<td>6</td>
<td>2.2</td>
<td>7.72</td>
</tr>
<tr>
<td>Si</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.07</td>
</tr>
</tbody>
</table>

The neutron spin asymmetry plot in Figure 4.8 confirms the suitability of the model representing the magnetic feature of the sample. The parameters extrapolated from the model found for the iron-coated silicon substrate were used for the subsequent fitting procedure.
After the characterisation of the solid substrate, the cell was assembled and filled with the oil phase. A series of spin up and spin down neutron reflectivity profiles were measured as a function of increasing palmitic acid concentration in deuterated hexadecane at room temperature. Because of the long acquisition time required for the full characterisation of each solution (about 16 hours for measuring the reflectivity profiles for both spin up and spin down neutrons), only three concentrations could be measured during this experiment. The concentration of palmitic acid in hexadecane used in this experiment was well above the CMC, which is about 3.9 mM (~1 ppm) [18]. The reason for this was to investigate a range of realistic concentration which is typical of that used in formulations.

The spin up ($\delta^{\uparrow}$) and spin down ($\delta^{\downarrow}$) reflectivities ($R^+$ and $R^-$) were measured for palmitic acid concentrations of 150, 500 and 1000 ppm in d-hexadecane at room temperature. An example of a pair is shown for 150 ppm palmitic acid concentration in Figure 4.9.
Figure 4.9. Spin up (●) and spin down (○) reflectivity profiles for the 150 ppm solution in d-hexadecane at the oil-metal interface.

Figure 4.10. Reflectivity profiles for spin up neutrons for 1000 (∆), 500 (●) and 150 (○) ppm of palmitic acid in d-hexadecane, the solid line is the fit to the 150 ppm of palmitic acid. There are no measurable differences within the three reflectivity profiles and one fit is satisfactory for all concentrations. This confirms that the spin up neutrons lead to reflectivity profiles with insufficient sensitivity to the adsorption processes at the metal-oil interface.
As discussed earlier, the reflection from the silicon-iron interface using spin up ($\delta^{\uparrow}$) neutrons dominates the total measured reflectivity and no measurable differences were observed within the different profiles. The spin up reflectivity profiles are shown in Figure 4.10, where the solid line is the fit shown in Figure 4.9 for the 150 ppm concentration. The same fit can adequately represent all three concentrations. Because of the lack of sensitivity to the adsorbed layer using spin up ($\delta^{\uparrow}$) neutrons, henceforth only the spin down ($\delta^{\downarrow}$) reflectivity profiles are discussed for convenience.

The reflectivity profiles for spin down neutrons ($\delta^{\downarrow}$) for all concentrations are shown in Figure 4.11; the solid lines represent the fits to the data. The contrast scheme adopting spin down neutrons was expected to lead to measurable differences within the reflectivity profiles and such differences are shown in Figure 4.11 Insert. The fitted parameters for the fits to the data are given in Table 4.3 and the scattering length density profiles of the fitted models are shown in Figure 4.12.

![Graph showing reflectivity profiles for spin down neutrons for 1000 (Δ), 500 (●), and 150 (○) ppm of palmitic acid in d-hexadecane, with solid lines representing fits to the data. The profiles are shifted by a factor of 10 for clarity. Details of non-shifted profiles are shown in the Figure Insert to highlight differences. The fitted parameters are given in Table 4.3.](image-url)

**Figure 4.11.** Reflectivity profiles for spin down neutrons for 1000 (Δ), 500 (●), and 150 (○) ppm of palmitic acid in d-hexadecane, the solid lines represent the fits to the data. The profiles are shifted by a factor of 10 for the purpose of clarity. Details of the non-shifted profiles are shown in the Figure Insert to highlight the differences within the profiles. The fitted parameters are given in Table 4.3.
Table 4.3. Structural parameters determined from the fits to the silicon-iron-palmitic acid-d-hexadecane reflectivity profiles.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Thickness / Å (± 2)</th>
<th>Roughness / Å</th>
<th>Magnetic moment $\mu / \mu_B$</th>
<th>$Nb \times 10^{-6} / \text{Å}^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common layers to all three concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Si</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>2.07</td>
</tr>
<tr>
<td>Fe</td>
<td>300</td>
<td>1</td>
<td>2.2</td>
<td>7.72</td>
</tr>
<tr>
<td>Fe$_2$O$_3$</td>
<td>20</td>
<td>2</td>
<td>2.0</td>
<td>6.19</td>
</tr>
<tr>
<td>Oxide + PA</td>
<td>8</td>
<td>2</td>
<td>1.2</td>
<td>3.50</td>
</tr>
<tr>
<td>Palmitic acid concentration: 1000 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>16</td>
<td>5</td>
<td>-</td>
<td>-0.45</td>
</tr>
<tr>
<td>PA + d-oil</td>
<td>45</td>
<td>22</td>
<td>-</td>
<td>4.11</td>
</tr>
<tr>
<td>d-oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.70</td>
</tr>
<tr>
<td>Palmitic concentration 500 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>16</td>
<td>4</td>
<td>-</td>
<td>1.16</td>
</tr>
<tr>
<td>PA + d-oil</td>
<td>45</td>
<td>22</td>
<td>-</td>
<td>4.93</td>
</tr>
<tr>
<td>d-oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.70</td>
</tr>
<tr>
<td>Palmitic concentration 150 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>16</td>
<td>4</td>
<td>-</td>
<td>1.34</td>
</tr>
<tr>
<td>PA + d-oil</td>
<td>35</td>
<td>17</td>
<td>-</td>
<td>4.93</td>
</tr>
<tr>
<td>d-oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.70</td>
</tr>
</tbody>
</table>

Figure 4.12. Scattering length density profiles for spin down neutrons for the palmitic acid in d-hexadecane (150 ppm is the black line, 500 ppm the blue line and 1000 ppm the red line). To focus the attention on the metal-oil interface, a break has been introduced in the thickness axis.
4.3.4 Discussion

The scattering length density of the iron oxide layer indicated some inclusion of the palmitic acid to a depth of $8 \pm 2$ Å (the overall thickness of the oxide layer was $28 \pm 6$ Å), suggesting some degree of porosity. The overall composition of the oxide layer was the same for all the palmitic acid concentrations. A two layer model was then required to describe the structure of the adsorbed palmitic acid at the iron oxide-oil interface. The palmitic acid was found to be strongly adsorbed on the oxide-oil interface, resulting in a monolayer of thickness $16 \pm 4$ Å for 150 and 500 ppm palmitic acid concentrations ($16 \pm 5$ Å for the 1000 ppm solution). The fully extended molecular length for palmitic acid is $\sim 21$ Å; hence, the layer thickness suggests an apparent tilt of the fatty acid molecules with respect to the interface normal. An alternative explanation could be significant disorder within the monolayer. For an adequate representation of the data sets, the model also requires a second diffuse layer extending in the bulk oil. The thickness of this diffuse layer was $35 \pm 17$ Å for the 150 ppm solution and $45 \pm 22$ Å for 500 and 1000 ppm solutions.

![Image](image_url)

**Figure 4.13.** Volume fraction of hexadecane ($\Phi_h$) at the interface as a function of palmitic acid concentration (150 ppm is the solid line, 500 ppm the medium-dashed line and 1000 ppm the short-dashed line). The volume fraction of palmitic acid ($\Phi_{PA} = 1 - \Phi_h$) is shown in figure insert.
The relatively high roughness between the diffuse layer and the bulk hexadecane (however acceptable, less than half of the layer thickness) was adopted to represent a gradual change in composition of the palmitic acid away from the interface. This change in composition can be seen in the volume fraction profiles for the oil (hexadecane) and the corresponding palmitic acid as a function of distance away from the iron oxide (Figure 4.13 and Figure 4.13 insert respectively).

The composition profiles in Figure 4.13 suggest a depletion of the oil in the vicinity of the interface as the concentration of palmitic acid increases. The adsorbed amounts were calculated by integrating the volume fraction profiles of the palmitic acids and are shown in Figure 4.14. The inclusion of palmitic acid in the iron oxide layer was not considered in the calculations. These data show an almost linear increase for the adsorbed amount of palmitic acid as a function of concentration in the investigated range. However, a non-linear decrease to zero is normally expected as the palmitic acid concentration approaches zero.

![Figure 4.14. Adsorbed amount for palmitic acid at the oil-metal interface as calculated from the scattering length density profiles.](image)
4.3.5 Adsorbed amount of palmitic acid and wear profile: a tentative comparison

The adsorption profile of palmitic acid can be compared to the wear data reported for the same system at the metal-oil interface [12]. Since the pin-on-disc wear tests are rarely carried out under a hydrodynamic regime, and the adsorption data are taken from a system where no stress was applied, this is a tentative comparison and one should be cautious given the experimental designs are not fully comparable. The wear profile for the palmitic acid in \( n \)-hexadecane is shown in Figure 4.15, superimposed on the adsorption profile for palmitic acid in the same system. There seems to be a good correlation between this profile and the adsorbed amount for palmitic acid.

![Figure 4.15. Correlation between adsorbed amount and wear profile as a function of palmitic acid concentration. Error bars for the wear profiles are missing as they were not reported in the literature [12].](image)

The volume fraction profiles in Figure 4.13 suggest that as the adsorbed amount of palmitic acid increases with concentration, it results in the exclusion of the oil in the vicinity of the metal oxide surface. This exclusion is detrimental to the
hydrodynamic lubrication process provided by the oil. The role of fatty acids in these systems seems to arise from an increase in wettability of the oil at the metal oxide surface: at a low adsorbed amount of palmitic acid, the metal oxide-oil wettability increases providing the condition of good lubrication. As the adsorbed amount of acid increases prior to a full coverage there is depletion of the oil in the vicinity of the metal oxide-bulk oil interface and this is reflected in the increase of wear observed in the relative wear profile. However, when full coverage is reached, the lubrication property recovers. This indicates that a fully hydrophobic surface is now formed by the acid allowing a complete wetting of the surface by the hexadecane oil, hence a recovery in the lubrication process as suggested by the wear profile in Figure 4.15.

A macroscopic interpretation of the events occurring could be as follows. It has been observed that when metal surfaces in relative motion come in contact, they undergo stress and, as a result, the adsorbed layers could be partially sheared from the surfaces [6]. The oil wets the newly formed surfaces providing the formation of a new adsorbed layer. At a relatively low concentration of palmitic acid, the oil is able to wet these surfaces very fast, hence promptly restoring an efficient lubrication regime. Similarly, at a high concentration a uniform layer of palmitic acid is deposited and the oil is capable of quickly wetting the newly formed surfaces. At an intermediate concentration, however, the adsorbed layer has almost reached a full coverage: under these conditions, the wetting of the metal surface by the oil is slightly slowed down, leading to a delayed recovery of the lubrication properties. When surfaces are moving at such a high relative speed as in engines, it is not surprising that a small delay in the recovery of lubrication properties leads to greater wear.

4.4 Conclusions and future work

This chapter reports the first measurements of the adsorption of surfactants at the metal oxide-oil interface as a function of surfactant concentration using polarized neutron reflectometry. The adsorbed amount for palmitic acid was found to increase
almost linearly with the concentration and a connection between the adsorption processes at the oil-metal interface and the wear profiles observed for the same system was tentatively suggested. Further experiments have already been planned in collaboration with BP to investigate in more detail the adsorption processes taking place at the oil-metal interface. It is of interest to investigate other surfactants and oil systems, particularly multi-component mixtures, in addition to assessing the role of temperature on these adsorption profiles.

Polarised neutron reflectivity has proved to be an excellent technique to analyse adsorption processes on metal surfaces. Because of the presence of negative charges on the oxide, iron surfaces are considered good models for dirt particles. Interestingly, the presence of charges is pH dependent and the iron surface charge density can be, to some extent, tuned by adjusting the pH of the bulk solution. Our group has very recently applied polarised neutron reflectivity to study adsorption processes of surfactants at the iron oxide-water interface. The adsorption of positively charged C_{14}TAB from the aqueous solution was studied as a function of increasing pH. At high pH, when the surface is more negatively charged, the charged surfactant head groups present a strong affinity for the iron oxide and an adsorbed maximum was observed. At constant surfactant concentration, the adsorbed amount at the iron oxide-water interface decreases as a function of decreasing surface charge density. Further studies are required and have been planned in the near future to comprehend in greater detail the adsorption processes and to prove the suitability of the model to mimic dirt particles.
4.5 References for Chapter 4

Neutron reflectivity at the oil-water interface

5.1 Introduction

The oil-water interface is relevant to many important chemical, physical and biological processes. Countless processes occur at the oil-water interface [1]; for example separation techniques are based on the selective transport of species from a polar to a non-polar phase, or vice-versa, across an oil-water interface (this subject is discussed in detail in Chapter 8). Understanding the nature of these interfaces is fundamental to elucidate, for example, the transport mechanisms across a membrane (see Chapter 7 for more details). Protein folding processes are also highly affected by interactions with the water phase and a hydrophobic environment. Emulsions, which are mixtures of two immiscible liquids stabilised by interfacial phenomena, are commonly used in key areas such as medicine, cosmetics and the food industry.

5.2 Experimental techniques to probe the liquid-liquid interface

The oil-water interface separates two immiscible liquids; therefore surfactants adsorbed at such interfaces interact with both of the bulk phases. Particularly, van der Waals interactions occur between the oil molecules and surfactants' hydrophobic tail groups. As a result of these interactions, the interfacial structure of molecules at the oil-water interface is very different from that in the bulk or at other interfaces.
such as the air-water interface. Because of the buried nature of the oil-water interface, it has been experimentally very difficult to study these systems. Most spectroscopic techniques, such as absorption or emission spectroscopy, are not applicable because of their sensitivity to both the bulk phase and the interface. The analysis of these systems is limited to techniques that are strictly surface-sensitive. As a result there is limited understanding of the important processes that occur at the oil-water interface. In the past two decades several techniques have been developed or adapted to probe the buried oil-water interface. These techniques are briefly summarised.

5.2.1 Surface second harmonic generation

In surface second harmonic generation (SSHG) a laser beam is focused onto the liquid-liquid interface, where it generates a second beam with twice the incident frequency [2]. Second-order processes are forbidden in isotropic media and only take place in interfacial regions, hence making this technique surface-specific. SSHG has been used since 1988 to probe the structure of adsorbed layers at liquid-liquid interfaces [3]. The technique is capable of probing the liquid-liquid interface at the molecular level and it has been extensively used to analyse a variety of systems [4]. Its main limitation is that it is only applicable to probe adsorption of molecules with a chromophore sensitive to the deployed wavelength.

5.2.2 Vibrational sum-frequency spectroscopy

Vibrational sum-frequency spectroscopy (VSFS) was developed shortly after SSHG [5]. In VSFS two laser beams, of which one is visible and the second one is tunable in the infrared region, are overlapped onto an interface and generate a third beam, whose frequency is the sum of the two frequencies of the incident beams. VSFS is much more versatile than SSHG because it can be used to analyse molecules with vibrations within the range of tunability of the incident infrared beam.
5.2.3 Total internal reflection fluorescence spectroscopy

Total internal reflection fluorescence spectroscopy (TIRFS) enables the analysis of buried interfaces using a fluorescent dye as a probe. An s-polarised laser beam is directed onto the oil-water interface so that total external reflection occurs. The beam generates an evanescent wave vertical to the interface which decays exponentially with distance. Molecules from the bulk medium and those at the interface can contribute to the measured signal. By measuring fluorescence from both the bulk medium and the interface and applying the appropriate signal subtraction, molecules at the interface can be probed. The technique has been extensively used to examine dynamics of fluorophores at hydrocarbon oil-water interfaces [6,7] and, despite the experimental difficulties associated to the technique, it seems quite promising in the area of molecular recognition in biological-mimicking systems.

5.2.4 Ellipsometry

Ellipsometry is a technique that allows for the investigation of interfacial structures by means of changes in the polarisation state of an incident light beam. A polarised beam travels through the lighter bulk phase and is reflected from the interface. The comparison of the polarisation state before and after reflection enables the determination of properties such as interfacial thickness and refractive index. Ellipsometry has been used for over two decades to probe liquid-liquid interfaces. It has been used, for example, to study surface freezing at the alkane-water interfaces [8], for the analysis of Pickering emulsions stabilised by silica particles [9], block copolymers [10] or surfactant-protein mixtures [11] at fluid-fluid interfaces. The major limitation of the technique is that refractive index and layer thickness are coupled and cannot be independently determined. Separate measurements to determine one of the two parameters are, therefore, essential in order to obtain a unique fit to the experimental data.
5.2.5 Brewster angle microscopy

Brewster angle microscopy (BAM) is an optical technique that allows visualisation of adsorbed layers at interfaces. The technique relies on a $p$-polarised light beam approaching the interface at the Brewster angle. When no species are adsorbed at the interfaces, no reflection is observed (section 2.2.1.1). The presence of an adsorbed layer changes the refractive index of the interface, allowing a small portion of the beam to be reflected. The reflected portion of the beam can be collected by a camera and domain patterns in the adsorbed layer can be visualised. BAM was applied in the late 90s for the first time to probe adsorption processes at the oil-water interface [12]. BAM microscopy is an excellent technique for the visualisation of adsorbed monolayers and allows detailed description of domains but lacks the appropriate sensitivity on atomic length scale.

5.2.6 X-ray reflectivity and the structure of bare liquid-liquid interfaces

X-ray reflectivity (XRR) is an optical technique that allows the characterisation of surface properties of materials, including adsorption processes of thin films or multilayer structures. XRR can measure the electron density of a sample perpendicular to the surface and delivers information regarding surface roughness, film thickness and density of interfaces. As in neutron reflectivity, the reflection is measured as a function of momentum transfer perpendicular to the interface, $Q$. The interface is generally probed with an X-ray beam with a selected wavelength and a large $Q$ range is achieved by varying the incident angle.

XRR has been extensively used to resolve the structure of bare liquid-liquid interfaces [13]. The interfacial width $\sigma$ of the interface is characterised by a combination of intrinsic interfacial width $\sigma_0$ and capillary wave contribution $\sigma_{CW}$ [14] which depends on the instrumental resolution. The interfacial width $\sigma$ can be written as:

$$\sigma^2 = \sigma_0^2 + \sigma_{CW}^2$$

Equation 5.1
The interfacial width of the water-\(n\)-alkane interface has been systematically studied as a function of \(n\) increasing from 6 to 22 carbon units [15,16,17]. The results are tabulated in Table 5.1 and shown in Figure 5.1.

Table 5.1. Interfacial width of a series of water-alkane interfaces obtained with XRR. Temperature and interfacial tension \(\gamma\) are also reported. A detailed discussion can be found at [17].

<table>
<thead>
<tr>
<th>Alkane</th>
<th>(T) °C</th>
<th>(\gamma) mN m(^{-1})</th>
<th>(\sigma_{CW}) Å</th>
<th>(\sigma_{meas}) Å</th>
<th>(R_g) Å</th>
<th>((\sigma_{CW}^2 - R_g^2)^{1/2}) Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>25</td>
<td>51.3</td>
<td>3.45</td>
<td>3.5 ± 0.2</td>
<td>2.00</td>
<td>3.99</td>
</tr>
<tr>
<td>Heptane</td>
<td>25</td>
<td>51.7</td>
<td>3.44</td>
<td>4.2 ± 0.2</td>
<td>2.28</td>
<td>4.13</td>
</tr>
<tr>
<td>Octane</td>
<td>25</td>
<td>51.8</td>
<td>3.44</td>
<td>5.5 ± 0.2</td>
<td>2.54</td>
<td>4.28</td>
</tr>
<tr>
<td>Nonane</td>
<td>25</td>
<td>52.5</td>
<td>3.41</td>
<td>4.5 ± 0.2</td>
<td>2.80</td>
<td>4.43</td>
</tr>
<tr>
<td>Decane</td>
<td>25</td>
<td>52.5</td>
<td>3.41</td>
<td>4.6 ± 0.2</td>
<td>3.05</td>
<td>4.58</td>
</tr>
<tr>
<td>Dodecane</td>
<td>25</td>
<td>53.5</td>
<td>3.38</td>
<td>5.0 ± 0.2</td>
<td>3.54</td>
<td>4.89</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>25</td>
<td>54.4</td>
<td>3.35</td>
<td>6.0 ± 0.2</td>
<td>4.43</td>
<td>5.55</td>
</tr>
<tr>
<td>Docosane</td>
<td>44.6</td>
<td>54.4</td>
<td>3.46</td>
<td>5.7 ± 0.2</td>
<td>5.60</td>
<td>6.58</td>
</tr>
</tbody>
</table>

Figure 5.1. Interfacial width determined by X-ray reflectivity from the water-alkane interface as a function of \(n\)-alkane carbon number [17]. The solid line is determined using Equation 5.2 by combining the capillary wave contribution and the gyration radius of the alkane; the horizontal, short-dashed line is determined combining the capillary wave contribution and the intrinsic width determined by the bulk correlation length of the alkane. The long-dashed line indicates the capillary wave contribution to the interfacial width (the rise for docosane is because of higher temperature).
The capillary wave contribution to the interfacial width $\sigma_{CW}$ is a function of interfacial tension $\gamma$. As there is very little increase in interfacial tension with increasing numbers of carbon atoms ($\gamma = 51.3$ mN m$^{-1}$ for the hexane-water interface, $\gamma = 54.4$ mN m$^{-1}$ for the docosane-water interface), the capillary wave contribution to the interfacial width does not vary significantly. According to capillary wave theory, such interfacial tension is consistent with a capillary wave contribution $\sigma_{CW} \sim 3.4$ Å [18,19]. The capillary wave contribution is shown by the long-dashed line in Figure 5.1. The intrinsic interfacial width, $\sigma_0$, was found to increase with increasing alkane chain length. Mitrinovic et al. [17] observed that by substituting the intrinsic interfacial width $\sigma_0$ with the gyration radius $R_g$ for the shorter alkanes (chain length up to C18), a satisfactory relationship could be established between theoretical model and experimental data. Equation 5.1 can be thus expressed as:

$$\sigma^2 = R_g^2 + \sigma_{CW}^2$$

Equation 5.2

The radius of gyration $R_g$ can be calculated as:

$$R_g^2 = c(N)Nl^2$$

Equation 5.3

Where $l$ is the carbon-carbon bond length ($l = 1.54$ Å), $N$ is the number of bonds and $c(N)$ is a correction factor that varies with the number of bonds [20]. Such correlation is shown by the solid line in Figure 5.1. This relationship fails for alkanes with $n > 18$, where the intrinsic interfacial width seems to be driven by the bulk correlation length (see Figure 5.1, short-dashed line).

Several studies regarding surfactant adsorption have been performed using XRR. Because of the significant difference in electron density between fluorinated surfactants and both oil and water, XRR has proved to be an excellent technique to study adsorption processes regarding fluorinated alcohol surfactants. Adsorption processes at the hexane-water interface [21,22,23] revealed that while at room temperature surfactants form a highly packed monolayer, they tend to desorb and diffuse into the bulk oil phase at higher temperature. A less ordered monolayer is observed when long chain alcohols are adsorbed at the hexane-water interface [24]. Whereas the tail group region seems to be nearly close-packed, water penetration into the head group region leads to significant disorder in this layer. XRR has also been deployed to study a di-palmitoyl phosphocholine (DPPC) monolayer at the
hexadecane-water interface [25]. The results are consistent with a DPPC monolayer at the interface, with two distinct regions corresponding to the lipid tail group region and the head group region. More recently Wojciechowski et al., studied the adsorption of alkylated azacrown ether at the hexane-water interface [26]. Their results are discussed in detail in Chapter 8.

The main disadvantage of XRR to probe adsorption processes at buried interfaces is often the lack of suitable contrast. Most surfactants present an electron density that does not differ significantly from that of the bulk phases, preventing the determination of structural conformation of adsorbed species. The lack of contrast can be overcome when buried interfaces are probed using neutron reflectivity instead of XRR. Because of the nature of neutron scattering, neutrons interact with nuclei instead of electrons. By using H-D substitution both for the bulk phases and the adsorbed species, a methodology known as contrast variation (see section 2.3.1), neutron reflectivity has recently emerged as a fundamental technique to probe such buried interfaces.

5.3 Neutron reflectivity to probe the liquid-liquid interface

Neutron reflectivity has been used for decades to study processes occurring at various interfaces. It has been successfully applied to study air-liquid and solid-liquid interfaces, as well as solid-air interfaces. However, there is a major difficulty for the application of neutron reflectivity to resolve structures at the buried liquid-liquid interface. Namely, the neutron beam is strongly attenuated when passing through an organic or an aqueous phase.

Three possible approaches can be undertaken to measure neutron reflectivity from the buried liquid-liquid interface. An in-depth review was published by Schlossman in 2002 [27].
5.3.1 Neutron reflectivity measurements with bulk oil phase

With the experimental setting shown schematically in Figure 5.2, the neutron beam travels through a bulk oil phase to reach the interface. After being reflected at the interface, the beam travels through the oil phase a second time to reach the detector. The large attenuation of the neutron beam upon crossing the oil-phase renders this approach impractical with the current reflectometers. To our knowledge no neutron reflectivity experiments have been carried out using truly bulk phases.

![Figure 5.2. Possible configuration for a neutron reflectivity experiment at the liquid-liquid interface: the neutron beam travels entirely through the oil phase to reach the interface.](image)

5.3.2 Neutron reflectivity measurements with thin oil phase: condensation method

In this setting, shown schematically in Figure 5.3, a thin (nm to µm thick) layer of volatile oil is condensed onto the aqueous sub-phase. The first neutron reflectivity experiments at the oil-water interface were reported in the early 90s [28,29] adopting this setting.
Figure 5.3. Possible configuration for a neutron reflectivity experiment at the liquid-liquid interface: the neutron beam travels through a very thin oil layer deposited onto the water phase. The deposition takes place by condensation from the vapour-saturated air phase. The attenuation of the beam is generally tolerable if the oil layer is nm to µm thick.

Lee et al., measured the thickness of a decyl-ethoxylate layer adsorbed at the interface between d-octane and D$_2$O [28]. A thin oil layer was deposited by condensation. The beam attenuation upon travelling through the oil phase was not considered in their data reduction. The reason may be a combination of two factors, which together lead to a rather small attenuation.

i. The oil used was fully deuterated: attenuation through deuterated media is considerably smaller compared to hydrogenous media. Attenuation of a neutron beam upon passing through an oil phase is discussed in detail later in this chapter.

ii. The oil phase was ultra-thin ($d \sim 3000$ Å) and the neutron beam incident angle was $\theta = 1.0^\circ$. The neutron beam path on crossing the oil phase twice can be calculated as:

$$l = \frac{2d}{\sin \theta}$$

leading to a particularly short neutron path through the oil phase, $l \sim 35$ µm.

Neutron reflectivity measurements of β-casein [29] and copolymers [30] at the hexane-water interface were reported in 1993. The same methodology was used by Clifton et al., in 1998 [31] to analyse the adsorption of block copolymers at the hexane-water interface. The measurements were performed using a PTFE trough.
containing the aqueous solutions which was placed in a vapour-sealed compartment. The oil layer was kept in contact with a hexane pool adjacent to the aqueous phase. The thickness of the oil layer was maintained by balancing the film drainage rate and the condensation rate. This setting allowed the formation of a hexane layer with a thickness \( d \sim 10-20 \mu m \). A schematic diagram of the experimental setting adopted is shown in Figure 5.4.

![Sealed thermostatic vessel diagram](image)

**Figure 5.4. Experimental setting for the first neutron reflectivity experiments at the air-oil-water interface.**

The reflectivity was calculated using the thick-film approximation: the measured reflectivity \( R_{Total} \) is a function of the reflectivities from the air-hexane interface (\( R_1 \)) and from the hexane-water interface (\( R_2 \)) and is given by:

\[
R_{Total} = \frac{R_1 + R_2 A - 2R_1 R_2 A}{1 - R_1 R_2 A}
\]

Equation 5.5

\( A \) represents the attenuation of the neutron beam upon travelling through the oil phase:

\[
A = \exp \left( \frac{-2\chi d}{\sin \theta} \right)
\]

Equation 5.6

In Equation 5.6, \( \chi \) is the attenuation coefficient of hexane which was experimentally measured.
Because of the oil layer being formed by condensation, the procedure was only applicable to volatile oils. Besides, maintaining a thin, homogeneous oil layer was time-consuming and the lower $Q$-range of the data needs to be measured several times to ensure the stability of the oil layer.

5.3.3 Neutron reflectivity measurements with thin oil phase trapped between a solid substrate and the aqueous phase

A newer, more general methodology to perform neutron reflectivity measurements at the oil-water interface was reported by Zarbakhsh et al., in 1999 [32] when they measured the reflectivity profiles from the cyclohexane-water and hexadecane-water interface. In their approach the thin oil layer was spread on a suitably hydrophobed silicon substrate and was formed by spin coating. The oil layer was then frozen and trapped between the silicon substrate and the aqueous phase (Figure 5.5). The oil layer was allowed to melt after the cell had been assembled. This methodology is known as “spin-freeze-thaw” method.

Figure 5.5. Third possible configuration for a neutron reflectivity experiment at the liquid-liquid interface: the neutron beam travels through a very thin oil layer deposited onto a solid substrate. Oil layers with thickness in the order of a few µm can be deposited by means of spin-coating.

The thickness of the oil phase was directly measured from the reflectivity profiles as follows, by using suitable contrast by adjusting the $Nb$ of both oil and water. When a
silicon block \((Nb = 2.07 \times 10^{-6} \, \text{Å}^{-2})\) is in contact with oil whose \(Nb\) is contrast matched to \(Nb = 4.00 \times 10^{-6} \, \text{Å}^{-2}\), a critical edge at \(Q_c = 0.0098 \, \text{Å}^{-1}\) is observed. If a sufficiently thin layer of such oil is trapped between silicon and a D\(_2\)O aqueous sub-phase \((Nb = 6.35 \times 10^{-6} \, \text{Å}^{-2})\), a second critical edge at higher \(Q\) is observed (Figure 5.6). If the two interfaces are well separated, the resolution of the reflectometer does not allow the observation of fringes in the reflectivity profile and the reflectivity can be calculated using the thick-film approximation [31] (Equation 5.5):

\[
R_{\text{Total}} = \frac{R_1 + R_2A - 2R_1R_2A}{1 - R_1R_2A}
\]

*Equation 5.5*

![Graph showing reflectivity vs. wavelength](image)

*Figure 5.6. Data and solid line: oil thickness 3 µm; long-dashed line: oil thickness 1 µm; short-dashed line: oil thickness 5 µm. Measurement performed at an incident angle \(\theta = 0.29^\circ\). Printed with permission of the authors [33].*

The second critical edge presents a lower intensity because of the attenuation of the neutron beam passing through the oil phase twice. The difference in intensity
between the two critical edges is a function of the neutron path through the oil phase and can be used to estimate the oil layer thickness using Equation 5.6:

$$A = \exp\left(\frac{-2\chi d}{\sin\theta}\right)$$

Equation 5.6

The linear absorption coefficient $\chi$ was measured for both cyclohexane and hexadecane using a single crystal Hellma cell. An oil layer thickness of $16400 \pm 400 \, \text{Å}$ was found for cyclohexane, whereas a thinner layer of $8600 \pm 400 \, \text{Å}$ was found for hexadecane. Adjustments in the spin coating procedure allow the reproducible production of hexadecane layers ranging from 2000 to 24000 Å.

A similar approach was reported by Strutwolf et al., in 2000 [34]. They formed a thin aqueous layer ($\sim 10 \, \mu\text{m}$) by depositing a small aliquot of water onto a quartz substrate and spreading it by means of PTFE tape. The water layer was then trapped between the quartz substrate and a hydrophobic sub-phase. The methodology allows for the use of different types of oils and is very attractive for the analysis of oil-soluble surfactants. However, several ml of oil phase are required for a single measurement and the cost of deuterated oils is generally extremely high.

### 5.4 “Spin-freeze-thaw” methodology to perform neutron reflectivity at the oil-water interface

The “spin-freeze-thaw” methodology is currently the first choice for neutron reflectivity measurements at the oil-water interface. The cell used for the measurements is a standard cell for the investigation of solid-liquid interfaces; a schematic diagram is shown in Figure 5.7.
Figure 5.7. Schematic diagram of the cell use for neutron reflectivity experiments at the liquid-liquid interface.

The cell consists of a circular stainless steel trough with an external diameter of 100 mm, which contains the aqueous phase. A circular silicon block, with a diameter of 100 mm and thickness of 10 mm, is used as solid substrate. The block is rendered hydrophobic by means of silane coupling (deposition of C1-layer) prior to use to ensure wettability by the oil phase. After deposition of the oil layer on the silicon block surface, the solid substrate and the stainless steel trough are sandwiched and no leakage is ensured by an o-ring present in the trough. The water phase can be inserted or withdrawn from the cell by syringing it through two greaseless valves. A circulating water bath is connected to two thermostatic water chambers, one above the silicon block and one below the stainless steel trough, insuring a constant and adjustable temperature throughout the whole duration of the measurement. Background scattering arising from the cell is minimised by using a boron external wall that almost fully covers the inner cell, leaving just enough space for the incoming and outgoing neutron beam.
5.4.1 Sample preparation and spin coating procedure

The surface of a silicon block is hydrophilic because of the presence of a thin, native silicon oxide layer. In order to deposit a uniform oil layer on a silicon block, its surface must be rendered hydrophobic. Earlier attempts to render the silicon hydrophobic involved adsorbing a layer of eicosanoic acid layer on its surface [32]. The process led to a loosely bound, thick layer of adsorbed eicosanoic acid. Much better results were obtained using a chemically grafted trimethylsilane (C1). Such layers can be easily deposited by means of silanization and lead to uniformly coated surfaces with excellent reproducibility.

For the coating procedure, the silicon block is initially cleaned using the RCA method [35] and subsequently chemically dried [36]. The dried silicon block is transferred to a 1% v/v solution of chlorotrimethylsilane in chloroform under N$_2$ atmosphere for approximately one hour. The block is then rinsed with methanol and chloroform.

The oil layer is deposited onto the silicon substrate using the spin coating technique. Spin coating is a procedure that allows the deposition of uniform thin films onto solid substrates. The spin coater used, a programmable SCS P-6700, is shown in Figure 5.8.

![Spin coater](image)

Figure 5.8. Spin coater used to deposit the oil layer onto the silicon block.
An excessive amount of the spreading liquid is placed onto the substrate and a thick layer is spread, generally by means of a Pasteur pipette. The substrate is placed on the sample holder, where it is held in position by vacuum suction. When the substrate is rotated at high speed, the excess liquid is thrown off the edges by centrifugal forces, leaving a uniformly spread layer. The thickness of the layer mainly depends on the angular speed of spinning (the higher the angular speed, the thinner the spun film) and on the features of the solvent, such as volatility and viscosity. Rate of acceleration (RAMP), rotations per minute (RPM) and time, as well as the number of steps, are adjusted to produce an oil layer whose thickness can be calibrated using neutron reflectivity. Zarbakhsh et al., developed a spin coating procedure that leads to a reproducible and uniform hexadecane layer with thickness $d \approx 2.1 \, \mu m$ [32]. After spreading hexadecane onto silicon, the silicon block is accelerated to 2000 RPM in 10 s, is maintained at 2000 RPM for 12 s, then decelerated to 0 in 10 s. The spin coating profile is shown in Figure 5.9.

![Spin coating RPM profile](image)

**Figure 5.9.** Spin coating RPM profile adopted to deposit a 2.1 μm hexadecane layer onto a hydrophobed silicon substrate.
The silicon substrate is transferred into an ice container immediately after deposition to freeze the oil layer and maintain it on the surface. The cell is assembled with the oil still frozen and only after the cell has been filled with the aqueous phase (water excess is syringed through the valves to ensure that the cell is bubble free) is the oil allowed to melt. The spin coating procedure can be applied to relatively non-volatile oils if they have a suitable freezing point to be maintained solid for the cell-assemble procedure and to non-volatile oils. Besides cyclohexane and hexadecane, recently layers of decane and triolein have been successfully prepared.

5.4.2 $R_1$ and $R_2$ part of the reflectivity

As suggested from Equation 5.5, the measured reflectivity in an oil-water experiment is a combination of the reflectivity from the silicon-oil interface ($R_1$) and the reflectivity from the oil-water interface ($R_2$). The information regarding the structure of the layer adsorbed at the oil-water interface is contained in the $R_2$ component of the reflectivity. Therefore if the $R_1$ part of the reflectivity is not kept to a minimum its contribution to the $R_{\text{tot}}$ would be strongly dominant. Under these conditions it would be extremely difficult to detect small changes in the $R_2$ part of the reflectivity, hence loss in sensitivity to the interfacial structure.

To ensure that the $R_1$ part of the reflectivity is kept to a minimum, the $Nb$ of the oil phase is generally contrast-matched to that of silicon (CMSi oil). Despite this, some reflection arises from the silicon-oil interface because of the presence of the hydrophobic layer deposited by silanization. Two well distinct layers can be distinguished at the silicon-oil interface:

i. A thin, native silicon oxide layer is generally observed on the silicon surface. Its presence is essential for the silanization process.

ii. A hydrophobic trimethylsilane layer which is deposited by means of silanization.

The silicon-oil interface has been characterised in numerous occasions and a two layer model can adequately describe the interface [33,36,37]. The parameters for the two layer model are given in Table 5.2. These parameters have been used for all the fitting procedures for oil-water measurements presented in Chapter 6, 7 and 8. A
schematic representation of the model used to calculate the reflectivity from the buried oil-water interface is shown in Figure 5.10.

![Diagram](image)

**Figure 5.10. Schematic representation of the model used to calculate the structure at the oil–water interface. Fitting parameters for the $R_1$ reflectivity are also given.**

**Table 5.2. Fitting parameters for the $R_1$ part of the reflectivity**

<table>
<thead>
<tr>
<th>Layer</th>
<th>Thickness / Å</th>
<th>$Nb \times 10^{-6}$ / Å$^{-2}$</th>
<th>Roughness / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicon</td>
<td>-</td>
<td>2.07</td>
<td>-</td>
</tr>
<tr>
<td>Silicon oxide</td>
<td>8</td>
<td>3.40</td>
<td>2</td>
</tr>
<tr>
<td>$(\text{CH}_3)_3$ layer</td>
<td>5</td>
<td>-0.50</td>
<td>2</td>
</tr>
<tr>
<td>Oil</td>
<td>-</td>
<td>2.07</td>
<td>2</td>
</tr>
</tbody>
</table>
5.4.3 Suitable instruments to perform neutron reflectivity: the absorption coefficient problem

The number and choice of a suitable neutron reflectometer to perform liquid-liquid experiments is very limited. The reason for this is twofold:

i. Intuitively, if the sample cell is tilted the oil layer tends to drain significantly. For this reason oil-water experiments must be performed in horizontal geometry reflectometers.

ii. Although the attenuation of the neutron beam is minimised by using an ultra thin oil layer, still noticeable attenuation is observed. High flux reflectometers are preferable as they would permit the measurement of a reflectivity profile within a reasonable time.

So far only four reflectometers have been used to perform neutron reflectivity from the liquid-liquid interface. Namely:

- CRISP, SURF and more recently INTER at ISIS, Rutherford Appleton Laboratory, Didcot, UK.
- FIGARO in ILL, Grenoble, France.

These reflectometers are equally suitable to perform these experiments. Because of the different available wavelength range (namely $0.5 < \lambda < 7.0$ Å for CRISP and SURF, $1.5 < \lambda < 20$ Å for INTER and $2 < \lambda < 30$ Å for FIGARO) the accessible $Q$ range varies substantially. The SURF and CRISP reflectometers were until very recently the only reflectometers available for liquid-liquid interfaces and they have a relatively confined wavelength range. The linear absorption coefficient $\chi$, required to calculate the attenuation factor $A$ using Equation 5.6, changes as a function of H/D composition of the oil and as a function of wavelength $\lambda$. Zarbakhsh et al., [33] measured $\chi$ for hexadecane using the reflectometer SURF. Single crystal Hellma cells were filled with small volumes of hexadecane with different isotopic composition ($Nb$ varying from 0.0 to $6.8 \times 10^{-6}$ Å$^{-2}$). The results are plotted in Figure 5.11.
Figure 5.11. Attenuation coefficient, $\chi$, plotted as a function of wavelength $\lambda$, for different H-D hexadecane mixtures. $N_{b_{oil}} = 0.0$ (○), 2.1 (∆), 4.2 (∇) and 6.7 (□) × 10$^{-6}$ Å$^{-2}$. Measurements were taken with the instrument SURF at ISIS, RAL. The solid lines are polynomial fits to the data calculated using Equation 5.7.

By fitting a series of polynomials to the transmission data for the different oils and then interpolating between these polynomials, $\chi$ could be determined for any specific contrast of oil used. Such a polynomial is shown in Equation 5.7:

$$\chi(N_{b_{oil}}, \lambda) = [(0.021 N_{b_{oil}} - 0.135) \lambda^2$$
$$+ (-0.248 N_{b_{oil}} + 1.742) \lambda$$
$$+ (-0.177 N_{b_{oil}} + 1.617)] \times 10^{-4}$$

Equation 5.7

$N_{b_{oil}}$ is the scattering length density of the oil phase. Because of the wavelength-dependent nature of $\chi$, the data analysis has been traditionally carried out in wavelength instead of $Q$. All the reflectivity profiles discussed in this thesis in Chapter 6, 7 and 8 are, however, conventionally shown as a function of $Q$. 

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Because of the broader available wavelength range, when neutron reflectivity experiments were performed with INTER and FIGARO the absorption coefficient for different H/D hexadecane compositions had to be measured adopting the same procedure as above. We tried to use the previously determined polynomial fit to describe the obtained $\chi$ at higher wavelengths, but it was not possible to achieve a satisfactory fit to the data. The absorption coefficient $\chi$ for three hexadecane contrasts as measured for the reflectometer FIGARO and the fit to the data with the polynomial illustrated in Equation 5.7 are shown in Figure 5.12.

![Figure 5.12. Attenuation coefficient, $\chi$, plotted as a function of wavelength $\lambda$, for different H/D hexadecane mixtures. $N b_{oil} = -0.43$ (○), 2.07 (△), and 6.7 (□) × 10^{-6} Å^{-2}. Measurements were taken with the instrument FIGARO at ILL, Grenoble. The polynomial fits using Equation 5.7 are shown as solid lines and clearly are unable to describe the attenuation coefficient for $\lambda > 6.9$ Å.](image)

Initially it was attempted to fit the attenuation coefficient applying a similar polynomial fit, namely:

$$\chi(N b_{oil}, \lambda) = [a(N b_{oil})\lambda^2 + b(N b_{oil})\lambda + c(N b_{oil})]\times k$$  
Equation 5.8
where \( k \) is a constant. So far our efforts have not been successful and a similar, general polynomial fit has not been yet developed. However, the individual curves were successfully fitted as a function of \( \lambda \). The general Equation 5.9 shown below is not a function of \( Nb_{\text{oil}} \) and the three coefficients, \( a, b \) and \( c \) vary from contrast to contrast.

\[
\chi = a\lambda^2 + b\lambda + c 
\]

Equation 5.9

The values for the three coefficients are given in Table 5.3 for the three contrasts measured.

**Table 5.3. Parameters for the polynomial fitting to the attenuation coefficient.**

<table>
<thead>
<tr>
<th>Contrast</th>
<th>( Nb \times 10^6 / \text{Å}^{-2} )</th>
<th>( a )</th>
<th>( b )</th>
<th>( c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>h-hexadecane</td>
<td>-0.43</td>
<td>-0.01400</td>
<td>0.806</td>
<td>2.055</td>
</tr>
<tr>
<td>CMSi hexadecane</td>
<td>2.07</td>
<td>-0.00553</td>
<td>0.594</td>
<td>1.224</td>
</tr>
<tr>
<td>d-hexadecane</td>
<td>6.70</td>
<td>-0.00292</td>
<td>0.094</td>
<td>0.349</td>
</tr>
</tbody>
</table>

**Figure 5.13.** Attenuation coefficient, \( \chi \), measured with the instrument FIGARO. \( Nb_{\text{oil}} = -0.43 (\bigcirc), 2.07 (\bigtriangleup), \) and 6.7 (\( \square \)) \( \times 10^6 \text{Å}^{-2} \). Solid lines represent the updated fits using Equation 5.9 and the parameters given in Table 5.3.
The fitting to the new attenuation coefficients using Equation 5.9 and the values in Table 5.3 are shown in Figure 5.13.

As all the reflectivity profiles so far measured with FIGARO and INTER presented an oil phase which \( N_b \) was contrast-matched to that of silicon, and a satisfactory fit to the attenuation coefficient can be obtained by using the parameters given in Table 5.3, the lack of a general correlation between \( \chi \), \( \lambda \) and \( N_b \) does not currently represent a problem.

It is noteworthy that there is a small discrepancy between the attenuation coefficient \( \chi \) measured in SURF and that measured in FIGARO in the region \( 2.3 < \lambda < 6.9 \, \text{Å} \). Such a difference is shown in Figure 5.14 for the CMSi hexadecane.

![Figure 5.14. Difference in attenuation coefficient for hexadecane with \( N_b = 2.07 \times 10^{-6} \, \text{Å}^{-2} \) as measured with the reflectometer SURF (△) and FIGARO (▲). The small discrepancy between the two instruments is currently under investigation.]

The reason for such discrepancy is still unclear and further investigations have already been planned. It must be stressed, however, that such a difference does not affect in any way the quality of the data analysis. Because of the thin oil layer used
in these experiments, simulations adopting the two different attenuation coefficients are almost identical.

5.5 Previous observations at the oil-water interface using the “spin-freeze-thaw” methodology

The “spin-freeze-thaw” methodology was used for the first time in 1999 to measure the width of the bare hexadecane-water interface [32]. Accurate measurements of this system had already been performed using x-ray reflectivity [17], reporting an interfacial width $\sigma = 6.0 \pm 0.2$ Å, in close agreement with the theoretical value $\sigma = 5.55$ Å. The measured reflectivity profiles were consistent with an interfacial width $\sigma = 12 \pm 2$ Å. This result was confirmed in a subsequent experiment [36,38] where the interfacial width was found to be $\sigma \sim 15$ Å. The discrepancy between the interfacial width measured with neutron reflectivity and X-ray reflectivity was initially tentatively attributed to impurities in the oil, which was not purified before the experiments. This discrepancy was eventually resolved in 2005, when Zarbakhsh et al., published a paper presenting an updated methodology to perform neutron reflectivity at the oil-water interface [39]. Immediately after the sample formation, the interfacial width was found to be $\sigma \sim 9$ Å. When the sample was heated to about 71º C, the interfacial width was reported to decrease to $\sigma \sim 6$ Å. The same width, within error, was retained upon cooling to 25 º C. After thermal annealing, the interfacial width measured with neutron reflectivity was $\sigma = 6 \pm 1$ Å, in excellent agreement with the measurement reported with X-ray reflectivity.

The technique has been used for nearly a decade to investigate the structure of adsorbed surfactants at the oil-water interface. A brief overview of previous studies of systems at the oil-water interface shows how the nature of the surfactant significantly influences the structure of the adsorbed layer. Nonetheless, one common feature is that at the oil-water interface the adsorbed layer is generally much thicker, rougher and less organised as compared to the air-water interface.
5.5.1 Polybutadiene-poly(ethylene oxide) (PB-b-PEO) linear diblock copolymer at the air-water interface

PB-b-PEO copolymers with a molecular mass of $\sim 60000 \text{ g mol}^{-1}$, containing approximately 50% PB and 50% PEO, are known to form stable monolayers both at the air-water and oil-water interface [40]. A comparative study between the monolayers at the air-water and the oil-water interfaces was performed using neutron reflectivity.

The structural conformation of PB-b-PEO copolymer at the air-water interface was studied as a function of increasing surface pressure $\Pi$ [40]. At very low surface pressure, $\Pi \sim 0 \text{ mN m}^{-2}$ (corresponding to a surface coverage $\Gamma = 0.45 \text{ mg m}^{-2}$), the copolymer forms a very thin layer on the water surface. The reflectivity profiles were fitted to a two layer model. A first layer with thickness $d = 3 \text{ Å}$, in contact with air, represents the PB group, whereas the second layer, in contact with water with thickness $d = 6 \text{ Å}$, represents the PEO group. With increasing surface pressure, $\Pi \sim 2 \text{ mN m}^{-1}$ ($\Gamma = 0.83 \text{ mg m}^{-2}$), there is a decrease in the quality of the fits to the reflectivity data obtained with the two layer model. Also, an increase in thickness for both layers was observed ($d = 7$ and $11 \text{ Å}$, respectively). This observation suggested that the interface is more complex than can be represented with this simplified layer model. At surface pressure $\Pi \sim 9 \text{ mN m}^{-1}$ ($\Gamma = 2.06 \text{ mg m}^{-2}$), the two layer model fails to represent the reflectivity profiles and a third layer was required to adequately fit the data. The thickness of the multilayer structure increased to $d \sim 100 \text{ Å}$ with the PEO groups significantly extending into the water sub-phase. The reflectivity profiles also showed some degree of PB group penetration into the diffuse layer. The significant increase in layer thickness with increasing $\Pi$ could be because of formation of brushes, surface aggregates or a multi-layer structure. Similar polymers have been reported to form extremely thin layers at low surface pressure at the air-water interface and to form brushes with increasing surface pressure [41].
5.5.1.1 Oil-water interface

When a neutron experiment is carried out at the air-water interface, the surface pressure can be generally monitored by means of a Wilhelmy plate. This is not possible in an oil-water experiment as the thin oil-water phase is trapped between the solid substrate and the underlying water phase. Hence the structural conformation of PB-\(b\)-PEO copolymer at the hexadecane-water interface was studied as a function of increasing surface coverage instead of surface pressure \(\Pi\) [36]. Two values of surface coverage were investigated: 4.0 and 10.0 mg m\(^{-2}\), which are significantly higher compared to the air-water interface. This was chosen to mimic corresponding features in the surface pressure isotherms which appear to be shifted when moving from the air-water to the oil-water interface [40]. A surface coverage at the air-water interface \(\Gamma_{a/w} = 0.8\) mg m\(^{-2}\) (\(\Pi \sim 2\) mN m\(^{-1}\)) corresponds to a coverage at the oil-water interface \(\Gamma_{o/w} = 4.0\) mg m\(^{-2}\), similarly \(\Gamma_{a/w} = 2.0\) mg m\(^{-2}\) (\(\Pi \sim 9\) mN m\(^{-1}\)) corresponds to a coverage at the oil-water interface \(\Gamma_{o/w} = 10.0\) mg m\(^{-2}\). Hence \(\Gamma_{o/w} = 4.0\) mg m\(^{-2}\) should be compared with \(\Pi \sim 2\) mN m\(^{-1}\) at the air-water interface and \(\Gamma_{a/w} = 10.0\) mg m\(^{-2}\) with \(\Pi \sim 9\) mN m\(^{-1}\).

The reflectivity profiles were consistently fitted to a four layer model. At \(\Gamma_{a/w} = 4.0\) mg m\(^{-2}\) the layer thickness of the PB group was considerably thicker than at the air-water interface. Namely, it increased from 3-7 Å to 25 Å. Also, a dilute 40-60 Å layer protruding toward the oil-phase was observed. The water-side of the interface is also slightly thicker compared to the air-water interface, with a similar dilute layer protruding in the water phase. The structure of the adsorbed layer does not vary significantly moving to higher surface coverage, although there is a marked increase in adsorbed amount. The thickness of the overall structure at the oil-water interface is much thicker compared to the air-water interface. Particularly, there seems to be significant segregation between the hydrophilic PEO group, which resides in the water phase, and the hydrophobic PB group. This is because the oil is efficiently capable of solvating the hydrophobic PB part of the copolymer. Conversely, no significant changes were observed on the water side of the interface: the volume fraction of PEO did not vary significantly. The increased layer thickness was attributed to the higher surface coverage compared to the air-water interface.
5.5.2 Tetradecyl-trimethylammonium bromide (C\textsubscript{14}TAB) at the air-water interface

The structure of a C\textsubscript{14}TAB monolayer was studied at the air-water interface as a function of concentration above and below the CMC using neutron reflectivity [42]. The adsorbed monolayer could be adequately described by dividing it into two layers. The layer in contact with air represents the surfactant’s tail group; the second layer, in contact with water, represents a mixture of hydrated head group with a certain proportion of alkyl chain. At low concentration the thickness of the tail group region is slightly less than a fully extended C\textsubscript{14} chain, suggesting some degree of tilting in the tail groups. At concentrations higher than the CMC, the thickness of the tail group region roughly matches the fully extended length, suggesting either a staggered conformation or vertical orientation of the surfactants. The increase in thickness of the head group region as a function of concentration is significant and is probably because of electrostatic repulsion between the positively charged headgroups. Such repulsion had already been observed in C\textsubscript{10}TAB monolayers where the effect on the structure of the adsorbed layer was even more noticeable [43]. The increase in thickness of the head group region, combined with the small changes in thickness of the tail group region, suggest that the close packing of the C\textsubscript{14}TAB surfactants leads to significant roughening of the head group region.

5.5.2.1 Oil-water interface

Structural determination of a C\textsubscript{14}TAB monolayer at the CMC at the hexadecane-water interface was performed using neutron reflectivity [33]. The reflectivity profile could be fitted to a single layer model, with thickness lower than that of a fully extended molecule. However, the quality of the fitting was relatively poor and the interfacial region could be better represented by a two layer model. The layer in contact with the oil phase has a layer thickness \(d \sim 20\) Å and is consistent with a fully extended C\textsubscript{14}TAB molecule. The second layer is rather diffuse and it extends in the water phase with a thickness \(d \sim 50\) Å. The layer thickness is in the order of twice the molecular length, possibly suggesting that a dilute layer of micelles is
adsorbed underneath the primary monolayer on the water side of the interface. A sketch of the proposed interfacial structure is shown in Figure 5.15.

**Figure 5.15. Proposed model for the interfacial structure of a C$_{14}$TAB layer adsorbed at the hexadecane-water interface.**

Adsorption of micelles onto an already formed monolayer had already been observed in several occasions, particularly at the solid-liquid interface [44].

A similar two layer model was also deployed to describe the conformation of tetradecyl-trimethylammonium sulphate (C$_{14}$TAS) at the hexadecane-water interface, suggesting an analogue arrangement at the interface [45]. The thickness of the diffuse layer in contact with the water phase is comparable to that observed for C$_{14}$TAB (increases from 40 to 50 Å with increasing concentration). The layer in contact with the oil phase was thicker compared to that observed for C$_{14}$TAB (31 Å as opposed to 20 Å). This is reasonable as the increased charge in the counterion results in an increased efficiency to screen the electrostatic repulsion between the charged head groups. As a consequence, a decrease in area per molecule was observed, hence an increase in adsorbed amount.
5.5.3 Hexadecyl-phosphocholine (C\textsubscript{16}PC) at the air-water interface

The structural characterisation of alkyl-phosphocholines at the air-water interface as a function of increasing alkyl chain length was carried out using a combination of neutron reflectivity and surface tension [46,47,48]. Surprisingly, with the alkyl chain increasing from 12 to 16 carbon units, no detectable changes were observed in the thickness of the surfactant monolayer (d ~ 20 Å for all surfactants). Also, changes in concentration did not lead to significant changes in layer thickness. The phosphocholine head group aligns almost vertically, its hydration conditions not varying with increasing alkyl chain length, presence of salt or changes in temperature. The thickness of the tail group region does not vary with increasing carbon units. These results suggest that the tail groups’ tilt increases as the alkyl chain length increases. Namely, the angle of tilt increases from around 40º for C\textsubscript{12}PC to about 53º for C\textsubscript{16}PC. As opposed to C\textsubscript{n}TAB monolayers, increases in the alkyl chain length do not lead to increased monolayer roughness [42]. This could be because of the zwitterionic nature of the surfactants: as they do not possess an overall net charge, there is no need for the accommodation of counterions in the proximity of the adsorbed layer.

5.5.3.1 Oil-water interface

C\textsubscript{16}PC monolayers were studied at the hexadecane-water interface as a function of concentration above and below the CMC using a combination of neutron reflectivity and interfacial tension [37]. The overall thickness of the adsorbed layer was about 70 Å, which corresponds to approximately twice the fully C\textsubscript{16}PC molecular length. The scattering length density profiles suggested an increase in water presence on the oil side of the interface with increasing concentration. Such an observation is consistent with a multilayer conformation at the interface. The authors suggested a tri-layer arrangement to represent the interfacial region, which is shown in Figure 5.16.
Figure 5.16. Proposed model for the interfacial structure of a $\text{C}_{16}\text{PC}$ layer adsorbed at the hexadecane-water interface.

The first layer lies with the tail groups in contact with the bulk oil phase, where significant solvation by the hexadecane molecules occurs. The hydrophilic head groups of the third layer are instead in direct contact with the water phase. The second layer lies in a “reversed” conformation: the head groups are in contact with the head groups of the first layer while the alkyl chains interact with those of the third layer. Such arrangement would minimise the Coulombic interactions between charges in the head groups. The structure resembles that of a roughened monolayer with inclusion of surfactants pointing to the “wrong” side of the interface. The structure also suggests that the presence of hexadecane in the layer only allows a slight tilt relative to the interface normal, as opposed to the air-water interface where significant tilt was observed.
5.6 Neutron reflectivity experiments carried out during the PhD

One of the main aims of the PhD project was to use the recently developed “spin-freeze-thaw” methodology to analyse different surfactant systems at the oil-water interface. These studies are discussed in the following chapters.

In Chapter 6 the structure of non-ionic surfactants dodecanol ethoxylates, $C_{12}E_n$, was studied as a function of increasing head group size. The aim of the study was to understand how the size of the head group influences the structure of adsorbed layers at the oil-water interface.

In Chapter 7 the structure of a phospholipid was analysed as a function of increasing surface coverage to explore the possibility of using lipid monolayers at the oil-water interface as simple models for biological membranes.

In Chapter 8 a complex study of co-adsorption at interfaces is presented. Zarbakhsh et al., recently carried out structural studies of alkylated azacrown ether (ACE) at the air-water and oil-water interfaces [49]. Such compounds are currently studied as co-carriers in liquid-liquid extraction processes where the main carrier is a fatty acid. Co-adsorption processes of ACE and fatty acid at both the air-water and oil-water interface are discussed in the chapter.
5.7 References for Chapter 5

Chapter 6

Dodecanol ethoxylates (C_{12}E_n) surfactants at the oil-water interface

6.1 Introduction

The van der Waals interactions between the alkyl chains of a surfactant play a very important role in how surfactants assemble at the air-water interface [1]. It was discussed in the previous chapter how these attractive forces generally lead to a relatively ordered surfactant monolayer, with the hydrophilic head groups immersed into the bulk aqueous phase and the tail groups exposed to air, often tilted at an angle with respect to the normal. At the oil-water interface, on the other hand, the van der Waals interactions between the hydrophobic parts of the surfactants are much reduced as solvent molecules can diffuse between the chains. Under these conditions, the surfactant tails are able to adopt different, generally much less ordered conformations. Therefore, one would expect the nature of the head groups and their chemical environment to play a significant role in the final conformation of the interfacial region.

Our group has previously investigated the effect of the presence of charges in the surfactants head groups, both for cationic [2] and zwitterionic surfactants [3], on adsorption processes at the oil-water interface. It is of great interest to extend these studies to investigate systematically the role of head group size on the conformation of surfactants at the buried oil-water interface and to compare the results to the same systems at the air-water interface. This study would help to increase our understanding of the factors that affect the nature of the adsorbed layer at the oil-water interface.
This aim was achieved by studying the conformation of non-ionic surfactants dodecanol ethoxylates, $\text{C}_{12}\text{E}_n$, at the hexadecane-water interface. By increasing the number of ethylene oxide units, $n$, from 4 to 12, the size of the head group can be steadily increased. Furthermore, the non-ionic nature of the surfactant would eliminate any structural effect that could be caused by the presence of charges in the head group. Alkyl ethoxylates ($\text{C}_m\text{E}_n$) are extensively used in detergency, shampoo, fabric softening, cosmetics and pharmacy. Their interfacial adsorption and aggregation in solution have a key role in their industrial utilisation. Much is known about bulk properties of dodecanol ethoxylates such as cloud point as a function of increasing $n$ [4], or properties of mixed micelles containing $\text{C}_{12}\text{E}_n$ [5,6]. The addition of $\text{C}_{12}\text{E}_n$ to SDS mixtures efficiently reduces the solubilisation of zein proteins, thus reducing the extent to which the detergent formulations will provoke adverse skin reactions [7]. Interfacial behaviour at the solid-water interface has been reported at a number of different substrates such as graphite [8], silicon [9] and cellulose [10]. Dodecanol ethoxylates are also known to interact with phospholipids, thus altering parameters such as hydration of membranes or their structural properties [11]. In particular, $\text{C}_{12}\text{E}_2$ seems to stabilise efficiently the gel phase of 1-palmitoyl-2-oleoyl-phosphocholine (POPC) [12].

Besides their extensive use, there were two reasons for choosing dodecanol ethoxylates for this study:

1. The structural conformation of dodecanol ethoxylates at the air-water interface was fully characterised in the 90s. In a series of a neutron reflectivity experiments the size of the ethoxylate head group was gradually increased from $n = 1$ to 12. The interface was investigated for a series of concentrations above and below the CMC and the effects of the increasing head group on the structural conformation was studied. Extensive knowledge of the system at the air-water interface is essential to understand how the head group affects the surfactant conformation at the oil-water interface.

2. As the number of ethylene glycol units increases from $n = 1$ to $n = 12$, the thickness of the tail group region at the air-water interface, as determined by neutron reflectivity, varies very little. The thickness varies between 11 and 13 Å, suggesting a tilted conformation of the tail groups ($\theta \sim 40^\circ$). Hence, it is the size of the head group that plays a key role on the conformation at the
air-water interface. One would expect a similar behaviour on the final conformation at the oil-water interface.

### 6.2 Conformation of C$_{12}$E$_n$ surfactants at the air-water interface

The studies referenced here examined the conformation of dodecanol ethoxylates at the air-water interface for a range of concentrations above and below the CMC. With the purpose of making a direct comparison between different surfactants, the discussion here only refers to interfaces that have reached full coverage, i.e. at the CMC. It has already been mentioned how the thickness of the tail group region varies very little as the size of the head group is increased. Therefore, the discussion here will mainly focus on the role of the head group on the conformation of the adsorbed layer.

When the surfactant head group is relatively small ($n = 1$-4) there is very little difference between the thickness of the head group region and the length of the fully extended ethoxylate fragment [13,14]. This is an indication of either a very small tilt, with respect to the interfacial normal, or a slightly folded conformation.

The increase in thickness of the head group region is confirmed for dodecanol hexaethylene oxide ($n = 6$). However comparing the thickness of the head group region to the shorter analogues, the ethylene glycol chain is proportionally less extended. Deuterium labelling of the two halves of the head group region showed a large degree of overlap, compatible with a globular-like head group region [15]. The difference between the fully extended length and the measured thickness of the head group region is even more pronounced for C$_{12}$E$_8$ [16], suggesting that as the head group increases in size it acquires more marked globular-like features.

For C$_{12}$E$_{12}$ the reflectivity profiles were initially analysed using the optical matrix method. A slightly thinner layer was required for the tail group region compared to shorter head group analogues. This is because the division between the layers for the
$\text{C}_{12}\text{E}_{12}$ was taken at the position where head group and water end, whereas for $\text{C}_{12}\text{E}_6$ the boundary between the two layers was taken where there was still significant overlap between head group, tail group and water. When discussing the results in terms of partial structure factors, however, the thickness of the tail group region is comparable to the shorter members of the $\text{C}_{12}\text{E}_n$ series. The thickness of the head group region is less than half that of the fully extended dodecaethylene oxide fragment, suggesting a conspicuous globular nature of the head group for $\text{C}_{12}\text{E}_{12}$.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure61.png}
\caption{Thickness of the head group region for a series of $\text{C}_{12}\text{E}_n$ surfactants at the CMC at the air-water interface. Solid line corresponds to the length of the fully extended ethylene oxide fragment. A sketch of the head group progressively assuming a more globular-like structure is shown.}
\end{figure}
When the size of the head group is small, the thickness of the head group region decreases rapidly with decreasing surface coverage. It was observed that for C$_{12}$E$_3$ at low coverage ([C$_{12}$E$_3$] $\sim$ 0.02 × CMC) the head group lies almost flat on the interface [17]. As $n$ increases, however, the thickness of the head group region is little affected by surface coverage [18]. This confirms that as the size of the head group increases, it tends to assume a globular-like conformation. This can be observed in Figure 6.1 where the closed circles show the thickness of the head group region at the CMC as a function of increasing ethylene oxide units. The solid line indicates the length of the fully extended head group.

When the head group contains up to 4 ethylene oxide units, the thickness of the head group region is consistent with a slightly folded or tilted conformation. As the size of the head group increases, the ethylene oxide region is unable to adopt a fully extended conformation and begins to curl up. With increasing number of ethylene oxide units this behaviour becomes more marked, as can be observed from the increasing discrepancy between the thickness of the head group region and the fully extended length. Further evidence of the head group gradually assuming a globular shape with increasing size comes from the area per surfactant molecule at the CMC. Had the head group maintained an extended conformation upon addition of more ethylene oxide units, the head group cross-section would have been little affected and only small changes would have been observed in the APM. Instead, as can be seen from Figure 6.2, increasing head group size leads to a significant increase in APM; hence a larger cross-sectional area of the surfactant head group and indication of increased globular character. The values for the area per molecule as a function of increasing head group size are reported in Table 6.1.

<table>
<thead>
<tr>
<th>$n$</th>
<th>EO units</th>
<th>APM / Å²</th>
<th>$\Gamma$ / mol m$^{-2}$</th>
<th>$\Gamma$ / mg m$^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29 ± 1</td>
<td>5.73 × 10$^{-6}$</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>33 ± 1</td>
<td>5.03 × 10$^{-6}$</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>36 ± 2</td>
<td>4.61 × 10$^{-6}$</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>44 ± 1</td>
<td>3.77 × 10$^{-6}$</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>55 ± 3</td>
<td>3.02 × 10$^{-6}$</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>65 ± 2</td>
<td>2.55 × 10$^{-6}$</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>72 ± 3</td>
<td>2.31 × 10$^{-6}$</td>
<td>1.65</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.2. Area per molecule for a series of C\textsubscript{12}E\textsubscript{n} surfactants at the CMC at the air-water interface.

The adsorbed amount for C\textsubscript{12}E\textsubscript{n} surfactants at the air-water interface, expressed in mol m\textsuperscript{-2}, is also reported in Table 6.1 and is shown in Figure 6.3. Intuitively, as the size of the head group increases the number of molecules at the interface decreases. However, if the adsorbed amount at the interface is plotted in terms of g m\textsuperscript{-2}, small changes are observed with increasing size of head group (Figure 6.3 insert). This clearly shows how the decrease in adsorbed molecules is counterbalanced by the increased molecular weight of the surfactant: as a result, the total amount of material adsorbed at the interface varies very little.

It is noteworthy that as the number of ethylene oxide units increases, an enhanced overlap between the head group and tail group occurs, leading to significant mixing between the two regions and increased disorder within the monolayer. However, as there is no direct proof of constant and uniform hydration throughout the ethoxylated head group, there is no indisputable evidence of increased mixing between the water phase and the alkyl chain [19].
6.3 Conformational studies of $\text{C}_{12}\text{E}_n$ surfactants at the hexadecane-water interface

The systematic study of $\text{C}_{12}\text{E}_n$ surfactants at the hexadecane-water interface was performed adopting neutron reflectivity and interfacial tension. To study the role of the head group on the structural conformation, four surfactants were investigated. The surfactant head groups contained, respectively, 4, 6, 8 and 12 ethylene oxide units. The surfactants were synthesised by our collaborators in Oxford, England (Professor R. K. Thomas’s group) and were available both as fully hydrogenous and with the alkyl chain deuterated.

Two main objectives were set for the study:
1. To determine the structure of the adsorbed layer both as a function of concentration and increasing head group size.

2. To determine the adsorbed amount at the interface, again both as a function of concentration and increasing head group size. For this part of the investigation, a parallel interfacial tension study was carried out using the Du Nouy ring method.

The two parts will be individually discussed in the following sections. With regard to the structural determination element of the project, the analysis of C$_{12}$E$_4$ could not be carried out because of a lack of beam time. However, data regarding the determination of the adsorbed amount of C$_{12}$E$_4$ are here reported.

### 6.4 Determination of the interfacial structure for a series of C$_{12}$E$_n$ surfactants

When the structural determination of surfactants at interfaces is performed using neutron reflectivity, a large $\Delta N\!b$ between the two bulk phases is desirable. For instance, the structural determination of surfactant at the air-water interface is generally performed using D$_2$O as aqueous phase. At the oil-water interface, good results have been recently achieved using D$_2$O as aqueous sub-phase and oil contrast-matched to silicon (CMSi oil) to minimise the reflectivity between the silicon block and the oil phase. In general, it is preferable to use the hydrogenous surfactant as its $N\!b$ is quite different from that of the two bulk phases; hence it provides a good contrast.

Because of the magnitude of the investigation, a series of neutron reflectivity investigations were planned and three different reflectometers were used for this study. C$_{12}$E$_6$ was analysed using FIGARO, at ILL (see section 2.4.3.3), C$_{12}$E$_8$ was analysed using INTER, at ISIS (see section 2.4.3.2) and C$_{12}$E$_{12}$ was analysed using SURF, also at ISIS (see section 2.4.3.1). The three instruments are all time-of-flight reflectometers and the available wavelength range changes significantly between
them. The incident angle was therefore varied to cover a most suitable $Q$ range. The incident angle was 3.82° for $C_{12}E_6$, 2.3° for $C_{12}E_8$ and 1.5° for $C_{12}E_{12}$.

A series of reflectivity profiles were measured for all surfactants for a series of concentrations above and below the recorded CMC at the air-water interface. CMC values for the three surfactants at the air-water interface are given in Table 6.2. Reflectivity profiles for all concentrations are shown in Figure 6.4 for $C_{12}E_6$, Figure 6.5 for $C_{12}E_8$ and Figure 6 for $C_{12}E_{12}$. Because the attenuation of the neutron beam upon traversing the oil phase is wavelength-dependent, the data analysis must be carried out in wavelength. The data are, however, presented in $Q$ for clarity.

Table 6.2. CMC values at the air-water interface for the surfactants used.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>CMC / mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{12}E_6$ [15]</td>
<td>0.080</td>
</tr>
<tr>
<td>$C_{12}E_8$ [16]</td>
<td>0.091</td>
</tr>
<tr>
<td>$C_{12}E_{12}$ [18]</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Figure 6.4. Reflectivity profiles for a series of h-$C_{12}E_6$ at the CMSi oil-D$_2$O interface, solid lines correspond to the fit to the data. The concentration is shown in mM units. Profiles are shifted by a factor of $\times 10$ for the purpose of clarity.
Figure 6.5. Reflectivity profiles for a series of h-C_{12}E_{8} at the CMSi oil-D_{2}O interface, solid lines correspond to the fit to the data. The concentration is shown in mM units. Profiles are shifted by a factor of $\times 10$ for the purpose of clarity.

Figure 6.6. Reflectivity profiles for a series of h-C_{12}E_{12} at the CMSi oil-D_{2}O interface, solid lines correspond to the fit to the data. The concentration is shown in mM units. Profiles are shifted by a factor of $\times 10$ for the purpose of clarity.
All reflectivity profiles were fitted to a three layer model. The thickness of the first layer, in contact with the oil phase, was found to be constant (22 Å) regardless of the increasing size of the head group. On the other hand, the thickness of the second, intermediate layer increased with increasing head group size. A small decrease in thickness of the second layer was observed for C_{12}E_{12} when the concentration decreases to low values ([C_{12}E_{12}] < 0.104 mM). A third layer, with Nb close to that of D_{2}O, was required to model the reflectivity data. The thickness of this layer increased from C_{12}E_{6} to C_{12}E_{8} and no further changes were observed upon increasing the head group to 12 ethylene oxide units. The thickness of this layer decreased for C_{12}E_{6} with decreasing concentration ([C_{12}E_{6}] < 0.261 mM).

Changes in interlayer roughness between the oil phase and the first layer, between the first and the second layer, and between the third layer and the D_{2}O, affect very little the reflectivity profiles. Conversely, small variations in the roughness between the second and the third layer significantly affect the quality of the fitting. Roughness between the second and the third layer increases considerably between C_{12}E_{6} and C_{12}E_{8} and a further increase is observed between C_{12}E_{8} and C_{12}E_{12}. Roughness between the second and the third layer is particularly relevant in the fitting procedure and will henceforth be referred to as \( \sigma_3 \).

All the fitting parameters are shown in Table 6.3, Table 6.4 and Table 6.5 for C_{12}E_{6}, C_{12}E_{8} and C_{12}E_{12} respectively. The corresponding Nb profiles are shown in Figure 6.7, Figure 6.8 and Figure 6.9 for C_{12}E_{6}, C_{12}E_{8} and C_{12}E_{12}.
Table 6.3. Fitting parameters for $C_{12}E_6$ at the contrast-matched silicon hexadecane-$D_2O$ interface.

<table>
<thead>
<tr>
<th>Concentration / mM</th>
<th>0.523</th>
<th>0.261</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer thickness $\text{Å (± 2)}$</td>
<td>Nb $\times 10^6 / \text{Å}^2$</td>
<td>Roughness / Å</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>2.07</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>2.77</td>
<td>2.78</td>
</tr>
<tr>
<td>25</td>
<td>3.10</td>
<td>2.88</td>
</tr>
<tr>
<td>35</td>
<td>5.93</td>
<td>5.94</td>
</tr>
<tr>
<td>$D_2O$</td>
<td>6.35</td>
<td>2</td>
</tr>
</tbody>
</table>

Low concentration

<table>
<thead>
<tr>
<th>Concentration / mM</th>
<th>0.087</th>
<th>0.044</th>
<th>0.017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer thickness $\text{Å (± 2 Å)}$</td>
<td>Nb $\times 10^6 / \text{Å}^2$</td>
<td>Roughness / Å</td>
<td></td>
</tr>
<tr>
<td>Hexadecane</td>
<td>2.07</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>3.20</td>
<td>3.43</td>
<td>3.88</td>
</tr>
<tr>
<td>25</td>
<td>3.32</td>
<td>4.98</td>
<td>5.89</td>
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<tr>
<td>24</td>
<td>5.96</td>
<td>6.03</td>
<td>6.13</td>
</tr>
<tr>
<td>$D_2O$</td>
<td>6.35</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.4. Fitting parameters for $C_{12}E_8$ at the contrast-matched silicon hexadecane-$D_2O$ interface.

<table>
<thead>
<tr>
<th>Concentration / mM</th>
<th>0.273</th>
<th>0.091</th>
<th>0.068</th>
<th>0.046</th>
<th>0.018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer thickness $\text{Å (± 2)}$</td>
<td>Nb $\times 10^6 / \text{Å}^2$</td>
<td>Roughness / Å</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexadecane</td>
<td>2.07</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>2.46</td>
<td>2.87</td>
<td>3.24</td>
<td>3.36</td>
<td>3.66</td>
</tr>
<tr>
<td>30</td>
<td>3.29</td>
<td>3.95</td>
<td>4.33</td>
<td>4.59</td>
<td>5.08</td>
</tr>
<tr>
<td>42</td>
<td>5.70</td>
<td>5.76</td>
<td>5.77</td>
<td>6.09</td>
<td>6.15</td>
</tr>
<tr>
<td>$D_2O$</td>
<td>6.35</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.5. Fitting parameters for C₁₂E₁₂ at the contrast-matched silicon hexadecane-D₂O interface.

### High concentration

<table>
<thead>
<tr>
<th>Concentration / mM</th>
<th>0.418</th>
<th>0.139</th>
<th>0.104</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Layer thickness (Å (± 2))</strong></td>
<td>Nb × 10⁶ / Å⁻²</td>
<td>Roughness / Å</td>
<td></td>
</tr>
<tr>
<td>Hexadecane</td>
<td>2.07</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>2.33</td>
<td>2.47</td>
<td>2.54</td>
</tr>
<tr>
<td>42</td>
<td>2.57</td>
<td>2.86</td>
<td>2.98</td>
</tr>
<tr>
<td>D₂O</td>
<td>6.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Low concentration

<table>
<thead>
<tr>
<th>Concentration / mM</th>
<th>0.070</th>
<th>0.028</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Layer thickness (Å (± 2))</strong></td>
<td>Nb × 10⁶ / Å⁻²</td>
<td>Roughness / Å</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>2.07</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>2.87</td>
<td>2.96</td>
</tr>
<tr>
<td>35</td>
<td>4.44</td>
<td>4.49</td>
</tr>
<tr>
<td>D₂O</td>
<td>6.35</td>
<td>13</td>
</tr>
<tr>
<td>42</td>
<td>6.13</td>
<td>13</td>
</tr>
</tbody>
</table>
Figure 6.7. Nb profiles for a series of C_{12}E_{6} at the CMSi hexadecane-D_{2}O interface. Purple line: 0.523 mM; black line: 0.261 mM; blue line: 0.087 mM; red line: 0.044 mM; green line: 0.017 mM.

Figure 6.8. Nb profiles for a series of C_{12}E_{8} at the CMSi hexadecane-D_{2}O interface. Black line: 0.273 mM; blue line: 0.091 mM; gray line: 0.068 mM; red line: 0.046 mM; green line: 0.018 mM.
6.4.1 First layer: the tail group region

The interface was divided into three layers which represent three distinct regions of the interfacial area. The first layer, with a thickness of 22 Å for all contrasts, represents the region where the tail group inserts into the oil phase. The thickness is broader compared to the fully extended dodecyl chain length (~ 16.3 Å), indicating a relatively staggered conformation. This is not surprising given the rougher nature of the oil-water interface. The chain solvation by the hexadecane molecules prevents the perfect alignment of the tail groups, playing a key role in maintaining the staggered conformation in this region of the interface. Such broadening of the tail group region at the oil-water interface was also observed for the zwitterionic C₁₆PC molecules [3], indicating that solvation of the tail group occurs regardless of the presence or absence of charges in the head group. The Nb of the first layer is in all cases higher than that of the oil and the surfactant, indicating the presence of water.
(D\textsubscript{2}O) in the region. The \(N_b\) of the first layer decreases as a function of increasing head group size, therefore the presence (volume fraction) of D\textsubscript{2}O in this region must also decrease. The phenomenon might be related to surface activity: the surfactant with the smaller head group (C\textsubscript{12}E\textsubscript{6}) is more surface active and leads to better intermixing between oil and water. The increase in head group size leads to a decrease in the surface activity of the compound; hence the progressively worse intermixing between oil and water and the reduced presence of water (D\textsubscript{2}O) in the layer.

### 6.4.2 Second layer: the head group region

The second layer contains mostly surfactant head groups. The thickness of this layer increases as a function of increasing head group size which is to be expected as the head group gets bulkier. The thickness of the region is comparable to the length of the fully extended head group for all surfactants. It must be stated, however, that a reduction in layer thickness was observed for C\textsubscript{12}E\textsubscript{12} at low concentrations and as a result the thickness deviates from the fully extended length of the ethoxylated fragment.

Figure 6.10 shows the thickness of the head group region both at the air-water (●) and at the oil-water (○) interface. At the air-water interface the increase in thickness above \(n = 4\) was limited because of globular shape assumed by the ethoxylated region upon folding.

As a staggered conformation was already observed for the tail group region, it is not realistic to expect a perfectly aligned orientation for the head groups either. However, the staggered conformation alone cannot account for the significant increase in thickness compared to the air-water interface. A possible explanation for such an increase in thickness compared to the air-water interface is that at the oil-water interface the head group assumes a comparably less globular and more extended conformation, with no appreciable folding of the head group region.
6.4.3 Third layer: the diffuse region

The third layer, the one in contact with D$_2$O, represents a diffuse underlying structure. The $N_b$ of the layer is very close to D$_2$O in all cases, indicating that little surfactant is adsorbed in this region. It must be stressed that since the $\Delta N_b$ between the diffuse layer and D$_2$O is very small, the sensitivity of neutron reflectivity to this interfacial region is rather limited and the discussion regarding the structure of this layer is bound to be highly speculative. For all surfactants, as the concentration decreases below the CMC, the amount of surfactant in the underlying structure decreases (the $N_b$ of the underlying structure increases to almost that of D$_2$O). C$_{12}$E$_n$ surfactants form Gibbs monolayers where molecules from the bulk keep exchanging with those adsorbed at the interface. The underlying structure, adjacent to the adsorbed primary monolayer, represents the interfacial region where the exchange between the bulk phase and the monolayer takes place. This region could be interpreted as a surfactant-enriched area of the bulk phase in the vicinity of the interface; a necessary pathway for surfactant molecules to reach the interface. This hypothesis is also suggested by the observation that the $N_b$ of the third layer

Figure 6.10. Thickness of the head group region as a function of head group size at the air-water (●) and the oil-water interface (o). The straight line indicates the length of the fully extended head group.
consistently decreases as the size of the head group increases. In fact, surfactants with larger head groups present higher CMC, so that the concentration of free monomers in solution increases steadily with increasing head group size, hence increasing the concentration in the surfactant-enriched region and slightly diminishing its $Nb$.

### 6.4.4 Interlayer roughness

Interlayer roughness plays an important role in the data fitting for $\text{C}_{12}\text{E}_n$ surfactants. No interlayer roughness was required between the oil phase and layer 1, between layer 1 and layer 2 and between layer three and $\text{D}_2\text{O}$. A roughness of 2 Å was used solely to smooth the steps in the $Nb$ profiles in Figure 6.7, Figure 6.8 and Figure 6.9 and to improve the visual representation. Conversely, the roughness between the second layer and the diffuse layer ($\sigma_3$) increases significantly from 5 to 11 Å when moving from 6 to 8 ethylene oxide units in the head group. A further small increase was observed for $n = 12$ ($\sigma_3 = 13$ Å). The increased roughness indicates that the interdigitation between the primary monolayer and the diffuse region increases. The increased intermixing between the two regions could be caused by a combination of two factors:

1. Increased disorder in the head group region of the primary monolayer. This is not unlikely as it has already been observed at the air-water interface [18].
2. As the size of the head group increases, the CMC increases, and so does the concentration of free monomers in the aqueous phase. The surfactant molecules become more hydrophilic and progressively less surface active, consequently the interaction between the surfactant molecules and the interface becomes less favourable. The turnover between the primary monolayer and the diffuse region becomes much faster with increasing head group size, therefore it becomes progressively more difficult using neutron reflectivity to discern neatly between the two regions. An increase in roughness is then required to represent the imperfectly neat separation between the two regions.
6.4.5 Structural differences between the air-water and the oil-water interface

The structure of the primary monolayer in direct contact with the oil phase significantly differs from that at the air-water interface. At the air-water interface the tail region was constant and thinner than the fully extended length of the alkyl region, so that the tail groups were assuming a tilted conformation. At the oil-water interface the thickness of the tail group region also remains constant as a function of increasing head group size, but the thickness is now larger than the fully extended alkyl chain length. This effect is caused by the presence of hexadecane, which is now solvating the alkyl chains so that tail-tail interactions are reduced. The effect observed is a staggered conformation of the surfactant molecules where steric repulsion between the tail groups is much reduced. A similar increase in layer thickness for the tail group region when moving from the air-water to the oil-water interface was already observed for C$_{16}$PC surfactants and it was associated with a significant reduction in tilt angle [3].

Penfold et al., suggested a possible increase of water presence in the tail group region with increasing head group size at the air-water interface [19]. The opposite seems to happen at the oil-water interface: the Nb of the tail group region decreases with increasing head group size, clearly indicating that the presence of D$_2$O in the layer decreases as the head group gets bulkier.

Of particular interest is the increase of layer thickness for the head group region as a function of increasing head group size. It was shown earlier that the thickness of the head group region at the air-water interface increases linearly up to $n = 4$; after that, the increase is not as pronounced because of globular conformation assumed by the head group. At the oil-water interface, on the other hand, the thickness of the head group region seems to increase linearly with increasing size (Figure 6.10). The much faster increase observed at the oil-water interface can be caused by the head group assuming an almost fully extended conformation. One possible explanation for the extended conformation could be as follows. If some hexadecane molecules were to be present in this region, they could form van der Waals interactions with the hydrocarbon part of the head group preventing it from folding and assuming a
globular conformation. This is clearly highly speculative and more detailed contrast schemes would be required to prove it. However, some support for the validity of this theory comes from the presence of the diffuse underlying structure. The hydrocarbon tail groups of the surfactants in the underlying structure require a partially hydrophobic anchoring surface in order to adsorb onto the primary monolayer. As the diffuse region was not observed at the air-water interface, the surfactant head group alone is clearly not able to provide a suitable partially hydrophobic surface. At the oil-water interface, the presence of hexadecane molecules intermixed with the head groups could provide some degree of hydrophobicity to the head group region, hence the presence of the underlying structure.

The overall conformation of the primary monolayer is much more extended at the oil-water interface than it is at the air-water interface. The presence of hydrophobic hexadecane molecules provides solvation of the surfactant tail groups and the hydrophilic head groups also have some hexadecane molecules associated. The consequence of such solvation is a far more stretched conformation of the adsorbed monolayer compared to the air-water interface. The presence of the oil layer seems to pull the adsorbed surfactant molecules towards the more hydrophobic phase. This effect could clearly not be observed at the air-water interface as no solvation can be provided by air. To some extent, air can be considered as a “passive” hydrophobic medium. At the air-water interface, air behaves as a hydrophobic medium but it is clearly not capable of establishing any interactions with the substrates present at the interface. When a surfactant molecule in the aqueous phase reaches the interface it cannot interact with the air phase and the only favourable interactions left available for the hydrophobic part of the surfactant are van der Waals interactions between different tail groups. For this reason, the hydrophobic part of the adsorbed layer tends to be confined to a specific region in contact with the air phase where it forms a relatively well ordered structure. On the other hand, oil molecules can efficiently solvate surfactant molecules at the oil-water interface. When a surfactant molecule in water reaches the interface, its tail group can establish van der Waals interactions both with other surfactant tail groups and with oil molecules. As a result, the tail group region can assume a more relaxed and energetically favourable conformation
and the whole adsorbed layer results in a broader structure. For this reason, the oil surface can be considered as an “active” hydrophobic medium.

### 6.5 Adsorbed amount at the hexadecane-water interface: interfacial tension measurements

The adsorbed amount $\Gamma$ of C$_{12}$E$_n$ surfactants at the hexadecane-water interface was determined using a combination of neutron reflectivity and interfacial tension. A combination of the two techniques has already been reported in the literature to determine the adsorbed amount for C$_{16}$PC at the hexadecane-water interface and the results were in good agreement [3]. Interfacial tension measurements provide information regarding the adsorbed amount and the area per molecule only below the CMC, whereas neutron reflectivity can be used to measure these parameters at any given concentration.

#### 6.5.1 Surfactant partition between the oil phase and the water phase

Various water soluble surfactants may present some degree of solubility in oil. This is particularly the case of non-ionic surfactants where no charges are present in the hydrophilic head group. As a result, partition processes between oil and water may occur, thus lowering the aqueous concentration of the surfactant. It must be stressed that when the liquid-liquid interface is analysed with neutron reflectivity the volume of the hydrophobic phase is much smaller than that of the aqueous phase (with our setting ~ 15 µl of hexadecane, ~ 25 ml of aqueous phase). As a result, the reduction in aqueous concentration caused by partition processes can be considered negligible in a neutron reflectivity experiment. Conversely, interfacial tension measurements adopting the De Nouy ring method are performed using comparable volumes of oil and water and partition processes between the two phases can drastically affect the measurements.
In a previous study, C_{16}PC was practically insoluble in hexadecane and no partition occurred between the two insoluble phases [3]. Thus, the surfactant concentration in the aqueous phase was not affected by the volume of oil deployed. The solubility of dodecanol ethoxylates in hydrophobic solvents, on the other hand, is far from being negligible. In fact, it is known that they present a higher solubility in hydrocarbons than in water [18]. Therefore, when the interfacial tension is measured with the ring method the initial concentration of surfactant in the aqueous phase decreases after the hydrophobic phase has been deposited. The decrease in concentration is a function of the partition coefficient of the surfactant $K_{ow}$, which represents the ratio between the concentration of surfactant in the hydrophobic and the hydrophilic phases, respectively.

$$K_{ow} = \frac{[\text{surfactant}]_{\text{octanol}}}{[\text{surfactant}]_{\text{water}}}$$  \hspace{1cm} \text{Equation 6.1}

Because of the limited availability of surfactants, which were custom-synthesised, the partition coefficient could not be experimentally determined; hence it was not possible to calculate the concentration in the aqueous phase after equilibration. However, computer simulations predict a rather high distribution coefficient for C_{12}E_4 (Log $K_{ow}$ ~ 2.2), decreasing linearly as a function of head group size (for C_{12}E_{12} Log $K_{ow}$ ~ 1.0) [20].

The partition of C_{12}E_n molecules between the two phases would clearly affect the measured CMC (because of surfactant dissolution into the oil phase, the measured CMC will be higher than the real CMC). However, the Gibbs equation (Equation 6.2) states that the adsorbed amount $\Gamma$ at the CMC is not dependent on the interfacial tension $\gamma$ but on its variation as a function of concentration $\delta\gamma/\delta \ln C$.

$$\Gamma = -\frac{1}{RT} \left( \frac{\delta \gamma}{\delta \ln C} \right)$$  \hspace{1cm} \text{Equation 6.2}

$T$ is the temperature in K, $R$ is the gas constant. As a result, measurements of $\Gamma$ will not be affected by partition processes: the overall isotherm would be subject to a shift to higher values of $\ln C$ with no effect on the gradient $\delta\gamma/\delta \ln C$ of the linear fit. An example is shown for clarity in Figure 6.11.
Chapter 6  Mario Campana

Figure 6.11. Partition processes between oil and water do not alter the gradient of the interfacial tension profile. However, the profile will be shifted to higher values of Log C. While the measured CMC will drastically be affected by such shift, the adsorbed amount $\Gamma$ and the area per molecule does not show any deviations.

6.5.2 Interfacial tension measurements

To limit the extent of partition between the two phases, the volume of the aqueous phase used for interfacial tension measurements was bigger than that of the hexadecane. For all samples, the volume of the aqueous solutions and hexadecane was 50 ml and 10 ml respectively. One hour was allowed for equilibration, and neither the solution nor the oil phase was recovered after each measurement. The interfacial tension was recorded with the Du Nouy ring method using a Krüss K-9 tensiometer. The interfacial tension was measured six times for each concentration. The temperature was maintained at 25 ± 0.1 °C by means of a circulating water bath.

The interfacial tension was measured for $C_{12}E_4$, $C_{12}E_6$, $C_{12}E_8$ and $C_{12}E_{12}$ as a function of concentration. The results are shown in Figure 6.12 - Figure 6.15 for $C_{12}E_4$, $C_{12}E_6$, $C_{12}E_8$ and $C_{12}E_{12}$ respectively. In all figures, a vertical dashed line
indicates the reported CMC for the corresponding surfactant at the air-water interface (the values for the CMC are reported in Table 6.2).

Figure 6.12. Interfacial tension measurements at the hexadecane-water interface for C_{12}E_4 surfactant as a function of concentration. This surfactant presents the highest $K_{ow}$ amongst those studied; hence little differences in the volumes of the two phases used would alter the aqueous concentration sensibly. This may have caused the very poor reproducibility observed.

Figure 6.13. Interfacial tension measurements at the hexadecane-water interface for C_{12}E_6 surfactant as a function of concentration.
Interfacial tension measurements for $C_{12}E_4$ show very poor reproducibility. This visibly affects the quality of the linear fit, which is clearly not satisfactory. Figure
6.13 - Figure 6.15 show that the quality of the linear fit increases as the size of the head group increases. The improved quality of the linear fits could be because of the decreasing $K_{ow}$ of the surfactant, which leads to less partition between the two phases and better reproducibility in the sample preparation.

The CMC cannot be determined in absence of reliable measurements of the $K_{ow}$. From Figure 6.13 to Figure 6.15 ($C_{12}E_6$ to $C_{12}E_{12}$) it seems that the CMC observed diverges from the recorded value at the air-water interface with decreasing head group size. This is indicative of increasing dissolution into the oil phase, as expected from the $K_{ow}$ predictions [20]. $C_{12}E_4$ has not been taken into consideration as the quality of the interfacial tension measurements is rather poor.

Using Equation 6.2, the adsorbed amount $\Gamma$ at the interface at the CMC can be calculated. Known $\Gamma$, the area per surfactant molecule can be also calculated using Equation 6.3:

$$APM = \frac{1}{N_A \Gamma}$$

$N_A$ is the Avogadro constant. The values for both $\Gamma$ and APM as determined from interfacial tension measurements are shown in Table 6.6.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>$\Gamma$ / mol m$^{-2}$</th>
<th>$\Gamma$ / mg m$^{-2}$</th>
<th>APM / Å$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{12}E_4$</td>
<td>$5.45 \pm 1.21 \times 10^{-6}$</td>
<td>$1.98 \pm 0.44$</td>
<td>$30.5 \pm 8.7$</td>
</tr>
<tr>
<td>$C_{12}E_6$</td>
<td>$2.65 \pm 0.18 \times 10^{-6}$</td>
<td>$1.19 \pm 0.08$</td>
<td>$62.7 \pm 4.5$</td>
</tr>
<tr>
<td>$C_{12}E_8$</td>
<td>$2.01 \pm 0.09 \times 10^{-6}$</td>
<td>$1.08 \pm 0.05$</td>
<td>$82.8 \pm 4.0$</td>
</tr>
<tr>
<td>$C_{12}E_{12}$</td>
<td>$1.53 \pm 0.05 \times 10^{-6}$</td>
<td>$1.09 \pm 0.04$</td>
<td>$108.3 \pm 3.4$</td>
</tr>
</tbody>
</table>

The adsorbed amount $\Gamma$ at the hexadecane-water interface is shown in Figure 6.16 by the filled circles; open signs correspond to $\Gamma$ at the air-water interface. The same results are shown in figure insert where, in a similar representation to Figure 6.3 insert, $\Gamma$ is plotted in form of mg m$^{-2}$. The area per molecule at the interface is shown
in Figure 6.17 by the filled circles. Again, the open circles correspond to the area per molecule at the air-water interface.

**Figure 6.16.** Adsorbed amount at the hexadecane-water interface as determined from interfacial tension (filled circles). Neutron reflectivity data at the air-water interface from the literature are shown for comparison (open circles). The adsorbed amount is also shown in mg mol$^{-2}$ units in figure insert.

**Figure 6.17.** Area per molecule at the hexadecane-water interface as determined by interfacial tension (filled circles). The area per molecule at the air-water interface as determined with neutron reflectivity from the literature is also shown (open circles).
6.6 Adsorbed amount at the hexadecane-water interface:
neutron reflectivity measurements

6.6.1 Determination of the adsorbed amount: from the air-water to the oil-water interface

Before discussing the results obtained with neutron reflectivity measurements, it is convenient to explain in detail the procedure adopted for the data analysis. The standard procedure to determine the adsorbed amount of a surfactant at the air-water interface involves the adsorption of deuterated surfactants at the air-null reflecting water interface (water with $Nb = 0$, same as air). This way, a large $\Delta Nb$ can be generated between the two bulk phases and the adsorbed layer, and the entire detected signal arises from the deuterated surfactant adsorbed at the interface [21]. Therefore, the reflected intensity is a function of the adsorbed amount $\Gamma$ at the interface. The data analysis is carried out adopting a well known methodology [21]: all profiles are fitted to a single layer with zero roughness and the adsorbed amount $\Gamma$ is a function of the integrated area under the $Nb$ profile curve. For the air-water interface, since the $Nb$ of air is 0 and the aqueous phase is null reflecting water, the adsorbed amount $\Gamma$ can be written as [22]:

$$\Gamma = \frac{m}{N_A b}$$  \hspace{1cm} \text{Equation 6.4}

$N_A$ is Avogadro’s number and $b$ is the scattering length of the adsorbed specie. The parameter $m$ corresponds to the scattering length density profile perpendicular to the interface integrated over the whole adsorbed layer:

$$m = \int_{-\infty}^{+\infty} Nb(z) \, dz = Nb_{\text{layer}} \cdot d$$  \hspace{1cm} \text{Equation 6.5}

$Nb_{\text{layer}}$ and $d$ represent the experimentally determined $Nb$ and thickness of the layer respectively. The adsorbed amount at the air water interface can be then calculated by combining the two fitted parameters $Nb$ and $d$ into Equation 6.6 [21]:

$$\Gamma = \frac{Nb_{\text{layer}} \cdot d}{N_A b}$$  \hspace{1cm} \text{Equation 6.6}
Analogous to the determination of $\Gamma$ at the air-water interface, at the oil-water interface the procedure also relies on adsorbing the surfactant onto a non-reflecting interface. However, the $Nb$ of both oil and water are generally contrast-matched to the solid substrate onto which the oil layer has been spun (different from 0) and Equation 6.5 must be written as:

$$\int_{-\infty}^{+\infty} Nb(x) \, dz = |Nb_{\text{layer}} - Nb_b|d$$

Equation 6.7

$Nb_b$ is the scattering length density of the bulk phases.

In order to ascertain the adsorbed amount $\Gamma$ of the surfactant from the scattering length density profile, a simplifying assumption is required. This reasonable, simplifying assumption is that the scattering length density of the adsorbed layer ($Nb_{\text{layer}}$) varies in a linear fashion with composition i.e. with the volume fraction of the surfactant in the layer [2]. As a consequence, the volume fraction ($\Phi$) of the adsorbed layer can be related to the experimentally determined $Nb_{\text{layer}}$, as:

$$\Phi = \frac{Nb_{\text{layer}} - Nb_b}{m}$$

Equation 6.8

where $Nb_b$ is the scattering length density of the bulk phase (in our case silicon, as both the oil and water are matched to silicon, $Nb_b = 2.07 \times 10^{-6} \text{Å}^{-2}$) and $m$ is the gradient of the linear regression line in Figure 6.18 ($m = \frac{\Delta y}{\Delta x} = \frac{Nb_{\text{surf}} - Nb_b}{1}$).

Figure 6.18. Relationship between layer scattering length density ($Nb$) profile (left) and volume fraction of the surfactant in the layer (right). An increase in $Nb_{\text{layer}}$ corresponds to an increase in $\Phi$, hence higher adsorbed amount.

For a known scattering length density of the adsorbed surfactant ($Nb_{\text{surf}}$), the volume fraction in Equation 6.8 can be rewritten as:
The volume fraction profile can then be used to determine the adsorbed amount ($\Gamma = \frac{d \rho \Phi}{MW}$):

$$\Gamma = \frac{d \rho}{MW} \times \frac{Nb_{layer} - Nb_b}{Nb_{surf} - Nb_b}$$  

Equation 6.10

$\Gamma$ is expressed in units of mol m$^{-2}$, $d$ is the layer thickness (determined experimentally), $\rho$ is the surfactant density and $MW$ is the surfactant molecular weight. The area per molecule can be then calculated using Equation 6.3:

$$APM = \frac{1}{N_A \Gamma}$$  

Equation 6.3

### 6.6.2 Choice of the contrasts for the determination of the adsorbed amount for C$_{12}$E$_n$ surfactants

As the procedure relies on measuring reflectivity from a single layer adsorbed onto an otherwise non-reflecting interface, the choice of contrast is essential. The $\Delta Nb$ between the adsorbed surfactant and the bulk media must be sufficiently big to be detectable. The adsorbed surfactant is generally deuterated so that its $Nb$ is relatively high compared to the media.

The case of C$_{12}$E$_n$ surfactants here reported presents some complications. The $Nb$ of both hexadecane and water was contrast-matched to silicon ($Nb = 2.07 \times 10^{-6}$ Å$^{-2}$) and the surfactants were only available as fully hydrogenous or partially (dodecyl, d$_{25}$) deuterated. For the deuterated compounds the $\Delta Nb$ between the surfactant and the bulk phases decreases very rapidly with increasing head group size. $\Delta Nb$ is relatively large for d-C$_{12}$E$_4$ and d-C$_{12}$E$_6$, it is rather small for d-C$_{12}$E$_8$ and clearly insufficient for the analysis of d-C$_{12}$E$_{12}$. On the other hand, the $\Delta Nb$ for the fully hydrogenous surfactants is relatively steady, showing very little decrease as the size of the head group increases. The $Nb$ of both partially deuterated and fully hydrogenous forms for all surfactants are reported in Table 6.7. The $\Delta Nb$ between the surfactant and the bulk phases is also shown.
Table 6.7. Nb values for the fully hydrogenous and partially deuterated surfactants. The density of h-C_{12}E_4 is 0.95 g cm^{-3}. Since no data regarding the density of C_{12}E_6, C_{12}E_8 and C_{12}E_{12} were found in the literature, the density was assumed to be the same as for C_{12}E_4. The Nb values for the deuterated surfactants were calculated considering 98% deuteration. The ∆Nb between the surfactant and silicon is also shown in the table.

| EO units | Nb d-C_{12}E_n (× 10^{-6} \text{ Å}^{-2}) | | ∆Nb | Nb h-C_{12}E_n (× 10^{-6} \text{ Å}^{-2}) | | ∆Nb |
|----------|----------------------------------------|-----------|----------------------------------------|-----------|----------------------------------------|
| 4        | 3.67                                   | 1.60      | 0.07                                   | 2.00      |
| 6        | 3.05                                   | 0.98      | 0.15                                   | 1.92      |
| 8        | 2.63                                   | 0.56      | 0.20                                   | 1.87      |
| 12       | 2.10                                   | 0.03      | 0.27                                   | 1.80      |

Therefore, Γ was determined by using the partially deuterated surfactant for C_{12}E_4, C_{12}E_6 and C_{12}E_8. The reflectivity from solutions of partially deuterated C_{12}E_{12} was not measured at all; the fully hydrogenous form was used instead. The hydrogenous forms of C_{12}E_8 and C_{12}E_4 were also analysed for comparison. The plan also involved the measurement of the hydrogenous form of C_{12}E_6 but it was not possible to analyse it because of insufficient beam time. Table 6.8 shows the summary of the surfactants analysed along with the reflectometer used for the analysis.

Table 6.8. Summary of the contrasts used for the determination of adsorbed amount for the four surfactants. In all cases both oil and water were contrast-matched to silicon (Nb = 2.07 × 10^{-6} \text{ Å}^{-2}). The reflectometer used for the measurements is also shown in the Table.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>h-surfactant</th>
<th>d-surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{12}E_4</td>
<td>INTER</td>
<td>INTER</td>
</tr>
<tr>
<td>C_{12}E_6</td>
<td>-</td>
<td>FIGARO</td>
</tr>
<tr>
<td>C_{12}E_8</td>
<td>INTER</td>
<td>INTER</td>
</tr>
<tr>
<td>C_{12}E_{12}</td>
<td>SURF</td>
<td>-</td>
</tr>
</tbody>
</table>
6.6.3 Neutron reflectivity measurements

The reflectometer FIGARO at ILL (section 2.4.3.3) was used to analyse the partially deuterated C$_{12}$E$_6$. The incoming beam was at an incident angle $\theta = 3.82^\circ$. The fully hydrogenous form of C$_{12}$E$_8$, analysed with INTER (section 2.4.3.2), was measured without any deflections of the incoming beam so that the incident angle was $\theta = 2.3^\circ$. The $Q$ range available with this setting is very broad but relatively little information is gained in the interesting region of $Q < 0.05$ Å$^{-1}$. The $Q$ range can be shifted to lower values by reducing the incident angle. This was achieved for both the hydrogenous and partially deuterated C$_{12}$E$_4$ and the partially deuterated C$_{12}$E$_8$ by deflecting the incident beam to $\theta = 1.4^\circ$ by use of a supermirror. C$_{12}$E$_{12}$ was analysed with the reflectometer SURF at ISIS (section 2.4.3.1) with an incident beam $\theta = 1.5^\circ$. The reflectivity profiles for the partially deuterated surfactants are shown in sequence for C$_{12}$E$_4$, C$_{12}$E$_6$, and C$_{12}$E$_8$ in Figure 6.19 - Figure 6.21. The solid lines represent the one layer fit to the data. The fitted parameters for the one layer fits are given in Table 6.9, Table 6.10 and Table 6.11 for d-C$_{12}$E$_4$, d-C$_{12}$E$_6$ and d-C$_{12}$E$_8$ respectively. No roughness was used for these one layer fits.

![Reflectivity profiles](image)

**Figure 6.19.** Reflectivity profiles for a series of d-C$_{12}$E$_4$ solutions as a function of concentration at the CMSi oil-CMSi water interface. Solid lines correspond to the one layer fit to the data. Concentration is in mM units, profiles are shifted by a ×10 factor for the purpose of clarity.
Figure 6.20. Reflectivity profiles for a series of d-C_{12}E_{6} solutions as a function of concentration at the CMSi oil-CMSi water interface. Solid lines correspond to the one layer fit to the data. Concentration is in mM units, profiles are shifted by a x10 factor for the purpose of clarity.

Figure 6.21. Reflectivity profiles for a series of d-C_{12}E_{8} solutions as a function of concentration at the CMSi oil-CMSi water interface. Solid lines correspond to the one layer fit to the data. Concentration is in mM units, profiles are shifted by a x10 factor for the purpose of clarity.
Table 6.9. Parameters used for one layer fit to the reflectivity profiles shown in Figure 6.19 for d-C\(_{12}\)E\(_4\) surfactant at the hexadecane-water interface. Both oil and water are contrast matched to silicon (\(Nb = 2.07 \times 10^6 / \text{Å}^2\)).

<table>
<thead>
<tr>
<th>Concentration mM</th>
<th>0.446</th>
<th>0.207</th>
<th>0.069</th>
<th>0.052</th>
<th>0.035</th>
<th>0.014</th>
</tr>
</thead>
<tbody>
<tr>
<td>(d / \text{Å})</td>
<td>85 ± 3</td>
<td></td>
<td>57 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nb \times 10^6 / \text{Å}^2)</td>
<td>2.55</td>
<td>2.55</td>
<td>2.68</td>
<td>2.54</td>
<td>2.44</td>
<td>2.26</td>
</tr>
</tbody>
</table>

Table 6.10. Parameters used for one layer fit to the reflectivity profiles shown in Figure 6.20 for d-C\(_{12}\)E\(_6\) surfactant at the hexadecane-water interface. Both oil and water are contrast matched to silicon.

<table>
<thead>
<tr>
<th>Concentration mM</th>
<th>0.523</th>
<th>0.261</th>
<th>0.087</th>
<th>0.044</th>
<th>0.017</th>
</tr>
</thead>
<tbody>
<tr>
<td>(d / \text{Å})</td>
<td></td>
<td>50 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nb \times 10^6 / \text{Å}^2)</td>
<td>2.64</td>
<td>2.73</td>
<td>2.69</td>
<td>2.42</td>
<td>2.34</td>
</tr>
</tbody>
</table>

Table 6.11. Parameters used for one layer fit to the reflectivity profiles shown in Figure 6.21 for d-C\(_{12}\)E\(_8\) surfactant at the hexadecane-water interface. Both oil and water are contrast matched to silicon.

<table>
<thead>
<tr>
<th>Concentration mM</th>
<th>0.273</th>
<th>0.091</th>
<th>0.046</th>
<th>0.018</th>
</tr>
</thead>
<tbody>
<tr>
<td>(d / \text{Å})</td>
<td></td>
<td>58 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nb \times 10^6 / \text{Å}^2)</td>
<td>2.42</td>
<td>2.34</td>
<td>2.22</td>
<td>2.18</td>
</tr>
</tbody>
</table>

For C\(_{12}\)E\(_4\) solutions at the highest concentration, good fits to the data are obtained adopting a layer thickness of 85 ± 3 Å; as the concentration drops to lower values, the thickness decreases to 57 ± 2 Å. The reduction in layer thickness is significant, possibly suggesting a structural change as a function of concentration. For the other surfactants, the fitted layer thickness is similar to that obtained for C\(_{12}\)E\(_4\) with low concentration. For C\(_{12}\)E\(_6\) a layer thickness of 50 ± 2 Å was found to adequately fit all the reflectivity profiles, whereas the thickness required for C\(_{12}\)E\(_8\) was \(d = 58 ± 3\) Å.

With regard to the fully hydrogenous surfactants, the reflectivity profiles for C\(_{12}\)E\(_4\), C\(_{12}\)E\(_8\) and C\(_{12}\)E\(_{12}\) are shown in Figure 6.22, Figure 6.23 and Figure 6.24 respectively. Again, the solid lines represent the one layer fit to the data. It is worth noticing the slight shift in the \(Q\) range towards lower values between C\(_{12}\)E\(_8\) and C\(_{12}\)E\(_4\) caused by the decrease in beam incident angle \(\theta (\theta = 2.3^\circ \text{ for C}_{12}\text{E}_8, \theta = 1.4^\circ \text{ for C}_{12}\text{E}_4)\). As a
result, it was also observed that the error bars significantly improve in the important low-$Q$ region. The fitted parameters for the one layer fits are given in Table 6.12, Table 6.13 and Table 6.14 for $h$-$C_{12}E_4$, $h$-$C_{12}E_8$ and $h$-$C_{12}E_{12}$ respectively.

Figure 6.22. Reflectivity profiles for a series of $h$-$C_{12}E_4$ solutions at the CMSi oil- CMSi water interface. Profiles are shifted by a factor of $\times10$ for the purpose of clarity. Solid lines correspond to the one layer fit to the data. Concentrations, expressed in mM units, are shown in the figure.

Figure 6.23. Reflectivity profiles for a series of $h$-$C_{12}E_8$ solutions at the CMSi oil- CMSi water interface. Profiles are shifted by a factor of $\times10$ for the purpose of clarity. Solid lines correspond to the one layer fit to the data. Concentrations, expressed in mM units, are shown in the figure.
Figure 6.24. Reflectivity profiles for a series of h-C$_{12}$E$_{12}$ solutions at the CMSi oil-CMSi water interface. Profiles are shifted by a factor of ×10 for the purpose of clarity. Solid lines correspond to the one layer fit to the data. Concentrations, expressed in mM units, are shown in the figure.

Table 6.12. Parameters used for one layer fit to the reflectivity profiles shown in Figure 6.22 for h-C$_{12}$E$_{4}$ surfactant at the hexadecane-water interface. Both oil and water are contrast matched to silicon.

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>0.207</th>
<th>0.069</th>
<th>0.052</th>
<th>0.035</th>
<th>0.014</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ / Å</td>
<td>85 ± 3</td>
<td>57 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N_b \times 10^{-6}$ / Å$^{-2}$</td>
<td>1.73</td>
<td>1.75</td>
<td>1.81</td>
<td>1.99</td>
<td>1.99</td>
</tr>
</tbody>
</table>

Table 6.13. Parameters used for one layer fit to the reflectivity profiles shown in Figure 6.23 for h-C$_{12}$E$_{8}$ surfactant at the hexadecane-water interface. Both oil and water are contrast matched to silicon.

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>0.273</th>
<th>0.091</th>
<th>0.046</th>
<th>0.018</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ / Å</td>
<td></td>
<td></td>
<td>25 ± 2</td>
<td></td>
</tr>
<tr>
<td>$N_b \times 10^{-6}$ / Å$^{-2}$</td>
<td>1.42</td>
<td>1.49</td>
<td>1.58</td>
<td>1.72</td>
</tr>
</tbody>
</table>
Table 6.14. Parameters used for one layer fit to the reflectivity profiles shown in Figure 6.24 for h-C$_{12}$E$_{12}$ surfactant at the hexadecane-water interface. Both oil and water are contrast matched to silicon.

<table>
<thead>
<tr>
<th>Concentration mM</th>
<th>0.418</th>
<th>0.139</th>
<th>0.104</th>
<th>0.028</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d / \text{Å}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Nb \times 10^6 / \text{Å}^{-2}$</td>
<td>1.34</td>
<td>1.37</td>
<td>1.26</td>
<td>1.35</td>
</tr>
</tbody>
</table>

Similarly to what has been reported for the d-C$_{12}$E$_4$, for h-C$_{12}$E$_4$ also a variation in fitted layer thickness is observed as the concentration decreases. A good fit to the reflectivity profile is obtained using a layer thickness of $85 \pm 3 \text{ Å}$ for the sample with the highest concentration; as the concentration drops to lower values, the layer thickness also decreases to $57 \pm 2 \text{ Å}$ as observed for d-C$_{12}$E$_4$.

The fitted layer thickness for h-C$_{12}$E$_8$ was found to be much smaller than that for d-C$_{12}$E$_8$. The thickness reduced significantly to $d = 25 \pm 2 \text{ Å}$. An even smaller layer thickness was required for h-C$_{12}$E$_{12}$, where the fitted layer thickness was found to be $22 \pm 2 \text{ Å}$. The differences in the reflectivity profiles as a function of concentration for C$_{12}$E$_{12}$ are very subtle. The minor changes in reflectivity profiles could be because of small variations in $\Gamma$ as a function of concentration, however the effect could also be caused by the small $\Delta Nb$ between the surfactant and the bulk phases.

### 6.6.4 Calculation of the adsorbed amount

Adsorbed amount $\Gamma$ and area per molecule at the interface can be calculated using Equation 6.10 and Equation 6.3 respectively. For the application of Equation 6.10, thus the calculation of $\Gamma$, a main issue arises, that is: for C$_{12}$E$_8$, the fitted layer thickness varies significantly between the two contrasts. We believe that the variation observed must be attributed to sensitivity of the neutron reflectivity technique to different parts of the interface. As will be discussed later, this seems to be the case in this situation.

$\Gamma$ and area per molecule at the interface were calculated both for the hydrogenous and deuterated surfactants as a function of concentration. $\Gamma$ for each surfactant is shown in Figure 6.25, Figure 6.26, Figure 6.27 and Figure 6.28 for C$_{12}$E$_4$, C$_{12}$E$_6$, and C$_{12}$E$_8$. 

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C\textsubscript{12}E\textsubscript{8} and C\textsubscript{12}E\textsubscript{12} respectively. Results for both hydrogenous and deuterated surfactants are grouped together for C\textsubscript{12}E\textsubscript{4} and C\textsubscript{12}E\textsubscript{8}. The horizontal dashed lines correspond to $\Gamma$ from interfacial tension, as discussed in the previous section.

**Figure 6.25.** Adsorbed amount $\Gamma$ as measured from interfacial tension (dashed lines) and neutron reflectivity. The large uncertainty in the interfacial tension results must be attributed to the low reproducibility observed in the measurement. Results for d-C\textsubscript{12}E\textsubscript{4} (○) and h-C\textsubscript{12}E\textsubscript{4} (●) are both shown.

**Figure 6.26.** Adsorbed amount $\Gamma$ as measured from interfacial tension (dashed lines) and neutron reflectivity for d-C\textsubscript{12}E\textsubscript{6} (○).
Figure 6.27. Adsorbed amount $\Gamma$ as measured from interfacial tension (dashed lines) and neutron reflectivity. Results for d-C$_{12}$E$_8$ (o) and h-C$_{12}$E$_8$ (●) are both shown.

Figure 6.28. Adsorbed amount $\Gamma$ as measured from interfacial tension (dashed lines) and neutron reflectivity for h-C$_{12}$E$_{12}$ (●) are both shown.
Figure 6.25 shows the large disagreement in $\Gamma$ between the results obtained with neutron reflectivity for d-C$_{12}$E$_4$ and h-C$_{12}$E$_4$. Much larger values are obtained when d-C$_{12}$E$_4$ is adsorbed at the interface. Furthermore, despite the large error bars, interfacial tension measurements are in disagreement with both the values obtained with neutron reflectivity. Interfacial tension measurement results are intermediate with respect to the two sets of neutron reflectivity measurements.

An even larger discrepancy between neutron reflectivity and interfacial tension results can be observed in Figure 6.26 for C$_{12}$E$_6$ (however, the larger discrepancy could simply be attributed to the significant improvement in the error bars for the interfacial tension measurements). Interfacial tension measurements lead, as observed for C$_{12}$E$_4$, to lower values for $\Gamma$ than NR measurements relative to the deuterated surfactant. Unfortunately no results are available for h-C$_{12}$E$_6$.

Neutron reflectivity results for d-C$_{12}$E$_8$ are also in disagreement with interfacial tension results. However, interfacial tension data are comparable to neutron reflectivity results for h-C$_{12}$E$_8$ (Figure 6.27). A reasonable agreement is also observed between interfacial tension and neutron reflectivity for h-C$_{12}$E$_{12}$ (Figure 6.28).

### 6.6.5 Discrepancy between hydrogenous and deuterated surfactants: the influence of the diffuse layer

The adsorbed amount was determined using neutron reflectivity for both the hydrogenous and partially deuterated forms of C$_{12}$E$_4$ and C$_{12}$E$_8$ and the results are shown in Figure 6.25 and Figure 6.27. One striking feature is that there is significant disagreement between $\Gamma$ determined from the hydrogenous and partially deuterated surfactants.

For C$_{12}$E$_4$ there is a substantial decrease in the fitted layer thickness for both contrasts as the concentration decreases and the possibility of a structural change occurring in the adsorbed layer was suggested. At room temperature, C$_{12}$E$_4$ solutions in water form lamellar phases [23,24] and the appearance of the solutions is rather opaque. The increase in fitted layer thickness could then be attributed to the
formation of multi-layer structures at the oil-water interface. These structures tend to disappear with decreasing concentration, hence the decrease in fitted layer thickness.

A significant decrease in fitted layer thickness also occurs between the hydrogenous and deuterated C$_{12}$E$_8$ for all concentrations. It was discussed in the previous section how the interface can be divided into two regions: the first region, in contact with the oil phase, is composed of a staggered surfactant monolayer, while the second region represents a rather diffuse layer extending towards the aqueous phase. The described interfacial conformation seems to be supported by the reduction in thickness observed between the two contrasts for C$_{12}$E$_8$. When h-C$_{12}$E$_8$ is adsorbed at the interface the fitted layer is rather thin, suggesting that the neutron reflectivity technique is only sensitive to the primary monolayer in contact with the oil phase. On the other hand, the fitted layer thickness is much larger for d-C$_{12}$E$_8$, suggesting that the sensitivity is extended to the whole interfacial structure, including the diffuse layer. One possible explanation for the difference in sensitivity of the technique between the two contrasts can be found in the scattering length density of the tail groups between hydrogenous and deuterated surfactants. The $Nb$ of the surfactant tail groups can be calculated using the density of the whole surfactant and they are shown in Table 6.15 along with the $\Delta Nb$ between the fragments and the bulk phases. A 98% deuteration was considered for the calculations.

| Fragment    | $Nb$ ($\times 10^{-6}$ Å$^{-2}$) | $|\Delta Nb|$ ($\times 10^{-6}$ Å$^{-2}$) |
|-------------|----------------------------------|-------------------------------------|
| C$_{12}$H$_{25}$ | -0.46                           | 2.53                                |
| C$_{12}$D$_{25}$ | 8.15                            | 6.08                                |

The underlying structure is rather diffuse, the volume fraction of surfactant in the layer is very little and the surfactant molecules present a significant hydration shell: the combination of these factors leads to a very small $\Delta Nb$ between the layer and the bulk phases. In absence of fragments that scatter neutrons significantly, as in the case of the hydrogenous surfactants, very little scattering arises from the underlying
structure, leading to a strict sensitivity to the material adsorbed in the more compact primary monolayer. The deuterated surfactants, however, present tail groups with an exceptionally high $N_b$. Much more scattering is expected from the diffuse layer in presence of these fragments, thus the signal may arise not only from the material adsorbed in the primary monolayer, but also by the material in the nearest proximity, i.e. the underlying structure.

With regard to $C_{12}E_4$, the fitted layer thickness does not change between the h and d-surfactant, whereas the discrepancy in $\Gamma$ is significant ($\Gamma$ was lower for the hydrogenous surfactant than the partially deuterated). In the case of $C_{12}E_8$, on the other hand, the decrease in $\Gamma$ between the deuterated and hydrogenous surfactant was coincidental with a significant reduction in layer thickness, suggesting that the underlying structure was not at all detected.

The fitted layer thickness for d-$C_{12}E_6$ is comparable to that obtained for d-$C_{12}E_8$ and d-$C_{12}E_4$ (at low concentration), suggesting that the contrast is sensitive to the underlying structure. The fitted layer thickness for h-$C_{12}E_{12}$ is much thinner and comparable with h-$C_{12}E_8$ instead, suggesting a lack of sensitivity to the underlying structure for the hydrogenous surfactant as the size of the head group increases.

The question that must be addressed is whether the underlying structure participates in the interfacial activity or not, i.e. whether its presence perceptibly affects the interfacial tension of the system. One would expect that if the surfactant molecules in the layer are strongly bound to the interface, they would significantly affect the interfacial tension; on the other hand if they were loosely bound to the interface their effect would be negligible. Because of the nature of the adsorption process taking place at the interface (Gibbs adsorption), the adsorbed molecules are expected to continuously exchange with the solution. One would expect a molecule that exchanges rapidly with the bulk to interact weakly with the interface, hence to present lower surface activity.

$C_{12}E_4$ presents the lowest CMC in the series (Table 6.2). It is the most hydrophobic surfactant and presents the most pronounced surface activity. The interface reaches saturation at very low concentration and the surfactant molecules are strongly bound to the interface. Surfactant molecules in the underlying structure are expected to
exchange relatively slowly with the solution, because of more hydrophobicity of the molecule and low concentration in the bulk phase. This suggests that the underlying structure interacts rather strongly with the interface, noticeably affecting the interfacial tension of the system. \( \text{C}_{12}\text{E}_8 \), on the other hand, is less surface active compared to \( \text{C}_{12}\text{E}_4 \). The CMC is higher and, because of the bigger head group, the surfactant is more hydrophilic. This implies that the interaction between the molecules in the underlying structure and the interface are weaker compared to \( \text{C}_{12}\text{E}_4 \), hence they exchange faster and have little effect on the interfacial tension of the system.

If the proposed hypothesis is valid, most of the material in the underlying structure affects the interfacial tension of the system when the head group is small (\( \text{C}_{12}\text{E}_4 \)). As the size of the head group increases, the material in the underlying structure tends to progressively interact more and more weakly with the interface, consequently losing its surface activity. This hypothesis is consistent with the relationship between interfacial tension and neutron reflectivity measurements:

- For surfactants with a big head group (\( \text{C}_{12}\text{E}_8 \) and \( \text{C}_{12}\text{E}_{12} \)) the interfacial tension is mainly affected by the primary monolayer and the effect of the underlying structure is marginal. Hence the results from interfacial tension measurements should more closely correspond to neutron reflectivity results for hydrogenous surfactants. This can be seen in Figure 6.27 and Figure 6.28 relative to \( \text{C}_{12}\text{E}_8 \) and \( \text{C}_{12}\text{E}_{12} \): \( \Gamma \) from interfacial tension corresponds reasonably well with \( \Gamma \) as obtained from neutron reflectivity measurements of the hydrogenous surfactants.
- When the head group is small, i.e. \( \text{C}_{12}\text{E}_4 \), the underlying structure actively participates in lowering the interfacial tension at the oil-water interface. The stronger the interaction between the underlying structure and the primary monolayer, the more \( \Gamma \) as determined from interfacial tension will shift to higher values to match \( \Gamma \) determined from neutron reflectivity studies of the deuterated surfactant. As a result of this shift, \( \Gamma \) as determined from interfacial tension would now be intermediate between the values obtained with neutron reflectivity for the hydrogenous and partially deuterated surfactant (Figure 6.25).
6.6.6 Estimated adsorbed amount for h-C_{12}E_6

The adsorbed amount determined from neutron reflectivity from h-surfactants with \( n \geq 8 \) has proved to be in good agreement with interfacial tension data. For \( n = 4 \), interfacial tension data seem to lead to intermediate results between neutron reflectivity for the two contrasts. Since the underlying structure shows distinct surface activity for C_{12}E_4 but has little or no effect for C_{12}E_8, the behaviour of C_{12}E_6 becomes of particular interest. Unfortunately, as already mentioned, neutron reflectivity data regarding h-C_{12}E_6 could not be recorded. We have here tentatively tried to extrapolate \( \Gamma \) at the CMC for the hydrogenous C_{12}E_6 from the neutron reflectivity results relative to the other h-surfactants of the C_{12}E_n series.

\( \Gamma \) at the CMC for h-surfactant as measured from neutron reflectivity is shown in Figure 6.29 by the filled black circles. \( \Gamma \) expressed in mg m\(^{-2}\) is also shown in Figure 6.29 insert.

![Figure 6.29](image)

Figure 6.29. Adsorbed amount at the CMC as determined from neutron reflectivity for the hydrogenous surfactant is shown by the black circles. The highlighted red circle corresponds to the extrapolated adsorbed amount for the fully hydrogenous C_{12}E_6. The same notation is used in the figure insert where the adsorbed amount is shown in mg m\(^{-2}\) units: the size of the error bar is such that the gap measured in \( \Gamma \) between C_{12}E_4 and C_{12}E_8 is fully covered.
There is little change in $\Gamma$ at the CMC when the size of the head group decreases from 12 to 8 units, whereas $\Gamma$ increases significantly for C$_{12}$E$_4$. The extrapolated $\Gamma$ for h-C$_{12}$E$_6$ must take into account the fact that it is not possible to speculate whether the change in $\Gamma$ at the CMC between C$_{12}$E$_4$ and C$_{12}$E$_8$ is gradual or quite abrupt. Therefore the theoretical $\Gamma$ must present marked error bars to account for the lack of knowledge regarding the system. The theoretical $\Gamma$ is highlighted in Figure 6.29 (mol m$^{-2}$) and Figure 6.30 insert (mg m$^{-2}$). The calculated $\Gamma$ at the CMC for h-C$_{12}$E$_6$ is shown in the shaded area in Figure 6.30 along with neutron reflectivity results for d-C$_{12}$E$_6$ and interfacial tension results.

![Graph showing adsorbed amount vs concentration](image)

**Figure 6.30.** Adsorbed amount as determined from neutron reflectivity for the deuterated C$_{12}$E$_6$ (open circles) and interfacial tension (dashed lines). The extrapolated value for $\Gamma$ at the CMC for the fully hydrogenous surfactant is shown by the shaded area. Although there does not seem to be a perfect match, there is clearly some degree of overlap between the interfacial tension measurement and the extrapolated $\Gamma$ for h-C$_{12}$E$_6$.

Despite the range of $\Gamma$ being relatively broad because of the high uncertainty, there is some correlation between the interfacial tension results and the estimated values for h-C$_{12}$E$_6$. This observation would place the interfacial behaviour of C$_{12}$E$_6$ in an
intermediate situation between C\textsubscript{12}E\textsubscript{4} and C\textsubscript{12}E\textsubscript{8}. Unlike C\textsubscript{12}E\textsubscript{8}, the underlying structure has some effect on the interfacial tension of the system; hence the non-perfect correlation between interfacial tension results and neutron reflectivity predictions for the hydrogenous surfactant. On the other hand, the significant deviation between interfacial tension and neutron reflectivity results regarding the d-C\textsubscript{12}E\textsubscript{6} suggest that the effect of the underlying structure on the interfacial tension is much smaller compared to C\textsubscript{12}E\textsubscript{4}.

6.7 Conclusions and future work

The conformation of C\textsubscript{12}E\textsubscript{n} surfactants at the oil-water interface was studied as a function of increasing head group size. It was found that the interface can be divided into two regions: a rough, staggered monolayer in contact with the oil phase and a diffuse region, extending towards the aqueous phase. The conformation of the head group significantly changes compared to the air-water interface, moving from a globular to an almost fully extended conformation. We believe that an important role in the stretching of the head groups should be attributed to the presence of hexadecane molecules in this region. The presence of oil molecules in the head group region also seems to be responsible for the existence of the diffuse underlying structure, which was not observed at the air-water interface. The combination of neutron reflectivity and interfacial tension measurements suggest that the interaction between the primary monolayer and the diffuse region increases in strength as the size of the head group decreases. This is intuitive as the molecules become progressively more hydrophobic and surface active.

It was believed that the interfacial roughening observed at the oil-water interface for ionic and zwitterionic surfactants was mainly caused by the necessity to accommodate the charges in the head groups. We have shown in this chapter that a broader and rougher adsorbed layer is also observed for C\textsubscript{12}E\textsubscript{n} surfactants at the oil-water interface. Since these surfactants do not carry any charges in the head group, the broadening of the interface must be attributed to the presence of the oil phase, which efficiently solvates the adsorbed layer.
To confirm these results, we have very recently studied the conformation of $C_{12}$PC surfactants at the hexadecane-water interface as a function of concentration above and below the CMC. The conformation of the longer alkyl chain analogue $C_{16}$PC is known [3]: because of the shorter chain, the interaction between such compounds and the oil phase may change considerably, thus affecting significantly the structure of the adsorbed layer. To this date, the results are largely incomplete and are not discussed in this dissertation.
6.8 References for Chapter 6

Chapter 7

Lipid monolayer at the oil-water interface

7.1 Introduction

A biological membrane acts as a barrier separating the inner part of a cell or an organelle from the surroundings. By partitioning the enclosed space, membranes usually maintain a chemical environment that significantly differs from the outside. In order to understand important cell processes and functions, it is important to resolve the structure at the membrane interface with its surrounding environment. The structure of the cell membrane was described for the first time in 1972 by Singer and Nicholson [1] when they found that proteins are embedded in a phospholipid bilayer, which consists of two weakly coupled monolayers. Proteins are a significant component of membranes (up to 50% of the membrane weight) and they are essential for a series of processes such as signal transduction and selective transport across the membrane. Nonetheless, it is the phospholipid bilayer that imparts stability and integrity to the membrane. Studying the interaction between biologically active compounds with membranes at molecular scale has been found to be very difficult because of the extreme complexity of membrane structure. Therefore, model systems have been developed for studying a variety of aspects such as dynamics, structure and interaction of biological membranes [2]. The most commonly deployed model systems are the solid-supported bilayers and lipid monolayers at the air-water interface.
7.1.1 Solid-supported lipid bilayers

These systems are deployed when a close mimic to a real biological membrane is required, i.e. to study interaction mechanisms responsible for the function and the resultant conformation of real membranes. The simplified and targeted composition of an artificial bilayer facilitates the study of processes involving the membrane. Many model lipid bilayer systems have been developed during the second part of 20th century, such as black lipid membranes [3] or vesicles. Because of their limited size or curvature, these systems are not suitable for probing interfacial phenomena using surface characterisation techniques such as neutron reflectivity and will not be further discussed in this dissertation.

Lipid bilayers deposited onto solid substrates have been extensively used as membrane models [4] and these have been successfully used for neutron reflectivity experiments [5,6]. Over the years many approaches have been adopted to produce solid-supported lipid bilayers, such as self-assembled tethered bilayers [7] or fusion of large unilamellar vesicles on a hydrophobic monolayer [8]. Although all these approaches lead to the formation of lipid bilayers with good reproducibility, they suffer from a common problem, namely lack of conformational freedom. The monolayer adjacent to the solid substrate is either covalently bound (chemisorbed) or strongly adsorbed (physisorbed) to the solid surface, thus limiting the molecules’ freedom to move in the plane of the membrane.

Adsorption of small unilamellar vesicles was found to produce more fluid like solid-supported lipid bilayers both at the quartz-water [9] and at the silicon-water interface [10]. It was shown that fluidity was conferred to the system by a thin layer of water trapped between the solid substrate and the lipid bilayer. These models proved to be good systems for the study of peripheral proteins and adsorption of molecules at the membrane surface [11]. However, this model is not very suitable for analysing the incorporation of integral membrane proteins as the water layer separating the solid substrate and the bilayer is too thin and it does not allow accommodation of the internal protein domains. Also, the lack of space between the deposited bilayer and the solid substrate makes this system impracticable to study diffusion processes.
Preferably, to meet the ideal requirements, a membrane model should fluctuate in the water phase at a known distance from the solid support, be highly hydrated and stable over time and resistant to vibrations. Additional features should include large sizes at low costs, controlled structures and the possibility of asymmetric lipid compositions. All these requirements are met by the so-called “floating bilayer”. The idea of the floating bilayer comes from the observation that lipid multilayers deposited on a solid substrate are suitable model membranes [12] and generally gaps of approximately 30 Å are left between each bilayer [13]. The floating bilayer is a simplification of these multilayer systems: it is composed of only two bilayers that are deposited onto a solid substrate as individual monolayers using a combination of Langmuir-Blodgett (for the first three layers) [4] and Langmuir-Schaefer techniques (last monolayer) [14]. In these systems, schematically shown in Figure 7.1, the second bilayer (the one in contact with the water phase) floats above the first one, which is in contact with the solid substrate [15].

**Figure 7.1.** Schematic representation of a floating bilayer system. The first three lipid monolayers (red) are deposited using Langmuir-Blodgett technique; the fourth monolayer (blue) is deposited using Langmuir-Schaefer technique. The composition of the fourth monolayer can be changed according to one’s needs.

Structural analysis using neutron reflectivity has shown that these systems are significantly stable over time [16]. An important aspect is that the separation between the two bilayers and the roughness of the floating bilayer can be controlled.
by varying the temperature of the system. Also, because of its nature, the floating bilayer provides different variable parameters that can be adjusted according to the experimental requirements (temperature, scattering length density of the water phase and the possibility of changing the composition of the individual lipid monolayers) [17]. The main disadvantage of these systems is that the deposition of the second bilayer is only possible for selected lipids, in particular for those that are in the gel phase above room temperature [18]. This problem was efficiently solved by eliminating the need of the first bilayer in contact with the solid substrate. This was achieved by functionalising the solid surface with a monolayer of octadecyltrichlorosilane (OTS), deposited by means of silanisation [19]. This grafted OTS layer substitutes the first monolayer (the one in contact with the solid substrate), whereas the same procedure as before is used to deposit the other three monolayers. It was found that the OTS layer was strongly interpenetrated with the first deposited monolayer and the quality of such interpenetration was essential for the stability of the whole floating bilayer structure. The strong dependency on the interpenetration quality was solved by replacing the whole first bilayer with a chemically grafted phosphatidyl-choline layer [20]. This approach was demonstrated to be efficient in solving the problem of interdigitation, preserving a membrane-like structure that maintains the floating bilayer in position. This approach not only leads to a structure that allows choosing between different lipids, but also, because of the first monolayer being chemically bound to the solid substrate, significantly speeds up the layer deposition process.

The observation that cationic lipid-DNA complexes (lipoplexes) readily interact with biological membranes [21] gave the opportunity to test the effectiveness of different floating bilayers as membrane-mimicking systems [22]. Whereas earlier versions of floating bilayers were either destroyed or underwent major structural changes (unreasonable roughening of the floating bilayer was observed, explained as undulations), floating bilayers deposited onto chemically grafted silicon substrates were found to be the most stable on exposure to lipoplexes. The overall conclusion was that floating bilayers deposited onto chemically grafted supports are extremely stable and can be considered as excellent models for biological membrane systems.
7.1.2 Lipid monolayers at the air-water interface

Floating bilayers are very complex systems. The preparation and the characterisation of the two bilayers prior to a neutron reflectivity experiment could be time consuming. Furthermore, the complexity of the structure could reduce the sensitivity of neutron reflectivity to small changes in the system. When studying adsorption processes that only involve the surface of the membrane, it may be convenient to use much simpler models. Phospholipid monolayers deposited at the air-water interface have been deployed as models for biological membranes for decades [23] and they are still used as simple model systems. Recently they have been used to study interaction between membranes and a variety of compounds such as proteins [24], antineoplastic drugs [25] or fluorinated compounds [26].

7.1.2.1 General pressure-area isotherm of lipids

Lipids are amphiphilic in nature and can be spread at the air-water interface from a volatile solvent such as chloroform. The polar head groups are found immersed in the aqueous phase, with the hydrophobic tail groups in contact with air; thus presenting a conformation comparable to a surfactant monolayer. A lipid monolayer closely mimics one half of the lipid membrane, and a molecule approaching the interface from the aqueous phase would interact with a surface resembling the surface of a membrane. Lipid monolayers at the air-water interface allow the study of interactions with virtually unlimited water-soluble molecules that are known to adsorb onto the membrane surface.

The general surface pressure-area isotherm of lipid molecules at the air-water interface as a function of surface pressure is well known and it shows features which clearly indicate phase transitions [27]. By spreading a known amount of lipid at the air-water interface and adjusting the surface pressure, the area per molecule of individual molecules can be monitored. Therefore it is possible to study lipid monolayers in different phases by just adjusting the surface pressure. A typical pressure-area isotherm for a lipid monolayer is shown in Figure 7.2, indicating the formation of several lipid phases. Understanding the conformation of a lipid monolayer as a function of surface pressure is of upmost importance when proposing
such systems as membrane models. The structure of the monolayer as a function of increasing surface pressure has been characterised using, amongst other techniques, neutron reflectivity, x-ray reflectivity and in-plane x-ray diffraction. The main structural details of a lipid monolayer will be briefly discussed as a function of increasing surface pressure.

![Graph showing pressure-area isotherm with phases](image)

**Figure 7.2. Typical pressure-area isotherm for a lipid monolayer as a function of increasing surface pressure.**

### 7.1.2.2 Gaseous phase

At very low surface pressure, \( \Pi \ll 1 \text{ mN m}^{-1} \), the spread monolayer is in the so-called gaseous (G) phase (Figure 7.3).

![Schematic of gaseous phase](image)

**Figure 7.3. Schematic representation of a lipid monolayer at the air-water interface in the gaseous phase.**

Under these conditions there is very little interaction between lipid molecules, and they lie almost flat on the water surface. Neutron reflectivity was used by Naumann
et al., to study a monolayer of di-palmitoyl phosphatidyl-choline (DPPC) at surface pressure $\Pi = 1.4 \text{ mN m}^{-1}$ [28]. Their results are consistent with a very disordered monolayer: the scattering length density of the adsorbed layer was found to be similar in both the hydrophobic and hydrophilic part of the monolayer, with significant hydration of the DPPC molecule. X-ray diffraction measurements on a di-myristoyl-phosphatidyl-ethanolamine (DMPE) monolayer were performed by Helm et al., [29] and the results confirmed significant disorder within the monolayer. As the requirements to mimic part of a biological membrane are clearly not required under these conditions, the behaviour of a lipid monolayer in the gaseous phase is not further discussed in this dissertation.

### 7.1.2.3 Liquid expanded phase

As the surface pressure of the lipid monolayer is increased, tail-tail interactions between different lipid molecules start taking place and the hydrophobic tail groups start to begin organising themselves perpendicularly to the surface. This is the so-called liquid expanded (LE) phase and it is schematically shown in Figure 7.4. Brumm et al., [30] analysed a monolayer of DPPC using neutron reflectivity. Measurements were taken at a fixed area per molecule of $\sim 95 \text{ Å}^2$, corresponding to a surface pressure of about 5 mN m$^{-1}$. They observed a layer thickness for the tail group region significantly lower than that of the extended molecule ($13.5 \pm 0.5 \text{ Å}$ as opposed to $\sim 21 \text{ Å}$ for the corresponded fully extended acyl group). This reduction can be explained by the acyl groups assuming a tilted conformation with respect to the interface. The change in the layer thickness is consistent with an angle of $\sim 40^\circ$ for the tail groups. Although in this phase it is believed that some degree of conformational order within the monolayer exists, in plane x-ray diffraction indicates a lack of major tail group orientation (data refer to a DMPE monolayer) [29].
Reflectivity profiles obtained from selectively deuterated DPPC molecules at the air-water interface were analysed to examine the conformation of the lipid head group [30]. The data suggest that the head group lies almost flat and mostly immersed in the water phase, hence significant hydration of lipid molecules as evaluated from the number of water molecules associated with each lipid molecule. Similar results were obtained for mixtures of di-myristoyl-phosphatidyl-choline (DMPC) and di-myristoyl-phosphatidyl-glycerol (DMPG) [31]. The considerable amount of water in the head group region suggested that the glycerol backbone was also significantly hydrated.

### 7.1.2.4 Liquid condensed phase

Upon further compression of the monolayer, the liquid condensed (LC) phase is reached (Figure 7.5). The area per molecule of the lipid considerably reduces and significant order appears in the tail group region. The increase in orientation is also suggested by x-ray in-plane diffraction for a DMPE monolayer [29].
7.1.2.5 Solid condensed phase

When approaching surface pressure over 30 mN m\(^{-1}\), any small changes in surface area are accompanied by large increases in surface pressure. This indicates that the solid condensed (S) phase has been reached (Figure 7.6).
There is further increase in the degree of ordering of the tail group region as this phase is reached [29], although a perfect ordering cannot be achieved because of inevitable annealing defects [32]. Neutron reflectivity data for a DPPC monolayer [30] confirm the further increase in the thickness of the head group region. The thickness increases up to 11.5 Å, which is slightly more than the length of the fully extended head group ($d_{hg} \sim 10.5$ Å) [33]. This small discrepancy could be explained if one includes the glycerol backbone in the head group region or considers a slightly staggered monolayer conformation. The increase in thickness in the head group region upon compression was attributed to the interactions between charges in the head groups. DPPC is a zwitterionic molecule carrying both a positive and a negative charge in the head group: at high surface pressure, when hydration in the head group is significantly decreased [34], the most stable conformation to accommodate such charges seems to be describable with head groups lying almost perpendicular to the surface. Hollinshead et al., [35] confirmed the vertical head group orientation in a distearoyl-phosphatidyl-choline (DSPC) at the air-water interface. Using several contrasts, they determined the partial structure of DSPC as a function of surface pressure. Their results suggested a slightly staggered conformation of the lipid molecules at high surface pressure, as observed from water penetration through the monolayer. The staggered conformation seems to be the most likely one for these lipids as it would help the accommodation of the charges in the head group.

### 7.2 Lipid multilayers at the oil-water interface

When lipids are adsorbed at the oil-water interface from bulk phases, they have the tendency to self assemble into bilayers separated by thin water layers [36]. Events such as thermally-induced waves or the application of electric fields across the interface destabilise the multilayer structure. Defects are then generated in the bilayers which eventually lead to their disintegration and dispersion in the oil phase as water-in-oil emulsions or reversed micelles. Both spherical and rod-like lecithin micelles have been reported in non-polar solvents, rod-like micelles being more stable at higher concentration [37]. The core of the micelles is very hydrophilic and is subject to significant hydration. It is believed that water transfers into the non-
polar solvent through hydration of the adsorbed lipid multilayer [38], triggering a series of phase transitions that, depending on the solvent, may lead to a marked increase in viscosity of the hydrophobic phase [39,40,41]. More recently, the spontaneous emulsification at a lipid-stabilised oil-water interface was reported [42] and was also attributed to the hydration of the adsorbed lipid multilayer.

7.2.1 Lipid monolayer at the oil-water interface as possible improved model for biological membranes

In lipid monolayers formed at the air-water interface the tail group region is in contact with air, which acts as the hydrophobic medium. As opposed to that, in a real membrane tail-to-tail entanglement occurs, hence the tail group region is in a much more hydrophobic environment and is in partially solvated conditions. We believe that the substitution of the air phase with a hydrophobic phase such as hexadecane would provide a more realistic model system. The oil-water interface would then represent a simple model of the membrane surface separating the aqueous compartment from the hydrophobic central region of the lipid bilayer [43].

Here is reported for the first time using neutron reflectivity the structure of a phospholipid, 1,2-di-stearoyl-sn-glycero-3-phosphocholine (DSPC), monolayer at the oil (hexadecane)-water interface. As DSPC is insoluble in both the oil and the water phase, the conformation of the lipid layer cannot be analysed as a function of concentration as was done for C_{12}E_{n} surfactants. Hence for the preparation of the sample a methodology that was recently deployed by our group was deployed [44]. Details are given in the experimental section. The principal objectives of this experiment were to measure the adsorbed amount as a function of increasing spread amount and to examine how the related interfacial phospholipid layer structure varies with concentration.
7.3 Neutron reflectivity experiment

7.3.1 Materials and methods

DSPC, available as fully hydrogenous (h) and with the two stearoyl chains deuterated (d$_{70}$) forms, was obtained from Avanti Polar Lipids. Neutron reflectivity measurements were carried out at ISIS using the SURF reflectometer (see section 2.4.3.1). The reflectivity profiles were measured at an incident angle of 1.5° to provide the most suitable Q-range possible and the highest sensitivity to interfacial structure. The hexadecane film was deposited as described in Chapter 5, was frozen in place and kept frozen for the cell-assembling procedure. In the current experiment, the water sub-phase was placed in the trough forming a meniscus and its surface was cleaned by vacuum suction. Before assembly of the cell, the level of the water surface was lowered by syringing through plug valves, until it was just lower than the O-ring seal. A known amount of DSPC was deposited on the bulk aqueous surface from a chloroform solution. After spreading, the solvent was allowed to evaporate (~15 minutes) prior to the introduction of the oil phase. Once the sample chamber was deemed to be bubble-free, the oil film was allowed to melt and the reflectivity profile was recorded.

When chain deuterated d-DSPC was studied, the hexadecane oil and the aqueous sub-phase were both contrast-matched to silicon ($N_b = 2.07 \times 10^{-6}$ Å$^{-2}$). A second contrast was also examined using h-DSPC lipid in which the oil phase was again contrast-matched to the silicon and D$_2$O was used as the aqueous sub-phase.

7.3.2 Results

In order to deduce the conformation of DSPC uniquely, two sets of contrasts were measured and the resulting reflectivity data fitted simultaneously to a consistent model. For the first contrast, a series of reflectivity profiles were measured as a function of spread amount of d-DSPC, with both the oil and water scattering length density being matched to that of the silicon. The reflectivity profiles as a function of
For the four lipid spread amounts used are shown in Figure 7.7. Note that the reflectivity profiles obtained for when the d-DSPC concentrations were $7.47 \times 10^{-6}$ and $9.96 \times 10^{-6}$ mol m$^{-2}$ overlay each other. The reflectivity from a bare oil-water interface was first measured as a reference benchmark and is also shown in Figure 7.7. The reflectivity data were first fitted to a single layer model of thickness $d = 38 \pm 2$ Å as the first step in the data analysis. No interlayer roughness was used to fit the data. This approach, discussed in Chapter 6 for C$_{12}$E$_n$ surfactants, is generally used to obtain the adsorbed amount $\Gamma$ at the interface adopting neutron reflectivity [45]. The fits are shown by the solid lines in Figure 7.7 and the fitted parameters are given in Table 7.1.

![Figure 7.7](image-url)

**Figure 7.7.** Reflectivity profiles for the system Si–CMSi hexadecane-CMSi water for a series of d-DSPC lipid spread amount and fitted using single layer fits. The solid lines are fits to the data. Figure insert shows the fit to the same set of reflectivity profiles adopting the multilayer model discussed later in this section.

**Table 7.1.** Parameters used for one layer fit (Figure 7.7) for contrast 1 with a layer thickness of 38 ± 2 Å.

<table>
<thead>
<tr>
<th>d-DSPC Spread amount $\times 10^{-6}$/ mol m$^{-2}$</th>
<th>1.87</th>
<th>5.60</th>
<th>7.47</th>
<th>9.96</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Nb \times 10^{-6}$/ Å$^{-2}$</td>
<td>2.67</td>
<td>2.91</td>
<td>3.40</td>
<td>3.40</td>
</tr>
</tbody>
</table>
As the reflectivity profiles for the higher lipid concentrations of $9.96 \times 10^{-6}$ and $7.47 \times 10^{-6}$ mol m$^{-2}$ were very similar (within error), one model could be used to represent both sets of data. The fitted layer thickness ($d$) and the scattering length density were used to estimate the adsorbed amount ($\Gamma$) of lipid and the area per lipid molecule ($A_{pm}$) as described in Chapter 6.

The area per lipid molecule as a function of spread amount was calculated from the single layer fit to the reflectivity data for the first contrast, and is shown in Figure 7.8.

![Figure 7.8](image)

**Figure 7.8. Area per molecule determined from a one layer fit to the data versus the spread amount.**

For the second contrast, a series of reflectivity profiles were measured for the h-DSPC lipid as a function of spread amount, with the oil contrast matched to silicon and D$_2$O as the aqueous sub-phase. The nature of model fitting of the neutron reflectivity data necessitates the finding of the simplest model (i.e. the model with the minimum number of layers) to represent the data. We found that at the lowest spread amount ($1.87 \times 10^{-6}$ mol m$^{-2}$) the reflectivity profiles for both contrasts could adequately be represented by a two layer model. Conversely, the reflectivity profiles obtained for the second contrast at higher spread amounts required a three layer
model to obtain a reasonable representation of the data sets. This result suggests the possibility of a conformational change of the lipid molecules at the buried interface as the amount of spread lipid increases. The reflectivity profiles for the second contrast (h-DSPC) are shown in Figure 7.9, solid lines correspond to the modelled reflectivities.

\[ \text{Reflectivity} \]

\[ Q / \text{Å}^{-1} \]

\[ 10^{-1} \]
\[ 10^{-2} \]
\[ 10^{-3} \]
\[ 10^{-4} \]
\[ 10^{-5} \]
\[ 10^{-6} \]
\[ 10^{-7} \]

\[ \diamond \ 7.47 \times 10^{-6} \text{ mol m}^{-2} \]
\[ \diamond \ 3.73 \times 10^{-6} \text{ mol m}^{-2} \]
\[ \triangle \ 1.87 \times 10^{-6} \text{ mol m}^{-2} \]

Figure 7.9. Reflectivity profiles from the Si–hexadecane contrast matched Si-D$_2$O for a series of h-DSPC lipid spread amount. The solid lines are fits to the data. The profiles are shifted by a factor $\times 10$ for the purpose of clarity.

This multilayer model could also be used to represent the data obtained for the first contrast (d-DSPC at the CMSi oil-CMSi water interface). All interlayer roughness in modelling the reflectivity profiles for both contrasts could be set to zero; however a step-like interface is not a realistic representation of the actual interface and a roughness of 2 Å was used to smooth the $Nb$ changes moving from one layer to the next. The multi-layer fits to the first contrast are shown in the insert of Figure 7.7. The fitted parameters obtained for both contrasts are given in Table 7.2. The scattering length density profiles for these fits are shown in Figure 7.10.
Table 7.2. Parameters used for multilayer fits to both contrast. The calculated fits are shown by the solid lines in Figure 7.7 (insert) and Figure 7.9.

Contrast 1: CM Si oil / d-DSPC / CM Si water, lowest spread amount

<table>
<thead>
<tr>
<th>d-DSPC mol m$^{-2}$</th>
<th>1.87 $\times$ 10$^{-6}$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ / Å (± 1)</td>
<td>$Nb$ $\times$ 10$^{-6}$ / Å$^{-2}$</td>
<td>Roughness / Å</td>
</tr>
<tr>
<td>55.0</td>
<td>2.59</td>
<td>2.0</td>
</tr>
<tr>
<td>15.0</td>
<td>1.78</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Contrast 1: CM Si oil / d-DSPC / CM Si water, higher spread amount

<table>
<thead>
<tr>
<th>d-DSPC mol m$^{-2}$</th>
<th>9.96 $\times$ 10$^{-6}$</th>
<th>7.47 $\times$ 10$^{-6}$</th>
<th>5.60 $\times$ 10$^{-6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ / Å (± 1)</td>
<td>$Nb$ $\times$ 10$^{-6}$ / Å$^{-2}$</td>
<td>Roughness / Å</td>
<td></td>
</tr>
<tr>
<td>35.0</td>
<td>2.61</td>
<td>2.61</td>
<td>2.36</td>
</tr>
<tr>
<td>20.0</td>
<td>4.31</td>
<td>4.31</td>
<td>3.55</td>
</tr>
<tr>
<td>15.0</td>
<td>1.72</td>
<td>1.72</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Contrast 2: CM Si oil / h-DSPC / D$_2$O, lowest spread amount

<table>
<thead>
<tr>
<th>h-DSPC mol m$^{-2}$</th>
<th>1.87 $\times$ 10$^{-6}$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ / Å (± 1)</td>
<td>$Nb$ $\times$ 10$^{-6}$ / Å$^{-2}$</td>
<td>Roughness / Å</td>
</tr>
<tr>
<td>55.0</td>
<td>2.62</td>
<td>2.0</td>
</tr>
<tr>
<td>15.0</td>
<td>4.90</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Contrast 2: CM Si oil / h-DSPC / D$_2$O, higher spread amount

<table>
<thead>
<tr>
<th>h-DSPC mol m$^{-2}$</th>
<th>7.47 $\times$ 10$^{-6}$</th>
<th>3.73 $\times$ 10$^{-6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ / Å (± 1)</td>
<td>$Nb$ $\times$ 10$^{-6}$ / Å$^{-2}$</td>
<td>Roughness / Å</td>
</tr>
<tr>
<td>35.0</td>
<td>2.90</td>
<td>2.80</td>
</tr>
<tr>
<td>20.0</td>
<td>1.75</td>
<td>2.56</td>
</tr>
<tr>
<td>15.0</td>
<td>4.60</td>
<td>4.82</td>
</tr>
</tbody>
</table>
Figure 7.10. Scattering length density profiles calculated from the fitted reflectivity profiles to both contrasts shown in Figure 7.7 (insert) and Figure 7.9.

The total layer thickness for these multilayer fits is about 70 Å, which is much larger than a fully extended DSPC molecule (~ 35 Å). This is indicative of rough molecular packing at the oil-water interface, in agreement with previous observations for surfactants at these buried interfaces. This roughened interface is suggested to be because of the solvation effect of the hydrocarbon tails and the resultant hydrophobic interactions. In addition, a minor contribution may originate from the accommodation of the charges in the head groups. The $N_b$ profiles for both contrasts were used to deduce the volume fraction profiles for all the three components of the interface, namely the oil, lipid and water, as follows. If each layer is solely composed of oil, water and lipid, the $N_b$ of the layer can be expressed as:

$$N_{b_{layer}} = N_{b_{oil}}\phi_{oil} + N_{b_{water}}\phi_{water} + N_{b_{lipid}}\phi_{lipid} \quad \text{Equation 7.1}$$

Where $\phi_i$ is the volume fraction of the specie $i$ and the sum of the volume fractions for the three species is 1:
\[ \sum_{i} \Phi_i = 1 \]  

Equation 7.2

The \( N_b \) profiles for both contrasts are then used, applying Equation 2, to estimate the volume fraction profiles for all the three components of the interface, namely the oil, lipid and water. These volume fraction profiles are shown in Figure 7.11.

![Figure 7.11](image_url)

Figure 7.11. Volume fraction profiles of the lipid distribution for two spread amounts (\( 7.47 \times 10^{-6} \) and \( 1.87 \times 10^{-6} \) mol m\(^{-2} \)) deduced from the two contrasts. The short-dash represents the lipid volume fraction profile, the solid line is the water and the long-dash is the oil.
7.3.3 Discussion

The scattering length density profiles obtained from the model fittings of all the reflectivity profiles are shown in Figure 7.10. The profiles for the higher spread amounts of h-DSPC with the D$_2$O forming the aqueous sub-phase are shown in Figure 7.10 (c). Regions (i) and (ii) of these profiles correspond, respectively, to the head group region and the tail group of the primary monolayer. The decrease in the $Nb$ in both regions as the spread amount increases indicates a combination of an increase in the lipid content coupled with an exclusion of D$_2$O from the layer. This is a consequence of an increased packing density of the lipid monolayer in this region. However a complete coverage was not achieved. On the contrary, the $Nb$ of region (iii) of the profile, in the vicinity of the oil phase, increases with increased spread amount, indicating the inclusion of D$_2$O. Such inclusion could indicate the formation of micelle-like aggregates: as more material is spread at the interface, more aggregates are formed in this region and the amount of water associated with the head groups also increases. The aggregates observed on the oil side of the interface could originate from spontaneous emulsification process [42]. However, because of the limited amount of lipid at the interface, the formation of aggregates seems more favourable than the formation of water-in-oil emulsion droplets.

There is an overall increase in the adsorbed amount in all three regions and this is also confirmed by the scattering length density profiles for the first contrast, shown in Figure 7.10 (a). Note that while the $Nb$ increases with increasing spread amount in regions (ii) and (iii), the trend is opposite in region (i) and $Nb$ decreases with increasing spread amount. The reason for this has to be found in the composition of region (i). Here, the $Nb$ is lower than that of silicon for all spread amounts, suggesting that only the non-deuterated DSPC head groups, whose $Nb$ is lower than that of silicon ($Nb_{hg} \sim 1.0 \times 10^{-6} \text{ Å}^{-2}$, $Nb_{Si} = 2.07 \times 10^{-6} \text{ Å}^{-2}$), are present in this region. We have shown in Chapter 6 that the volume fraction $\Phi$, hence the adsorbed amount $\Gamma$, is a function of $\Delta Nb$; therefore a decrease in $Nb_{layer}$ as a function of spread amount for layers with $Nb_{layer} < Nb_{Si}$ leads to an increase in $\Gamma$.

A similar trend in the scattering length density profile is also observed for the lowest spread amount (Figure 7.10 (b) and (d)). Again both these two sets of contrasts are fitted simultaneously to the same model.
Figure 7.12. Schematic representation of the conformation of DSPC at low (left) and high (right) spread amounts. A change in conformation is observed as the spread amount increases.

The overall results are shown schematically in Figure 7.12. The reflectivity data at low spread amount \((1.87 \times 10^{-6} \text{ mol m}^{-2})\) indicate a monolayer conformation of the lipid molecules at the interface. This monolayer is represented by two layers (Figure 7.10 (b) and (d)). The data further suggest that the monolayer is relatively rough with a staggered conformation. The first layer in contact with water is mainly composed of DSPC head groups and water. The second layer is a mixture of lipid, hexadecane and water. The thickness of the second layer is 55 Å, which is more than twice the fully extended stearoyl chain; hence some hydrated lipid head groups must also be included in the composition of this layer. The layer appears to be disordered with a significant amount of water penetration. This water inclusion in the hydrophobic side of the interface has been already observed adopting neutron reflectivity at the oil-water interface [46] as well as for phospholipid at the air-water interface [35].

As the spread amount increases, a different conformation of lipids at the oil-water interface is observed and the reflectivity profiles cannot be represented by a two layer model. The scattering length density profiles suggest that as a small volume of lipid solution is spread on the water surface, a monolayer is formed at the interface as was implied from the lowest spread amount data. As more and more of the
solution is spread subsequently on the top of this monolayer, the lipid molecules come into contact with a more hydrophobic environment composing of the lipid tail groups of the already formed monolayer. The spread lipids are now exposed to very little water and as a result there is a change in conformation of the newly spread lipids. Hence we move from a roughened monolayer to a monolayer plus lipid micelle-like aggregates. These aggregates reside on the oil side of the interface in the vicinity of the monolayer.

Because the nature of model fitting requires the simplest model, the structure of the lipid at the lowest spread amount \((1.87 \times 10^{-6} \text{ mol m}^{-2})\) has been discussed in terms of a two layer model. Yet one should keep in mind that if a reflectivity profile can be adequately represented by a two layer model, it is likely it could be also represented by a three layer model. Therefore it was tried to fit the reflectivity profiles for the lowest spread amount with the three layer model used to represent the profiles obtained for the higher spread amounts. As expected, the three layer model could adequately represent the reflectivity profiles. No changes were observed in the quality of the fitting, therefore the fits to the data are not shown here; however it is worth briefly discussing the newly derived scattering length density profiles, which are shown in Figure 7.13 along with the profiles for higher spread amounts.
Figure 7.13. Scattering length density profiles for Contrast 1 (a) and Contrast 2 (b) using the same model for all spread amounts. The solid lines represent the new profiles for lowest spread amount ($1.87 \times 10^{-6}$ mol m$^{-2}$), the other profiles are the same as in Figure 7.10 (a) and (c).

In Figure 7.13 (b), the increase in $Nb$ in regions (i) and (ii) as the spread amount decreases is confirmed for the lowest spread amount when the profiles are examined adopting the same model. The decrease in $Nb$ observed in region (iii) with
decreasing spread amount, interpreted as the presence of hydration water in reversed micelle-like aggregates residing in the oil phase next to the monolayer, is again confirmed for the lowest spread amount. When the three layer model is transferred to the first contrast (Figure 7.13 (a)) it can be seen that, as for the higher spread amounts, much of the material at the interface resides in region (ii), where the tail-to-tail entanglement between the lipid monolayer and the micelle-like aggregates takes place. As a consequence, the model proposed in Figure 7.12 for the higher spread amounts may also apply to the lowest spread amount. Although the two layer model adopted is technically the most appropriate to describe the structure of the monolayer at low spread amount, with the contrasts used we are not able to exclude the second possibility.

The Nb profiles for the two contrasts shown in Figure 7.10 were used to calculate the volume fraction (Φ) for the individual components (DSPC, water and hexadecane). This is a very simple estimation of the composition of each region of the interfacial layer. The volume fraction profiles for two spread amount values are shown in Figure 7.11. These volume fraction profiles suggest that the most of the lipid resides primarily on the aqueous side of the interface with a different degree of water penetration through the interface. However any oil in the interfacial region is confined to the layer adjacent to the bulk oil phase. The values for the area per molecule at the oil-water interface are shown in Figure 7.8. The values obtained are significantly higher than those reported in the literature [35] for the same DSPC lipid at the air-water interface (49 to 44 Å² with surface pressure increasing from 20 to 50 mN/m⁻¹). The values are also higher than those reported for di-palmitoyl-phosphocholine (DPPC) at the air-water interface [28,47]. This is not surprising given the lipid layer at the oil-water interface is formed by spreading from a volatile solvent. This is because it is not possible to compress this insoluble monolayer at the oil-water interface with the current experimental setup. The area per molecule plot in Figure 7.8 shows a plateau region for the spread amount above $7.47 \times 10^{-6}$ mol m⁻², indicating possible saturation at the interface with the current experimental procedure. Given the stability of micelle-like aggregates in hydrophobic phases, most of the excess material leaves the interface by going into the oil phase.
7.4 Conclusions and future work

The neutron reflectivity data for DSPC using two contrasts have shown the formation of a monolayer at the lowest lipid spread amount at the oil-water interface. The thickness of the monolayer is relatively high (about twice the molecular length) because of tail solvation. An increase in the amount of spread lipid results in the formation of a monolayer plus micelle-like aggregates at the interface. The results confirm that spreading molecules at high concentration from a volatile solvent onto a limited surface may not be ideal. At the air-water interface one would normally prepare the monolayer using a Langmuir trough, by spreading on a large area and then compressing the layer using a moveable barrier. This is not currently possible for the oil-water interface experiments using neutron reflectivity because of the drastic attenuation of the neutron beam through the bulk upper oil phase. A new experimental setting is required to determine whether the formation of aggregates is a consequence of the way in which the lipid film was prepared or it is due to the presence of oil for which it has a certain affinity.

The surface coverage achieved with the present experimental setting is lower than that of a real biological membrane, thus compromising the validity of such system as a model membrane. We are currently following an alternative approach to develop a more appropriate experimental setting and achieve a higher surface coverage. We intend to deposit the lipid monolayer onto the oil phase using Langmuir-Schaeffer deposition. The cell would be kept submerged in the water phase as the monolayer is prepared at the air-water interface and compressed to the required surface pressure. A monolayer would be deposited onto the oil-coated silicon block with the oil still frozen, with the temperature of the water phase kept below the freezing point of hexadecane (T < 16 °C). The cell would be assembled while still submerged, positioned in the beam line and the sample characterised using the same procedure. The idea originates from the observation that since stable lipid multilayers can be produced at the oil-water interface [36], the rate of spontaneous emulsification should be much reduced in presence of fully formed, densely packed lipid layers.
7.5 References for Chapter 7

Chapter 8

Mixtures of azacrown ether and fatty acid at the air-water and oil-water interface

8.1 Introduction

Traces of heavy metals are present in natural waters under various forms, such as hydrated ions or complexes. Some of them are known to be toxic and can cause environmental damage. The concentration of metals in natural waters is usually very low, but it can significantly increase in polluted water. However, even in highly polluted waters the concentration of contaminants is relatively low, i.e. in the order of nanomolar for metals such as nickel [1]. With the current instrumentation it is still complicated to measure accurately such concentrations. In addition to that, a complex composition of the sample (such as observed for example in seawater) or the presence of contaminants could compromise the analysis. Pre-concentration is universally recognised as an efficient process both for the analysis of samples with very low concentration and samples with a complex composition. The easiest and more thoroughly applied approaches, based on extraction techniques, are sometimes incompatible with analytical techniques because of difficulty introducing organic solvents into furnaces or flames [2]. Therefore extraction methods based on Permeation Liquid Membranes (PLM) have been developed [3].

There are several types of PLM devices that can be grouped into three categories [4,5]: Bulk (BLM), Emulsion (ELM) and Supported (SLM) Liquid Membranes. BLMs consist of a source solution and a strip solution separated by a hydrophobic phase immersed in a U-tube. The small size of the membrane separating the two
water phases and the considerable amount of hydrophobic phase required render these devices not very attractive. ELMs are characterised by a very high surface area available for the extraction processes and the extraction is generally very fast. However the emulsion must be stable throughout the process but easy to destroy after the separation, allowing the recovery of the extracted species. As the extraction process involves several steps and generally extensive manipulation of the sample is required, ELMs are not technologically very attractive.

8.1.1 Supported liquid membrane devices (SLM)

An SLM device consists of two aqueous phases, called the test (or source) and strip solution, which are separated by a membrane soaked in an immiscible organic solvent [6]. The metal ions transport is based on selective complexation by a hydrophobic ligand dissolved in the organic solvent, which works as a shuttle between the two phases. The metal ions are released in the strip solution, where they are extracted with a hydrophilic ligand which has a stronger affinity towards the ion than the carrier. The neutrality of the two solutions is generally ensured by $K^+$ or $Na^+$ ions antiport. The transport mechanism is shown in Figure 8.1 and the following steps are involved:

(i) Diffusion of metal ions ($M^{n+}$) through the source solution to the membrane interface.
(ii) Formation of the metal-carrier complex at the interface.
(iii) Diffusion of the complex to the membrane-strip solution interface. A co-carrier might be desirable for this step.
(iv) Release of the metal ion in the strip solution.
(v) Complexation of the metal ion in the strip solution.
(vi) $Na^+$ ions are transported from the strip solution to the source solution following the inverse path to maintain the neutrality of the solution.
The gradient of the metal ions’ chemical potential between the source and the strip solution works as the driving force for the transport. The chemical potential of the metal ion in the strip solution is kept at a minimum by using strong complexing agents, such as 1,2-diamino-cyclohexane-tetraacetic acid (CDTA) or pyrophosphates, which are known to efficiently remove free metal ions from solutions.

Supported liquid membranes (SLMs) offer a number of advantages, such as [5]:

- The reduced volume of the hydrophobic phase enables the use of expensive carriers;
- The choice of different complexing agents allows high selectivity;
- The devices are robust and allow extraction from very small to large volumes of source solution (easy scale-up);
- They are economic and easy to operate.

### 8.1.2 SLM device based on azacrown ether and fatty acid mixtures

Recently Salaun et al., proposed an SLM system for the selected transport of lead, cadmium and copper ions [7]. The bulk oil phase for the membrane was a 1:1 mixture of toluene and phenylhexane. $N$-$N’$-didecyl-4,13-diaza-18-crown-6 ether (didecyl-azacrown ether or ACE-10) and dodecanoic acid (lauric acid, LA) 0.1 M
were used as metal ion carriers. The structure of substituted azacrown ether is shown in Figure 8.2.

![Chemical structure of substituted azacrown ether.](image)

In a series of experiments Wojciechowski et al., aimed to assess the role of ACE-10 and LA in this system [8]. Cu\(^{2+}\) was used for all experiments. They reported that no transport was observed by using ACE-10 only, whereas it is known that in these systems Cu\(^{2+}\) ions can be transported by LA alone [9]. However the performance of membranes containing LA alone are only reproducible during the first few minutes of use and lead to the formation of a blue precipitate, composed of copper laurate dimers. The presence of ACE-10 in addition to LA prevents the formation of the copper laurate dimers, which are thought to be responsible for the poor reproducibility of the device. These observations led to the proposition of a mechanism for the Cu\(^{2+}\) transfer, shown in Figure 8.3.

Since the transport takes place in absence of ACE-10, LA must be involved in the transport of ions through the membrane. ACE-10, being amphiphilic in nature, seems first to have an important role at the water-membrane solution, where:

a) It attracts LA from the organic phase, possibly because of hydrogen bonding [10];

b) It attracts Cu\(^{2+}\) from the source solution by coordinating it.

The combination of the two events favours the formation of a more stable ternary complex (Cu\(^{2+}\), ACE-10 and LA) that eventually dissolves in the organic phase (Figure 8.3 (a)). The participation of ternary complexes in liquid extraction processes has already been reported in the literature [11].
Figure 8.3. Mechanism for metal ion transport in a recently developed SLM [7]. (a): formation of a ternary complex between LA-ACE-10 and Cu(II) at the oil-water interface. (b): formation of paddlewheel dimers between Cu(II) and LA. (c): the polymerisation between several dimers may cause the formation of a blue precipitate in absence of ACE-10. (d): ACE-10 seems to coordinate paddlewheel dimers slowing down their polymerisation. Published with permission of [8].

In such organic solvents “paddlewheel” dimers between Cu$^{2+}$ and fatty acids (Figure 8.3 (b)) seem to readily form [12] and they seem to be responsible for the transfer of Cu$^{2+}$ across the membrane. The “paddlewheel” dimers tend to polymerise in the oil phase and form the blue precipitate observed in absence of ACE-10 (Figure 8.3 (c)),
diminishing the efficiency of the transport after short times. Spectroscopic results [8] suggest that “paddlewheel” dimers are coordinated by ACE-10 in organic solvents (Figure 8.3 (d)). This seems to be the second important role of ACE-10 in these devices: the formation of the “paddlewheel” dimer - ACE-10 complexes significantly slows down the polymerisation and thus the formation of the precipitate, as experimentally observed, maintaining the efficiency of the system.

For the metal ions to reach the strip solution they have to undergo two consecutive extraction steps, both at the oil-water interface; hence it is of importance to resolve the adsorption behaviour of ACE-10 and LA, as individual molecules and as a mixture. Because of the complexity of the system, structural studies of interfacial processes in a SLM must be performed in model systems. Studies to elucidate the adsorption processes have been conducted in two different systems where the oil soaked-membrane was replaced by air or oil.

### 8.2 Azacrown ether and fatty acid studies at the air-water interface

ACE-10 monolayers at the air-water interface are not stable: the monolayer starts dissolving immediately after spreading onto a Langmuir trough. This can be observed upon compression of the monolayer, where the collapse is obtained at an unreasonably small area per molecule (between 10 and 20 Å$^2$) [13]. To study the interfacial properties of azacrown ethers more stability must be conferred to the monolayer. It was found that by replacing the ACE-10 with the longer aliphatic chain analogue $N$-$N'$-dihexadecyl-4,13-diaza-18-crown-6 ether (dihexadecyl azacrown ether, ACE-16), the stability of the spread monolayer increased significantly. Upon compression of the monolayer at the air-water interface to $\Pi < 30$ mN m$^{-1}$ almost no hysteresis was observed in the isotherm. Also no clear phase transition was observed for $\Pi < 30$ mN m$^{-1}$. A small shift in the isotherms appears upon consecutive compressions of the monolayer and this was attributed to small losses of material by dissolution in the water phase.
In attempt to study the co-adsorption of azacrown ether and fatty acid, mixed monolayers were prepared. Again, ACE-16 was used instead of ACE-10 to minimise the dissolution in the water phase. For the same reason lauric acid was replaced with hexacosanoic acid (palmitic acid, PA). Mixed PA-ACE-16 monolayers show less hysteresis compared to ACE-16 monolayers, even at high surface pressure ($\Pi > 30 \text{ mN m}^{-1}$). By simultaneous analysis of the pressure-area isotherm and BAM images, repulsive interactions between the two species were suggested [13]. The monolayer was found to collapse only after almost all the azacrown ether had been squeezed out into the water phase by palmitic acid domains. One should keep in mind that both azacrown ether and fatty acid are soluble in organic solvents, thus in presence of unfavourable interactions between them, desorption from the oil-side of the interface is likely to occur. This is clearly not possible when the hydrophobic phase is mimicked by air.

### 8.3 Azacrown ether and fatty acid at the oil-water interface

Azacrown ether readily adsorbs at the oil-water interface when dissolved in the oil phase. X-ray reflectivity studies at the hexane-water interface suggest the presence of a packed monolayer, with azacrown ether head-groups assuming a tilted conformation with respect to the normal to the interface [14]. Long aliphatic chain substituted azacrown ether (ACE-24) was used for this experiment because, by forming a thicker monolayer with respect to ACE-10 or ACE-16, it would allow higher spatial resolution for the X-ray measurements. Significant intermixing between hexane and the aliphatic chain was observed. Interfacial tension studies using a drop profile analysis tensiometer showed that ACE-10 and LA mixtures co-adsorb at the toluene-water interface in a broad range of concentration [10].
8.4 Recent neutron reflectivity measurements of azacrown ether at the air-water and oil-water interface

Recently neutron reflectivity was used to determine the structure of a palmitic acid layer at the air-water interface [15]. At a surface pressure of 30 mN m\(^{-1}\), the palmitic acid monolayer is extremely compact with a thickness corresponding to a fully extended molecule (~ 21.7 Å). Structural studies of azacrown ether (ACE-16) both at the air-water and oil-water interface were recently carried out by Zarbakhsh et al. [16]. The conformation of ACE-16 at the air-water interface was studied using simultaneously neutron reflectivity and surface pressure-area isotherm. Neutron reflectivity measurements were carried out in a Langmuir trough at fixed surface pressure, therefore the dissolution of ACE-16 molecules from the monolayer was counterbalanced by the movement of the barriers. The experimentally measured area per molecule was found to significantly differ from that calculated from the trough area, see Figure 8.4. Calculated and measured area per molecule were almost identical at low surface pressure, indicating little dissolution of ACE-16 in the aqueous phase. As the surface pressure increased, however, the two values significantly deviated. A linear dependence was observed between the variation in measured and calculated surface area (Figure 8.4).
Figure 8.4. Area per ACE-16 molecule in a monolayer at the air-water interface as determined from neutron reflectivity [16]. Area per molecule as calculated from neutron reflectivity and from the trough area are in good agreement at high APM values (low \( \Pi \)). With increasing \( \Pi \) significant deviation between the two values occurs, which is to be attributed to ACE-16 molecules dissolution from the monolayer.

No measurements were taken at \( \Pi > 30 \text{ mN m}^{-1} \) but from the linear dependence it is possible to extrapolate the area per molecule at which the monolayer collapses. The area per molecule, including the correction for loss of material from the monolayer, is approximately 40 Å\(^2\), which corresponds roughly to the cross-section of the two alkyl chains. This value is much more realistic than 10-20 Å\(^2\) observed previously [13] for the shorter chain analogue ACE-10.

Neutron reflectivity measurements suggest that at the oil-water interface the adsorbed ACE-16 layer is more diffuse compared to the air-water interface [16]. The interfacial area can be divided into two sections. ACE-16 forms a 17 Å thick densely packed monolayer on the oil side of the interface, whereas the aqueous side of the interface consists of a loosely bound, dilute layer of 38 Å. The adsorbed amount does
not increase as a linear function of the spread amount but it seems to reach a maximum with a spread amount between 2 and $3 \times 10^{-6}$ mol m$^{-2}$ (Figure 8.5).

![Figure 8.5. Adsorbed amount for ACE-16 at the hexadecane-water interface as determined with neutron reflectivity [16]. Note how maximum $\Gamma$ is observed for spread amount between 2 and $3 \times 10^{-6}$ mol m$^{-2}$.](image)

At high spread amount it seems that ACE-16 is expelled from the monolayer either onto the oil phase or the water phase. This phenomenon was interpreted as the corresponding collapse of the monolayer observed at the air-water interface.

### 8.5 Neutron reflectivity measurements of azacrown and fatty acid mixtures at the air-water and oil-water interface

Neutron reflectivity studies of such systems have only addressed the adsorption profile of ACE-16. It is important to extend the previous studies to systems that
closely mimic the conditions met in the actual SLM device. For this reason neutron reflectivity was used to understand the co-adsorption process of ACE-16 and fatty acids at both the air-water and oil-water interfaces. These studies would help to model the transport mechanism in such devices.

As a first part of the project, a 1:1 mixture of ACE-16 and fatty acid with increasing chain length (palmitic, stearic and hexacosanoic acid, C-16 to C-26) were studied at the air-water interface. In addition, the detailed conformation of ACE-16 and stearic acid was determined using contrast variation by using different mixtures of protonated and deuterated compounds. Once the co-adsorption mechanism was understood, a 1:1 mixture of ACE-16 and palmitic acid was analysed at the buried oil-water interface as a function of increasing spread amount.

8.5.1 Materials

The h-ACE-16 and d-ACE-16 $N,N'$-dihexadecyl-4,13-diaza-18-crown-6 ether ($C_{44}H_{70}N_2O_4$ and $C_{44}H_{24}D_{66}N_2O_4$, with hexadecyl chains deuterated) were custom synthesized by BDG Synthesis [17]. Protonated fatty acids: h-palmitic acid (h-PA, $C_{16}H_{32}O_2$), h-stearic acid (h-SA, $C_{18}H_{36}O_2$) and h-hexacosanoic acid (h-HA, $C_{26}H_{52}O_2$), as well as deuterated palmitic acid (d-PA) and stearic acid (d-SA) (98% D) were purchased from Aldrich.

8.5.2 Methods

8.5.2.1 Air-water interface

In order to understand the contribution of fatty acid to the stability of a spread monolayer of azacrown ether at the air-water interface, neutron reflectivity was used in combination with pressure-area isotherm. In this experiment, the d-ACE16 and the fatty acid solutions were prepared separately in distilled chloroform. Mixtures of d-ACE16 and fatty acid (1:1 mol/mol mixtures) were then prepared prior to the spreading. A standard Langmuir trough was used in these experiments and the average area of the trough could be varied in the range of 130-490 cm$^2$. Prior to use,
the trough was cleaned in a standard way. The trough was then filled with null reflecting water, the water surface was swept and aspirated before the deposition of the mixed azacrown ether and fatty acid film. After spreading, the solvent was allowed to evaporate for about 15 minutes. All measurements were conducted at $T = 25 \pm 0.5 \, ^\circ C$. The trough was situated on a vibration-isolation table covered with a perspex lid with two windows to prevent any perturbation of the surface. All profiles were measured at constant $\Pi$. Neutron reflectivity measurements were carried out at ISIS using the SURF reflectometer (see section 2.4.3.1). The reflectivity profiles were measured at an incident angle $\theta = 1.5^\circ$. The sample was under-illuminated with a constant resolution $\delta Q/Q \sim 3.0\%$.

In order to assess the effect of the chain length of the fatty acid on the conformation of the azacrown ether, the mixtures of d-ACE-16 with hydrogenous fatty acids were used. For each mixture of fatty acid and d-ACE-16, a number of reflectivity profiles were measured along the pressure-area isotherm. In the subsequent experiment, in order to fully characterize both the azacrown ether and the fatty acid conformation in the mixed monolayers on NRW, the following contrast mixtures were used: d-ACE-16 with h-SA and h-ACE-16 with d-SA.

### 8.5.2.2 Determination of the adsorbed amount for a surfactant mixture at the air-water interface using neutron reflectivity

All reflectivity profiles were fitted to a single layer model, thickness $d$, scattering length density $N_b$ layer, and zero roughness. It has been shown the adsorbed amount $\Gamma$ can be calculated using the following formula [18]:

$$\Gamma = \frac{N_b \text{layer} d}{N_A \sum_i b_i}$$  \hspace{1cm} \text{Equation 8.1}

where $\sum_i b_i$ is the sum of the coherent scattering length for each species (fatty acid and the ACE-16 molecules), $d$ is the layer thickness, and $N_A$ is Avogadro’s number.

For ACE-16 and SA mixtures, the adsorbed amount for the individual species could be calculated. In this case, the fitted $N_b$ values can be written as the sum of the...
contributions from each of the four species representing the layer: ACE-16, SA, air and water. With the contribution of air and water (NRW) equal to zero ($b_{air}=b_{nrw}=0$), the total $N b_{layer}$ can be written as:

$$N b_{layer} = N_{ACE-16} b_{ACE-16} + N_{SA} b_{SA}$$  \hspace{1cm} \text{Equation 8.2}

Two different values for $N b_{layer}$ were obtained from the single layer fit to the data for the two contrasts. These two values for the scattering length densities were then used to calculate the number densities, $N_{ACE-16}$ and $N_{SA}$ by solving Equation 8.2 for the two contrasts. The values for the scattering length ($b_{ACE-16}$ and $b_{SA}$) for both protonated and deuterated ACE-16 and SA are given in Table 8.1. The number density for the species $i$ ($N_i$) can be used to calculate the adsorbed amount $\Gamma_i$ using Equation 8.3:

$$\Gamma_i = \frac{N_i d}{N_A}$$  \hspace{1cm} \text{Equation 8.3}

### Table 8.1. Neutron scattering length for ACE-16 and SA

<table>
<thead>
<tr>
<th></th>
<th>ACE-16 $b_{ACE-16}$ / fm molec$^{-1}$</th>
<th>SA $b_{SA}$ / fm molec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deuterated</td>
<td>671.300</td>
<td>363.966</td>
</tr>
<tr>
<td>Hydrogenous</td>
<td>-2.122</td>
<td>-3.356</td>
</tr>
</tbody>
</table>

### 8.5.2.3 Oil-water interface

As for the experiment at the air-water interface, both the d-ACE-16 and palmitic acid solutions were prepared separately in distilled chloroform. 1:1 mixtures of d-ACE-16 and palmitic acid were then prepared prior to the spreading. The sample was prepared following the procedure discussed in Chapter 7. All measurements were conducted at $T = 25 \pm 0.5$ °C and the temperature was kept constant by means of a circulating water bath. The neutron reflectivity spectra were measured using the reflectometer FIGARO at ILL (see section 2.4.3.3). All the reflectivity profiles were measured at an incident angle $\theta = 3.78^\circ$ providing a suitable $Q$ range. The sample was under illuminated with resolution $\delta Q/Q \approx 5.0\%$. 

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In the first part of the experiment both oil and water were contrast-matched to silicon ($Nb = 2.07 \times 10^{-6} \text{Å}^{-2}$). Three contrasts, with different combinations of palmitic acid and ACE-16 (d-PA with d-ACE-16, h-PA with h-ACE-16, h-PA with d-ACE-16), were used to calculate the adsorbed amount of both species. The three contrasts used are given in Table 8.2. Two more contrasts, which will be discussed later, were used to determine the structural conformation of the adsorbed layer. The contrast schemes are given in Table 8.2.

**Table 8.2. Detailed summary of the contrasts used to study co-adsorption of ACE-16 and PA at the oil-water interface.** Contrasts 1-3 were used to calculate adsorbed amount for both ACE-16 and PA; contrasts 4 and 5 were used to determine the structure of the adsorbed layer.

<table>
<thead>
<tr>
<th>Contrast 1</th>
<th>Contrast 2</th>
<th>Contrast 3</th>
<th>Contrast 4</th>
<th>Contrast 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil phase</td>
<td>CMSi oil</td>
<td>CMSi oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-16</td>
<td>d-ACE-16</td>
<td>h-ACE-16</td>
<td>d-ACE-16</td>
<td>d-ACE-16</td>
</tr>
<tr>
<td>PA</td>
<td>d-PA</td>
<td>h-PA</td>
<td>h-PA</td>
<td>h-PA</td>
</tr>
<tr>
<td>Water phase</td>
<td>CMSi water</td>
<td></td>
<td>D$_2$O</td>
<td></td>
</tr>
</tbody>
</table>

The procedure described in Chapter 6 and adopted in Chapter 6 and 7 for the determination of the adsorbed amount $\Gamma$ is valid for adsorption of individual surfactants, but may be too simplistic in the case of co-adsorption processes. The procedure adopted in the following section leads to a more accurate determination of $\Gamma$ and involves the use of different contrast schemes (in this case three contrasts for two surfactants, in general $n+1$ contrasts for $n$ surfactants). The procedure is similar to that described for the air-water interface.

**8.5.2.4 Determination of the adsorbed amount for a surfactant mixture at the oil-water interface using neutron reflectivity**

The reflectivity profiles were fitted to a single layer model with thickness $d$, scattering length density $Nb$ and zero roughness. A two layer model was used to fully represent Contrast 2 (h-ACE-16 and h-PA). This will be discussed later.
Similarly to the situation encountered at the air-water interface, the fitted \( Nb \) values can be written as a sum of the contributions from each of the four species representing the layer: ACE-16, PA, oil, and water. However, when the adsorbed amount was determined at the air-water interface, the water phase was contrast-matched to air (null reflecting water, NRW) so that the contribution of both species to the fitted \( Nb \) was equal to zero \((b_{air}=b_{nrw}=0)\). When oil and water are contrast-matched to silicon, the contribution from the two liquid phases must be taken into account, hence the total \( Nb \) of the layer is written as:

\[
(Nb)_{layer} = N_{ACE-16}b_{ACE-16} + N_{PA}b_{PA} + N_{oil}b_{oil} + N_{water}b_{water}
\]

Equation 8.4

Since the oil and water phases have the same scattering length density, their contribution to the \((Nb)_{layer}\) can be grouped in:

\[
(Nb)_{liq} = N_{oil}b_{oil} + N_{water}b_{water}
\]

Equation 8.5

\((Nb)_{liq}\) is not the scattering length density of bulk oil or water \((Nb = 2.07 \times 10^{-6} \text{ Å}^{-2})\) but it is a function of the volume fraction of both liquids in the layer; hence it must be determined experimentally. This is because the number density \(N\) of both oil and water will be lower in the adsorbed layer than it is in the two bulk phases. Substituting Equation 8.5 in Equation 8.4 we obtain:

\[
Nb_{layer} = N_{ACE-16}b_{ACE-16} + N_{PA}b_{PA} + (Nb)_{liq}
\]

Equation 8.6

The number density \(N\) for ACE-16 and PA was calculated by simultaneously solving the above equation for three different contrasts. These were then used to calculate the adsorbed amount \(\Gamma\) for both species using Equation 8.3:

\[
\Gamma_i = \frac{N_i d}{N_A}
\]

Equation 8.3

In order to ascertain precisely the conformation of the adsorbed layer at the oil-water interface, two more contrasts were deployed (Contrast 4 and 5 in Table 8.2). For Contrast 4 it was decided to use h-ACE-16, h-PA and oil contrast-matched to silicon as in Contrast 2, but this time D\(_2\)O was used as water sub-phase. For Contrast 5 again D\(_2\)O was used as aqueous sub-phase, with protonated (h) ACE-16 and deuterated (d) PA.
8.6 Results obtained for the air-water interface

8.6.1 Effect of fatty acid chain length on ACE-16 adsorption at the air-water interface

The reflectivity profiles for mixtures of d-ACE-16 with h-palmitic and h-stearic acid for surface pressures in the range 11 to 40 mN m\(^{-1}\) are shown in Figure 8.6 and Figure 8.7. The respective mixture with the longest alkyl-chain derivative, h-hexacosanoic acid (Figure 8.8) could only be compressed to the surface pressure \(\Pi = 15\) mN m\(^{-1}\) using the current setup. The fits to the data are shown as solid lines. The relative changes in the reflectivity profiles as a function of increasing surface pressure are shown in Figure 8.6 insert for the ACE-16 - palmitic acid mixture.

![Figure 8.6. Reflectivity data for the mixed Langmuir monolayer of h-palmitic acid and d-ACE-16 (1:1 mol/mol) at the air-NRW interface for surface pressures in the range 11 to 40 mN m\(^{-1}\). The solid lines correspond to the one layer model fits with layer thickness 22 ± 1 Å. The \(N_b\) increases with surface pressure from 3.26 to 3.78 \(\times 10^{-6}\) Å\(^{-2}\). Profiles are shifted by a factor of \(\times 10\) for the purpose of clarity. Figure insert shows the variations in the reflectivity profiles with increasing surface pressure.](image-url)
Figure 8.7. Reflectivity data for the mixed Langmuir monolayer of h-stearic acid and d-ACE-16 (1:1 mol/mol) at the air-NRW interface for surface pressures in the range 11 to 40 mN m\(^{-1}\). The solid lines correspond to the one layer model fits with layer thickness 21 ± 1 Å. The \(N_b\) increases with surface pressure from 3.27 to 3.68 \(\times 10^{-6}\) Å\(^{-2}\). Profiles are shifted by a factor of \(\times 10\) for the purpose of clarity.

Figure 8.8. Reflectivity data for the mixed Langmuir monolayer of h-hexacosanoic acid and d-ACE-16 (1:1 mol/mol) at the air-NRW interface for surface pressures in the range 11 to 15 mN m\(^{-1}\). The solid lines correspond to the one layer model fits with layer thickness 19 ± 1 Å. The \(N_b\) increases with surface pressure from 3.19 to 3.43 \(\times 10^{-6}\) Å\(^{-2}\). Profiles are shifted by a factor of \(\times 10\) for the purpose of clarity.
The fitted layer thickness for the ACE-16 in the presence of palmitic acid was 22 ± 1 Å, which corresponds well to the length of a fully extended hexadecyl chain (∼21.7 Å) of the ACE-16. No significant changes in the layer thickness were observed for ACE-16 in the presence of SA (21 ± 1 Å). However, for the mixture of ACE-16 and hexacosanoic acid the fitted layer thickness dropped to 19 ± 1 Å. The fitted Nb for this layer was also lower than the values for the other two fatty acids. This is because of some degree of dissolution of the ACE-16 in presence of this longer chain fatty acid. As will be discussed later, this was caused by the larger mismatch of the alkyl chain of the two species involved. The respective fitted Nb and d were then used to estimate the total adsorbed amount of the ACE-16 in the mixed layers as a function of surface pressure. The corresponding adsorbed amounts of d-ACE-16 in the presence of different fatty acids are shown in Figure 8.9. Clearly, the highest adsorbed amount of ACE-16 is observed for the mixture with palmitic acid, pointing to the pivotal role of chain length matching for the optimum stabilization of the mixed monolayer.

![Figure 8.9. Adsorbed amount for ACE-16 in the mixed layers different chain-length fatty acids: palmitic acid (o), stearic acid (●) and hexacosanoic acid (▲) as a function of increasing surface pressure. The adsorbed amount for ACE-16 alone, reported from a previous study [16], is shown for comparison (□).](image-url)
8.6.2 Adsorption of stearic acid in the mixed monolayer

In a second experiment, the individual adsorbed amounts of both the SA and ACE-16 at the interface were determined using two different contrasts. For this purpose, the following contrast scheme was used: d-ACE-16 with h-SA (reflectivity profiles are shown in Figure 8.7) and h-ACE-16 with d-SA (profiles are shown in Figure 8.10) spread on the null-reflecting aqueous sub-phase.

![Figure 8.10](image_url)  
Figure 8.10. Reflectivity data for the mixed Langmuir monolayer of d-stearic acid and h-ACE-16 (1:1 mol/mol) at the air-NRW interface for surface pressures in the range 11 to 40 mN m⁻¹. The solid lines correspond to the one layer model fits with layer thickness 24 ± 1 Å. The Nb increases with surface pressure from 1.91 to 2.82 × 10⁻⁶ Å⁻². Profiles are shifted by a factor of ×10 for the purpose of clarity.

The adsorbed amounts of both components (SA and ACE-16) were then estimated from their number density using Equation 8.2 and Equation 8.3. The results are shown in Figure 8.11 (a) and Figure 8.11 (b), respectively.
Figure 8.11. Adsorbed amount for stearic acid (a) and ACE-16 (b) individually determined by simultaneous use of two contrasts. The solid lines show the maximum adsorbed amount as estimated form the area of the trough assuming zero dissolution. The comparison between the adsorbed amount for ACE-16 in presence of SA (●) and the case when ACE-16 is the only specie at the interface (□) is shown in figure insert.

The solid lines show the adsorbed amounts of the individual components estimated from the area of the trough as a function of surface pressure. For these estimations, it was assumed that no dissolution of any component takes place, thus the deviation of
experimental data from these lines is a measure of the extent of material dissolution from the monolayer. The inset in Figure 8.11 (b) clearly indicates an increase of the adsorbed amount of the ACE-16 in the presence of SA (●), compared to the case when ACE-16 is the only component of the spread monolayer (□, data taken from [16]).

8.6.3 Structure of mixed ACE-16 - fatty acid monolayers at the air-water interface

The separate contribution of SA and ACE-16 to the total adsorbed amount was calculated from the reflectivity profiles using two contrasts and is shown in Figure 8.11 (a) and (b) respectively. The solid lines show the estimated surface concentration, using the spread amount and the area of the trough under assumption of no dissolution taking place into the bulk phase. Figure 8.11 (a) clearly shows the stability against dissolution of the fatty acid in the mixed monolayer up to a surface pressure of 20 mN m$^{-1}$. However, above this surface pressure the calculated and estimated values for the adsorbed amount of SA start to deviate, indicative of partial dissolution of the fatty acid into the aqueous sub-phase. Dissolution of SA from Langmuir monolayer has already been reported in the literature [19]. In the present system, this process is further complicated by the presence of ACE-16, because of the mutual interactions between the two molecules. It has been shown previously that ACE and fatty acid can share a proton of the carboxylic acid and can form hydrogen bonding both in bulk and at the interface [20]. Figure 8.11 (b) shows the adsorbed amount of ACE-16 in the presence of SA. In contrast to the corresponding surface concentration of SA (Figure 8.11 (a)), a deviation from the no-dissolution line is observed even at very low surface pressures. Taken together, the data from Figure 8.11 indicate a much higher dissolution of ACE-16 than that of SA from the mixed monolayer. Nevertheless, it must be stressed that the extent of ACE-16 dissolution from the spread Langmuir monolayer is significantly reduced by the presence of co-adsorbed SA (a comparison is shown in Figure 8.11 (b) inset).

The effect of fatty acid chain length on the retention of ACE-16 at the interface was deduced from the reflectivity profiles shown in Figure 8.6 - Figure 8.8. The adsorbed amounts for the d-ACE-16 in presence of the three fatty acids (palmitic, stearic, and
hexacosanoic acids) were individually obtained from the fits to the reflectivity profiles and are shown in Figure 8.9. The highest adsorbed amount for the ACE-16 was observed in the presence of palmitic acid, closely followed by stearic acid. Hexacosanoic acid performs very poorly: the mixed monolayer could not be compressed to $\Pi > 15 \text{ mN m}^{-1}$ because of excessive dissolution of ACE-16 into the aqueous sub-phase. This result points to the importance of matching the lengths of alkyl chains of ACE-16 to those of the fatty acid. Similar observations have already been reported for several types of molecules forming mixed monolayers, leading to the conclusion that the less the difference in surfactant chain length, the stronger the interactions between surfactants [21].

![Graph showing adsorbed amount for ACE-16 in presence of different fatty acids](image)

**Figure 8.12.** Adsorbed amount for ACE-16 in presence of different fatty acids as determined from the trough area is plotted against the adsorbed amount determined from neutron reflectivity (palmitic acid (o), stearic acid (●), hexacosanoic acid (▲) and ACE-16 alone (□) from previous data [16]). The solid line, referred to as “no dissolution line” in the text, shows the estimated adsorbed amount assuming that no dissolution into the aqueous phase is taking place.

The overall results can be observed in Figure 8.12. Both palmitic acid and stearic acid are very efficient in retaining the ACE-16 in the mixed Langmuir monolayer up to the adsorbed amount of about $2 \times 10^{-6} \text{ mol m}^{-2}$, corresponding to the surface
pressure of about 20 mN m\(^{-1}\). This is indicated by relatively minor deviations from the “no-dissolution” line in Figure 8.12. The line is drawn with the assumption that the amount of material deposited does not change because of the dissolution in the sub-phase. Deviations from the line are indicative of dissolution from the monolayer. Bare d-ACE-16 monolayers show marked deviations from the “no-dissolution” line and the corresponding data are also shown in Figure 8.12 for comparison. Up to the adsorbed amount of \(2.0 \times 10^6\) mol m\(^{-2}\), the interactions between ACE-16 and fatty acid are sufficient to maintain the integrity of the monolayer. However, above this value both the fatty acid and d-ACE-16 start desorbing and the monolayer becomes more and more depleted. The slopes of the curves for d-ACE-16 in mixture with palmitic acid and stearic acid become comparable to that of bare d-ACE-16 (Figure 8.12) suggesting that above the threshold surface pressure of 20 mN m\(^{-1}\) the fatty acid is not able to maintain the azacrown ether at the interface anymore. The deviation of the experimental points from the “no-dissolution” line for SA in the mixed monolayer (Figure 8.11 (a)) suggests that the fatty acid may dissolve together with the ACE-16, e.g., as a hydrogen-bonded complex. The formation of such complexes partially masks the hydrophilic head groups of both molecules, thus delaying the dissolution into the aqueous sub-phase. The interaction between the two molecules is, therefore, beneficial for retaining the azacrown ether at the interface, but only at low and intermediate surface pressure regions. With increasing \(\Pi\), the tendency of both azacrown ether and fatty acid to dissolve into the aqueous sub-phase drastically increases, hence the availability of the two carrier molecules to take part in ion complexation.

8.7 Results obtained for the oil-water interface

In a previous neutron reflectivity experiment Zarbakhsh et al., found that spread amounts \(\geq 4.50 \times 10^6\) mol m\(^{-2}\) result in a collapse of the ACE-16 monolayer [16]. For this reason the spread amount of the ACE-16 and PA mixture at the oil-water interface was kept to lower values at all times. The investigated spread amount of the individual components ranged from 0.80 to 3.20 \(\times 10^6\) mol m\(^{-2}\). The same range of
spread amount was also adopted for the analysis of PA alone at the oil-water interface.

8.7.1 Adsorbed amount for palmitic acid at the oil-water interface

The adsorbed amount for palmitic acid at the oil-water interface was first determined as a reference. In order to do so, both oil and water were contrast-matched to silicon and increasing amounts of d-PA were spread at the interface. Neutron reflectivity profiles for d-PA at the oil-water interface for spread amount ranging from 0.80 to $3.20 \times 10^{-6}$ mol m$^{-2}$ are shown in Figure 8.13. The fits to the profiles are shown by solid lines. All the profiles were fitted to a single layer model with a thickness of $26 \pm 2$ Å, with no roughness. The fitted parameters, layer thickness ($d$) and scattering length density ($Nb$) are given in Table 8.3. No significant changes were observed in the reflectivity profiles with increasing spread amount for d-PA. To visualise the lack of changes within the profiles, the un-shifted profiles are shown in Figure 8.13 insert.

Table 8.3. Parameters used for one layer fit to the reflectivity profiles shown in Figure 8.13 for d-PA at the hexadecane-water interface. Both oil and water are contrast-matched to silicon.

<table>
<thead>
<tr>
<th>Spread amount $\times 10^{-6}$ mol m$^{-2}$</th>
<th>0.80</th>
<th>1.20</th>
<th>1.60</th>
<th>2.40</th>
<th>3.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ / Å ($\pm 2$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil</td>
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<td></td>
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<td>d-PA</td>
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<td>2.63</td>
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</tr>
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<td>-</td>
<td>2.07</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Nb \times 10^{-6}$ / Å$^{-2}$</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Oil</td>
<td>2.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-PA</td>
<td>2.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Water</td>
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</tr>
<tr>
<td>d-PA</td>
<td>0.0</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Water</td>
<td>0.0</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 8.13. Reflectivity profiles for a series of d-PA spread amount at the CMSi oil-CMSi water interface. Solid lines correspond to the one layer fit to the data, the fitted parameters are given in Table 8.3. Profiles are shifted by a factor of $\times 10$ for the purpose of clarity. The un-shifted profiles are shown in figure insert to highlight the lack of significant differences. Labels are in figure insert (units: $\times 10^6$ mol m$^{-2}$).

8.7.2 Individual adsorbed amount for ACE-16 and PA in mixed layers at the oil-water interface

For the ACE-16 and PA mixture at the oil-water interface, three contrasts with both oil and water contrast matched to silicon were simultaneously required to calculate the adsorbed amount for the individual components. As previously observed for the palmitic acid at the oil-water interface, very small changes were observed in the reflectivity profiles for the ACE-16 and PA mixtures with increasing spread amount. The reflectivity profiles for Contrast 1 and Contrast 3 for spread amount ranging
from 0.80 to $3.20 \times 10^{-6}$ mol m$^2$ are shown in Figure 8.14 and Figure 8.15. Solid lines correspond to the one layer fit to the data. A fitted layer thickness $d = 30 \pm 2$ Å was found for spread amount of $2.40 \times 10^{-6}$ mol m$^2$. The layer thickness increased to $34 \pm 2$ Å for the highest spread amount ($3.20 \times 10^{-6}$ mol m$^2$). Figure 8.14 insert and Figure 8.15 insert highlight how very little difference was observed within the reflectivity profiles as a function of increasing spread amount.

Figure 8.14. Reflectivity profiles for a series of d-ACE-16 and d-PA spread amounts (Contrast 1) at the CMSi oil-CMSi water interface. Solid lines correspond to the one layer fit to the data, the fitted parameters are given in Table 8.4 and Table 8.7. Profiles are shifted by a factor of $\times 10$ for the purpose of clarity. The un-shifted profiles are shown in figure insert to highlight the lack of significant differences. Labels are in figure insert (units: $\times 10^{-6}$ mol m$^2$).
Figure 8.15. Reflectivity profiles for a series of d-ACE-16 and h-PA spread amounts (Contrast 3) at the CMSi oil-CMSi water interface. Solid lines correspond to the one layer fit to the data, the fitted parameters are given in Table 8.6 and Table 8.7. Profiles are shifted by a factor of $\times 10$ for the purpose of clarity. The un-shifted profiles are shown in figure insert to highlight the lack of significant differences. Labels are in figure insert (units: $\times 10^6$ mol m$^{-2}$).

As opposed to Contrast 1 and Contrast 3, the one layer model for Contrast 2 required a much smaller layer thickness ($d = 21 \pm 2$ Å for all five spread amounts). This suggests that the one layer model is not sufficient to adequately represent the interfacial region. Therefore it was decided to apply a two layer model to fit the reflectivity curves for Contrast 2. When the number of layers is increased, the number of fitting variables is also increased; hence in order to reduce the ambiguity in the fitting procedure certain constraints were applied:

(i) The overall layer thickness for all spread amounts was kept the same as observed for Contrast 1 and Contrast 3.

\[
d_{layer\, 1} + d_{layer\, 2} = d_{C1\, and \, 3}
\]  

Equation 8.7
Where $d_{\text{layer } 1}$ and $d_{\text{layer } 2}$ are the thicknesses of the two layers in which the interfacial region has been divided, whereas $d_{C1 \text{ and } 3}$ is the thickness observed for the one layer fit to the reflectivity profiles for Contrasts 1 and 3.

(ii) The integrated scattering length density profile must give the same result as that for the one layer fit, i.e. constant adsorbed amount.

\[
d_{1\text{layer fit}} \times (N_{b_{\text{layer}}} - N_{b_b}) = d_{\text{layer } 1} \times (N_{b_{\text{layer } 1}} - N_{b_b}) + d_{\text{layer } 2} \times (N_{b_{\text{layer } 2}} - N_{b_b})
\]

Equation 8.8

Where $N_{b_{\text{layer}}}$ is the scattering length density observed for the one layer fit to the profiles, $N_{b_b}$ is the scattering length density of the liquid phases (both contrast matched to silicon) and $N_{b_{\text{layer } 1}}$ and $N_{b_{\text{layer } 2}}$ are the scattering length densities of the first and second layer respectively.

The reflectivity profiles for Contrast 2 are shown in Figure 8.16; solid lines correspond to the two layer model fits. The parameters adopted for the fits for Contrast 1, Contrast 2 and Contrast 3 are shown in Table 8.4, Table 8.5 and Table 8.6 for spread amount up to $2.40 \times 10^{-6}$ mol m$^{-2}$. The fitting parameters for all the three contrasts for spread amount $3.20 \times 10^{-6}$ mol m$^{-2}$, with layer thickness $d = 34 \pm 2$ Å are grouped in Table 8.7.

<table>
<thead>
<tr>
<th>Spread amount $\times 10^{-6}$ mol m$^{-2}$</th>
<th>0.80</th>
<th>1.20</th>
<th>1.60</th>
<th>2.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ / Å ($\pm 2$)</td>
<td>$N_b$ $\times 10^{-6}$/ Å$^2$</td>
<td>Roughness / Å</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Oil</td>
<td>-</td>
<td>2.07</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>d-ACE-16, d-PA</td>
<td>30</td>
<td>2.58</td>
<td>2.72</td>
<td>2.66</td>
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<tr>
<td>Water</td>
<td>-</td>
<td>2.07</td>
<td></td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 8.4. Fitted parameters for the reflectivity profiles for Contrast 1 (Figure 8.14). Both oil and water are contrast-matched to silicon. The fitted parameters relative to the highest spread amount are given in Table 8.7.
Figure 8.16. Reflectivity profiles for a series of h-ACE-16 and h-PA spread amount (Contrast 2) at the CMSi oil-CMSi water interface. Solid lines correspond to the two layer fit to the data, the fitted parameters are given in Table 8.5 and Table 8.7. Profiles are shifted by a factor of $\times 10$ for the purpose of clarity. The un-shifted profiles are shown in figure insert to highlight the lack of significant differences. Labels are in figure insert (units: $\times 10^{-6}$ mol m$^{-2}$).

Table 8.5. Fitted parameters for the reflectivity profiles for Contrast 2 (Figure 8.16). Both oil and water are contrast-matched to silicon. The fitted parameters relative to the highest spread amount are given in Table 8.7.
Table 8.6. Fitted parameters for the reflectivity profiles for Contrast 3 (Figure 8.15). Both oil and water are contrast-matched to silicon. The fitted parameters relative to the highest spread amount are given in Table 8.7.

<table>
<thead>
<tr>
<th>Spread amount $\times 10^{-6}$ mol m$^{-2}$</th>
<th>0.80</th>
<th>1.20</th>
<th>1.60</th>
<th>2.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ / Å $(\pm 2)$</td>
<td>$N_b \times 10^{-6}$ / Å$^{-2}$</td>
<td>Roughness / Å</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil</td>
<td>-</td>
<td>2.07</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>d-ACE-16, h-PA</td>
<td>30</td>
<td>2.56</td>
<td>2.57</td>
<td>2.60</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>2.07</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 8.7. Fitted parameters for the reflectivity profiles for Contrast 1, 2 and 3 for the highest spread amount ($3.20 \times 10^{-6}$ mol m$^{-2}$).

Contrast 1
d-ACE and d-PA

<table>
<thead>
<tr>
<th>$d$ / Å $(\pm 2)$</th>
<th>$N_b \times 10^{-6}$ / Å$^{-2}$</th>
<th>Roughness / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>-</td>
<td>2.07</td>
</tr>
<tr>
<td>d-ACE-16, d-PA</td>
<td>34</td>
<td>2.68</td>
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<tr>
<td>Water</td>
<td>-</td>
<td>2.07</td>
</tr>
</tbody>
</table>

Contrast 2
h-ACE and h-PA

<table>
<thead>
<tr>
<th>$d$ / Å $(\pm 2)$</th>
<th>$N_b \times 10^{-6}$ / Å$^{-2}$</th>
<th>Roughness / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>-</td>
<td>2.07</td>
</tr>
<tr>
<td>h-ACE-16, h-PA</td>
<td>17</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>1.95</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>2.07</td>
</tr>
</tbody>
</table>

Contrast 3
d-ACE and h-PA

<table>
<thead>
<tr>
<th>$d$ / Å $(\pm 2)$</th>
<th>$N_b \times 10^{-6}$ / Å$^{-2}$</th>
<th>Roughness / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>-</td>
<td>2.07</td>
</tr>
<tr>
<td>d-ACE-16, h-PA</td>
<td>34</td>
<td>2.54</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>2.07</td>
</tr>
</tbody>
</table>

To ascertain whether the two layer model is an adequate representation of all three contrasts, the same model was adopted to fit the reflectivity profiles for Contrast 1 and Contrast 3. The two layer model adequately represents the reflectivity profiles for both contrasts. As an example, the reflectivity profiles for spread amount...
1.60 \times 10^{-6} \text{ mol m}^{-2} \) are shown in Figure 8.17 (a) (Contrast 1) and Figure 8.17 (b) (Contrast 3) using the two layer model.

![Figure 8.17](image)

**Figure 8.17. Transposition of the two layer model adopted for Contrast 2 to the other two contrasts.** Only one spread amount is shown (1.60 \times 10^{-6} \text{ mol m}^{-2}).

Figure 8.17 (a): Contrast 1, (o); Figure 8.17 (b): Contrast 3, (\Delta).

For the purpose of calculations of the adsorbed amount, it is more convenient to handle a one layer model. Therefore, for Contrast 2 the two layers were averaged to give an equivalent single layer. This is possible because the adsorbed amount \( \Gamma \) is a function of the integrated area in the scattering length density profile [22]:

\[
\Gamma \sim \int_{-\infty}^{+\infty} N b(x) \, dz 
\]  

Equation 8.9

As a result, for Contrast 2 the two layers constituting the \( N b \) profile, each layer characterised by a certain \( N b \) value and thickness \( d \), were substituted with a single layer with the same overall thickness and a suitable \( N b \). This \( N b \) value must lead to the same integrated area and was calculated using the weighted average, Equation 8.10, from the contribution of the two layers, whose values are shown in Table 8.5 and Table 8.7.

\[
N b_{\text{layer 2}} = \frac{d_1 N b_1 + d_2 N b_2}{d_1 + d_2} \]  

Equation 8.10

This transformation was only made for a more convenient handling of a one layer model. However, one must stress that this transformation is only valid when
calculating the adsorbed amount at the interface and is not valid when analysing the structure of the adsorbed layer. The updated values are given in Table 8.8.

Table 8.8. Contrast 2, oil and water CMSi, h-ACE and h-PA. The \( N\bar{b} \) values are obtained as weighted average from the values relative to two layer model in Table 8.6 and Table 8.7.

<table>
<thead>
<tr>
<th>Spread amount ( \times 10^6 ) mol m(^{-2} )</th>
<th>0.80</th>
<th>1.20</th>
<th>1.60</th>
<th>2.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d / \text{Å} ) ( \pm 2 )</td>
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<td>Oil</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>h-ACE-16, h-PA</td>
<td>30</td>
<td>1.42</td>
<td>1.50</td>
<td>1.50</td>
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<td>Water</td>
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<td></td>
<td>1.59</td>
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<table>
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<td>2.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h-ACE-16, h-PA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>2.07</td>
<td></td>
<td></td>
</tr>
<tr>
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<table>
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<th>Roughness / Å</th>
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<td>Oil</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>h-ACE-16, h-PA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spread amount ( \times 10^6 ) mol m(^{-2} )</th>
<th>3.20</th>
</tr>
</thead>
<tbody>
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<td>( d / \text{Å} ) ( \pm 2 )</td>
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<tr>
<td>Oil</td>
<td>2.07</td>
</tr>
<tr>
<td>h-ACE-16, h-PA</td>
<td>1.54</td>
</tr>
<tr>
<td>Water</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
</tr>
</tbody>
</table>

8.7.2.1 The sign of \( \Delta N\bar{b} \) for Contrast 3: the problem of reciprocity

It is worth analysing in detail the reflectivity profiles for Contrast 3. According to Babinet’s reciprocity principle, in a two phase system the scattering length densities of the two phases can be interchanged without affecting the scattering curve [23]. This implies that, considering negligible the \( R_1 \) part of the reflectivity from the silicon-oil interface, two profiles such as those shown in Figure 8.18, presenting \( \Delta N\bar{b} \) with same modulus but opposite signs with respect to the two liquid phases, will give the same reflectivity profiles.
Figure 8.18. Schematic representation of two Nb profiles presenting $\Delta Nb$ with the same modulus between the adsorbed layer and the bulk phases. According to Babinet’s reciprocity principle, such Nb profiles lead to identical reflectivity profiles.

In Contrast 1, where there are two deuterated species at the interface, one can without any doubt assess that the Nb of the adsorbed layer will be higher than silicon ($\Delta Nb > 0$). Likewise, in Contrast 2 two protonated species are adsorbed at the interface and the Nb of the adsorbed layer will be lower than silicon ($\Delta Nb < 0$).

When both deuterated and protonated species are adsorbed at the interface, such as in Contrast 3, one cannot establish a priori whether the Nb of the adsorbed layer will be higher or lower than that of silicon. One way to assess whether the $\Delta Nb$ between the adsorbed layer and the silicon will be positive or negative is to compare it to other contrasts where this has already been determined with absolute certainty.

Let us consider the situation when the spread amount is $2.40 \times 10^{-6}$ mol m$^{-2}$. The Nb values for Contrast 1 and Contrast 2 are summarised in Table 8.9, along with the $\Delta Nb$ between silicon and the adsorbed layer in Contrast 3.
Table 8.9. \( \Delta Nb \) between the bulk phases and the adsorbed layers for the three contrasts (spread amount \( 2.40 \times 10^{-6} \) mol m\(^{-2} \)). Signs are omitted. The \( Nb \) of the adsorbed layer can be unequivocally assigned for Contrast 1 and 2, but this is not true for Contrast 3 where two situations are possible. However, one must discard the second possibility (\( Nb_{layer} = 1.50 \times 10^{-6} / \text{Å}^{-2} \)): because of the substitution of hydrogenous PA with deuterated PA, \( Nb_{layer} \) for Contrast 3 cannot be lower than that for Contrast 2.

| Contrast | \( |\Delta Nb| \times 10^{-6} / \text{Å}^{-2} \) | \( Nb_{layer} \times 10^{-6} / \text{Å}^{-2} \) |
|----------|---------------------------------|---------------------------------|
| 1        | d-ACE-16 and d-PA               | 0.60                            | 2.67                            |
| 2        | h-ACE-16 and h-PA               | 0.48                            | 1.54                            |
| 3        | d-ACE-16 and h-PA               | 0.57                            | 2.64                            |

We can assign with certainty the \( Nb_{layer} \) values for Contrast 1 and Contrast 2 because of the presence of only deuterated surfactants (the former) or hydrogenous surfactants (the latter). The adsorbed layer at the interface in Contrast 3 is composed of a mixture of d-ACE-16 and h-PA. If the d-ACE-16 contribution to the reflectivity is stronger than h-PA contribution, the \( Nb_{layer} \) will be higher than that of silicon. On the other hand, if the contribution of h-PA is dominating, then the \( Nb_{layer} \) will be lower than silicon. It is important to note that in no case the \( Nb_{layer} \) for Contrast 3, which contains some deuterated material (d-ACE-16) can be higher than that observed for Contrast 1 (all deuterated) or lower than that for Contrast 2 (all hydrogenous). Hence, the only acceptable \( Nb_{layer} \) value for Contrast 3 is \( 2.64 \times 10^{-6} \) Å\(^{-2} \). Should \( \Delta Nb \) be small enough that both possibilities were acceptable, one more contrast would be required to over constrain the calculations (for example, h-ACE-16 and d-PA could be used with oil and water both contrast matched to silicon).

### 8.7.3 Variation in adsorbed amount between individual components and their mixtures

The number densities for ACE-16 and PA, \( N_{ACE-16} \) and \( N_{PA} \), obtained from the Contrasts 1-3 were used to calculate the adsorbed amount at the oil-water interface.
for the individual compounds using Equation 8.6. In Figure 8.19 (a) the adsorbed amount of ACE-16 as a function of its spread amount at the oil-water interface in the presence and absence of PA are compared. An analogous comparison is shown for PA in Figure 8.19 (b).

Figure 8.19 (a): the adsorbed amount for ACE-16 in presence (○ from [16]) and absence (●) of PA is presented. The straight line represents the maximum adsorbed amount at the interface assuming total retention at the interface. Figure 8.19 (b): a similar representation is shown for PA alone (●) and PA in presence of ACE-16 (○).
The values for PA alone were measured in the first part of the experiment and refer to the reflectivity profiles in Figure 8.13. The adsorbed amount for both species when they co-adsorb at the oil-water interface does not change significantly as a function of spread amount. This was not surprising for PA, where no changes were observed also when it was the only species at the interface; however this steady adsorption was unexpected for ACE-16. For both species there is generally a significant decrease in adsorbed amount when they move from being the only component at the interface to the condition when they are part of a mixture.

8.7.4 Fitting to the reflectivity profiles for Contrast 4 and Contrast 5 for the structural determination of the adsorbed layer

D$_2$O was used as the aqueous sub-phase for Contrast 4 and Contrast 5 for the determination of the structure of the adsorbed layer. D$_2$O was chosen as the aqueous phase to maximise the difference in scattering length density between the two bulk phases, thus allowing the detailed structural characterisation of the adsorbed layer. As no significant changes were observed within the reflectivity profiles for Contrast 1-3 with increasing spread amount, very few changes were also expected for Contrast 4 and Contrast 5. The reflectivity profiles for Contrast 4 (h-ACE-16 and h-PA at the CMSi oil-D$_2$O interface) and Contrast 5 (d-ACE-16 and h-PA at the CMSi oil-D$_2$O interface) are shown in Figure 8.20 and Figure 8.21 respectively, solid lines correspond to the fit to the data. As expected, very few changes were observed in the reflectivity profiles with increasing spread amount. Such small changes are shown in Figure 8.20 insert and Figure 8.21 insert.

For Contrast 4, all the reflectivity profiles were found to be adequately represented by a two layer model consisting of a 28 Å layer on the oil side of the interface and a rather diffuse 49 Å layer on the aqueous side of the interface. The parameters for the fitting procedure are shown in Table 8.10. The same model used to represent the reflectivity profiles for Contrast 4 failed to adequately represent the profiles for Contrast 5 unless an unreasonable value for interlayer roughness was used (roughness > ½ d). A similar two layer model was therefore deployed: the thickness of the layer adjacent to the oil phase was kept constant between Contrast 4 and
Contrast 5, whereas a slight reduction in thickness was observed for the layer in contact with the aqueous side of the interface (from 49 Å to 42 Å). The parameters used for the fitting procedure are shown in Table 8.11.

The Nb profiles for both Contrast 4 and Contrast 5 are shown in Figure 8.22. As small differences are observed for the profiles at different spread amounts, for each contrast only the average Nb values are shown for clarity.

![Reflectivity profiles for a series of h-ACE-16 and h-PA spread amount (Contrast 4) at the CMSi oil-D$_2$O interface. Solid lines correspond to the fit to the data, the fitted parameters are given in Table 8.10. Profiles are shifted by a factor of ×10 for the purpose of clarity. The un-shifted profiles are shown in figure insert to highlight the lack of significant differences. Labels are in figure insert (units: × 10^{-6} mol m^{-2}).](image-url)
Table 8.10. Fitted parameters for the reflectivity profiles for Contrast 4 (Figure 8.20).

<table>
<thead>
<tr>
<th>Spread amount × 10^6 mol m^-2</th>
<th>d / Å (± 2)</th>
<th>Nb × 10^-6 / Å^-2</th>
<th>Roughness / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>-</td>
<td>2.07</td>
<td></td>
</tr>
<tr>
<td>Layer 1</td>
<td>28</td>
<td>3.31 3.25 3.44 3.41 3.36</td>
<td>5.0</td>
</tr>
<tr>
<td>Layer 2</td>
<td>49</td>
<td>5.38 5.61 5.61 5.48 5.54</td>
<td>5.0</td>
</tr>
<tr>
<td>D_2O</td>
<td>-</td>
<td>6.35</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Figure 8.21. Reflectivity profiles for a series of d-ACE-16 and h-PA spread amount (Contrast 5) at the CMSi oil-D_2O interface. Solid lines correspond to the fit to the data, the fitted parameters are given in Table 8.11. Profiles are shifted by a factor of ×10 for the purpose of clarity. The un-shifted profiles are shown in figure insert to highlight the lack of significant differences. Labels are in figure insert (units: × 10^-6 mol m^-2).
Table 8.11. Fitted parameters for the reflectivity profiles for Contrast 5 (Figure 8.21).

| Spread amount \( \times 10^{-6} \) mol m\(^{-2} \) | 0.80 | 1.20 | 1.60 | 2.40 | 3.20 |
|------------------------------------------------|
| \( d / \text{Å} \) (± 2)                        | Nb \( \times 10^{-6} / \text{Å}^{-2} \) | Roughness / Å |
|------------------------------------------------|
| Oil                                            | -    | 2.07 | -    |      |      |
| Layer 1                                        | 28   | 4.6  | 4.66 | 4.33 | 4.73 |
| Layer 2                                        | 42   | 5.78 | 5.77 | 5.61 | 5.89 |
| \( \text{D}_2\text{O} \)                      | -    | 6.35 | -    | 5.0  | 5.0  |

Figure 8.22. Nb profiles for Contrast 4 (black line) and Contrast 5 (red line). Given the small differences observed within the profiles for the individual contrasts, for each contrast only the average Nb values are shown.

8.8 Discussion

8.8.1 Adsorbed amount for the binary mixture: preferential adsorption of ACE-16

The adsorbed amount for palmitic acid at the oil-water interface is shown in Figure 8.19 (b) by the filled circles. For the spread amount equal to 0.80 \( \times 10^{-6} \) mol m\(^{-2} \) the adsorbed amount is slightly higher than the maximum theoretical value; the
difference is, however, very subtle. For all higher values of spread amount, the adsorbed amount is either equal to or lower than the spread amount. No significant changes were observed with increasing the spread amount, suggesting that for PA the full coverage at the oil-water interface is already reached at the lowest spread amount. This full coverage of PA at the oil-water interface is reached at rather low values of adsorbed amount ($\Gamma < 1 \times 10^{-6}$ mol m$^{-2}$), significantly lower than those observed for similar fatty acids (stearic acid) at the air-water interface (Figure 8.11: $\Gamma = 2.9 \times 10^{-6}$ mol m$^{-2}$ at the highest available surface pressure, 40 mN m$^{-1}$). The solubility of PA in water is negligible, whereas it has significant solubility in the oil phase. This suggests that the excess fatty acid dissolves in the bulk oil phase. The adsorbed amount of PA significantly drops when ACE-16 is co-adsorbed at the interface. This is shown also in Figure 8.19 (b), open circles. Again, very small changes are observed with increasing spread amount.

The adsorbed amount for ACE-16 in the absence of PA (Figure 8.19 (a)) shows an increase with spread amount up to $2.2 \times 10^{-6}$ mol m$^{-2}$. The layer is then depleted as the spread amount increases beyond this point. In presence of PA, however, the adsorbed amount for ACE-16 is remarkably constant with increasing spread amount (and smaller than in its absence). The fact that the adsorbed amount of both species at the oil-water interface is independent from the spread amount could be indicative of some ordering at the interface. Unfortunately, since neutron reflectivity is only sensitive to the $Nb$ profile normal to the interface averaged over the interfacial plane, any such ordering cannot be detected using the current setting. The association of PA and ACE-16 results in a monolayer of constant composition at the oil-water interface as a function of increasing spread amount. This result suggests the presence of a self-regulatory mechanism for the composition of the mixed adsorbed layer.

It is worth mentioning that at the lowest spread amount of the PA/ACE-16 mixture ($0.80 \times 10^{-6}$ mol m$^{-2}$ of each component), the spread amount and adsorbed amount for ACE-16 correspond well within error, thus indicating that ACE-16 is entirely retained at the interface. On the other hand, the adsorbed amount for PA is extremely low even at the lowest spread amount. In order to speculate about where the missing material from the interface resides, one should recall the behaviour of such mixed monolayers at the air-water interface. As previously mentioned, ACE-16 slowly dissolves from the water surface into the bulk aqueous phase [16]. It was discussed
in the previous section how the presence of PA is beneficial from the point of view of retaining ACE-16 at the surface. It is very likely that the dissolution of ACE-16 into the water phase in presence of PA is also slowed down at the oil-water interface. Nevertheless, whereas the solubility of ACE-16 in water is very small and that of PA is negligible, both species are readily soluble in hexadecane. Hence, even if some material may dissolve into the aqueous sub-phase (as reported at the air-water interface), it seems more likely that most of the adsorbed material leaves the interface by dissolving into the oil phase.

The small amount of PA retained at the oil-water interface stems probably from the fact that PA molecules readily form dimers when present in organic solutions. The driving force for the formation of these dimers is the fact that carboxylate groups are both good donors and acceptors of hydrogen bonds [24]. Dimerisation effectively shields the hydrophilic parts of PA, thus rendering it more oil-soluble and more prone to desorb from the interface. Analogously, formation of PA/ACE-16 interfacial complexes shields the hydrophilic parts of both molecules, by forming an H-bond between nitrogen atoms of ACE and carboxylate group of the PA [20]. As a result, the interfacial complex formed is more hydrophobic than the individual components and desorbs from the oil-water interface. While the fact that the azacrown ether and fatty acid do co-adsorb at the oil-water interface was evident from previous interfacial tension and surface rheology studies [10,25], the current experiment sheds new light on both the composition and surface activity of this complex. The adsorbed amount of PA in the mixed monolayer is very little and most of the material dissolves into the oil phase. However, the presence of PA both in the bulk and at the interface significantly affects the adsorption profile of ACE-16 (Figure 8.19 (a)). The present results show that the fatty acid may have a regulatory effect on the surface concentration of ACE-16.

The maximum adsorbed amount for both species seems to be already reached at spread amount $0.80 \times 10^{-6}$ mol m$^{-2}$. At this surface coverage ACE-16 is entirely retained at the interface, while most of the PA dissolves in the oil phase. As the surface coverage increases up to $1.60 \times 10^{-6}$ mol m$^{-2}$, no significant changes are observed in the adsorbed amount of both species. The presence of PA, which can be found both at the interface, although at very low concentration, and in the bulk oil phase, seems to enhance the adsorbed amount of ACE-16, which would be lower in absence of PA [16]. At spread amounts higher than $1.60 \times 10^{-6}$ mol m$^{-2}$, although no
changes are observed in the adsorbed amount for the individual species, the presence of PA lowers the adsorbed amount of ACE-16.

8.8.2 Structure of mixed ACE-16 - PA monolayers at the oil-water interface

The simultaneous analysis of Contrast 4 and Contrast 5 reflectivity data, with D$_2$O as sub-phase, confirms previous observations that at the oil-water interface the interfacial region is much broader compared to the air-water interface. The $\Delta N_b$ between the layers observed when moving from Contrast 4 to Contrast 5 must be attributed to the substitution of h-ACE-16 with d-ACE-16; hence the integration of the area comprised between the two $N_b$ profiles in Figure 8.22 can be used to obtain a rough estimation of the adsorbed amount of ACE-16 at the interface. The integrated area leads to an adsorbed amount $\Gamma \approx 1.1 \times 10^{-6}$ mol m$^{-2}$, which is slightly higher than the values calculated simultaneously using the first three profiles (section 8.7.3). Because of calculations being carried out using the average values between all the fitted reflectivity profiles and because of the presence of interfacial roughness in the current model, one does not expect to obtain exactly the same values calculated using the first three contrasts. The values are, however, of the same order of magnitude. The $N_b$ profiles in Figure 8.22 show that there is a significant $\Delta N_b$ in the first layer, adjacent to the aqueous phase, when moving from Contrast 4 to Contrast 5. The $\Delta N_b$ is much less pronounced in the second layer. This suggests that most of the ACE-16 is present in the first layer, where it forms a rough monolayer with the tail groups in contact with the oil phase. The thickness of the layer is $d = 28$ Å, more than a fully extended ACE-16 molecule ($\sim 25.7$ Å), suggesting a staggered conformation of the monolayer. The layer in contact with the aqueous phase contains less ACE-16 than the monolayer, as can be seen by the smaller $\Delta N_b$ in the profiles in Figure 8.22. The thickness, about twice the extended C-16 length, indicates the diffuse nature of the layer. The thickness of the diffuse layer was found to be slightly different between the two contrasts: it decreased from 49 Å for Contrast 4 to 42 Å for Contrast 5. In Contrast 5 the h-ACE-16 was replaced by d-ACE-16, whereas h-PA was used for both contrasts. The different layer thickness is indicative of non-homogeneous distribution of the two components throughout the
diffuse layer. At present it is not possible to speculate with regard to the distribution of the individual components in the diffuse layer. However, given the thicker layer and the rise in $Nb$ observed in Contrast 5, where ACE-16 is deuterated, we believe ACE-16 diffuses more toward the aqueous phase, while PA remains confined to the inner part of the interface.

The structure of d-ACE-16 monolayer in absence of PA at the hexadecane-water interface was previously described and a two layer model was deployed to represent the interfacial structure. The $Nb$ profiles as a function of d-ACE-16 spread amount at the CMSi oil-D$_2$O interface are shown in Figure 8.23 by the black lines. The arrow indicates the decrease in $Nb$ in the layer in contact with the oil phase as a function of increasing spread amount. The oil side of the interface showed a compact monolayer, with thickness slightly smaller than a fully extended molecule. A loosely aggregated, diffuse monolayer was found on the aqueous side of the interface and its presence suggested that depletion of ACE-16 from the interface occurs by dissolution in the aqueous phase. The decrease in $Nb$ in the layer in contact with the oil phase, representing the compact ACE-16 monolayer, is indicative of a decrease in the volume fraction of d-ACE-16 and D$_2$O (only an increase in volume fraction of oil CMSi would justify a decrease in $Nb$).

The red line in Figure 8.23 represents the $Nb$ profile for Contrast 5 and is the same reported in Figure 8.22. The profile has been slightly shifted to the left so that the separation between the two layers coincides with the other profiles. The conformation of ACE-16 at the oil-water interface in the presence (red line) and in the absence (black lines) of PA is remarkably similar. The only noticeable difference between the two data sets is the overall layer thickness, which increases when PA is co-adsorbed at the interface ($d \sim 55$ Å for ACE-16 alone, $d \sim 77$ Å for ACE-16 and PA mixtures). The increase in overall layer thickness could be an indication of a dynamic interface when ACE-16 and PA are co-adsorbed at the interface, as opposed to a static interface when ACE-16 is the only species adsorbed.
Figure 8.23. Nb profiles for d-ACE-16 at the CMSi-D$_2$O interface (the black lines), taken from a previous experiment [16]. The arrow indicates an increase in spread amount. The Nb profile for Contrast 5, reported from Figure 8.22, is also shown (red line). The latter profile has been shifted so that the boundary between the two layers coincides with that for ACE-16 alone (black lines).

8.8.3 Implications in the transport mechanism

The steadiness in the adsorption regime observed for both ACE-16 and PA suggests that the interface rapidly reaches saturation at low surface coverage and the excess material is ejected into the bulk phase. Hence, two scenarios are possible:

(i) The excess material in the bulk phase does not interact with the adsorbed layer and there is no exchange between the dissolved material and the interface.

(ii) The co-adsorption process at the oil-water interface is driven by equilibrium. The excess material which resides in the bulk phase exchanges with that in the adsorbed layer, leading to a dynamic exchange at the interface.

This system has been successfully used for metal ion transport in SLM systems; hence a static interface does not seem to be compatible with the efficient transport mechanism. We believe that the second scenario, whereby equilibrium between the material in solution in the oil phase and that adsorbed at the interface is established,
is more compatible with the transport mechanism. In fact, depletion of both ACE-16 and PA from the interface is desirable in a SLM device, where the extraction process takes place at the interfacial region. Only if the molecules at the interface rapidly exchange with those in the bulk is the extraction process highly efficient. A confirmation for the dynamic nature of the interface comes from the increase in overall layer thickness of the interfacial area observed when PA and ACE-16 are co-adsorbed at the interface. Exchange of material between the interface and the bulk phase would in fact lead to a thickening of the interfacial region. However, the experiments in the presence of metal ions in the water phase have not yet been performed; therefore one cannot exclude the possibility of a static interface, where the dynamic process is only generated by the presence of a metal ion gradient.

The mechanism of transport across the membrane has already been discussed [8]; however the mechanism for the formation of the metal-carrier complex at the interface is still under dispute. We tentatively suggest here that, because of the very small adsorbed amount of PA at the oil-water interface, it is more likely the ACE-16 that interacts with the metal ion first. The transport process can eventually proceed via “paddlewheel” formation in the bulk phase [8]. Another favourable effect on the transport mechanism is that the small adsorbed amount at the interface leads to a relative big area per molecule. This suggests that both ACE-16 and PA are in a spread conformation when adsorbed at the interface, which would favour the interaction between the metal ions and the carriers.

8.9 Conclusions and future work

Mixed monolayers of d-ACE-16 and fatty acids at the air-water and oil-water interface were analyzed using neutron reflectivity. First, the effect of the fatty acids chain lengths (palmitic, stearic, and hexacosanoic acid) on the extent of ACE-16 dissolution from mixed monolayers at the air-water interface was quantified. The optimum conditions were achieved when the chain length of the fatty acid was matched to that of the ACE-16 (palmitic acid). These results provide a clear experimental justification for the current empirical composition of the carrier in the SLM membrane for metal ion transport used in devices, where the chain length of
the fatty acid (dodecanoic acid) is closely matched to that of the azacrown ether (ACE-10) [26].

Mixed monolayers of ACE-16 and palmitic acid at the oil-water interface showed a remarkable uniformity in composition with increasing spread amount, where saturation of the interface was already achieved with low spread amount. A very little amount of palmitic acid is retained at the interface and it does not change with increasing spread amount. The excess material accommodates in the oil phase, where it plays an important role in equilibrating the interfacial concentration of ACE-16. In fact, in the absence of PA the adsorbed amount for ACE-16 increases up to a spread amount of about $2.5 \times 10^{-6} \text{ mol m}^{-2}$ (see Figure 8.19 (a)). The presence of PA increases the surface concentration of ACE-16 at low spread amount and facilitates the dissolution into the oil phase at the high spread amount.

The structure of the mixed layer is rougher and thicker than a pure ACE-16 layer, suggesting the instauration of a dynamic exchange between the bulk phase and the interface. Such exchange ensures a continuous turnover which reflects in more metal ions transported through the interface and increased efficiency of the SLM device.

The studies have been performed in the absence of metal ions, whereas in a real device a small concentration of metal ions would be present in the aqueous phase. The last step in the characterisation of these SLM devices involves the understanding of the addition of metal ions such as $\text{Cu}^{2+}$ to the bulk aqueous sub-phase. The presence of ions would not excessively alter the conformation of the adsorbed layer at the air-water interface (extraction is prevented by the lack of a hydrophobic solvent) but may significantly affect the conformation of the adsorbed layer at the oil-water interface.
8.10 References for Chapter 8

Chapter 9

Conclusions

Neutron reflectivity has been extensively used to study adsorption processes at different interfaces. The technique has proved to be extremely versatile with appropriate sensitivity to resolve structural conformations at different interfaces: i.e. from the molecular conformation at a simple air-water interface to a more challenging and complex buried oil-water interface. A summary of the main findings is here reported.

The structural studies of a complex multi-layer system, currently studied for the development of biosensors for the monitoring of water quality, was discussed in Chapter 3. These biosensors consist of several layers of phthalocyanine complexes, which exhibit a colour change in presence of NO\(_2\), trapped between a solid support and a lipid monolayer. This monolayer acts as the sensing region of the biosensor, triggering a colour change in the presence of contaminants in water as the result of damage to the lipid monolayer integrity. A simple design for such possible biosensors was demonstrated. Neutron reflectivity results showed the efficiency of the lipid monolayer in partitioning the phthalocyanine multi-layer structure from the bulk water, which is essential for the viability of these devices.

In Chapter 4 neutron reflectivity was used to study the adsorption processes at the oil-metal interface. The investigation aimed to assess the role of surfactants in lubricant systems. Polarised neutron reflectivity was deployed to provide additional contrasts and enhance the sensitivity to the adsorbed layer. The results showed that the adsorbed amount of palmitic acid increased almost in linear fashion as a function of concentration. A tentative connection between the adsorption processes at the oil-metal
interface and the wear profiles reported in the literature for the same system has been discussed. This work is continuing in collaboration with BP to achieve a deeper understanding of conformations of added surfactants and corrosion inhibitors in the oil lubrication formulation.

Extensive neutron reflectivity studies at the oil-water interface have only recently been achieved with the development of new experimental methodologies. From a general point of view, adsorbed layers at the oil-water interface are much broader and diffuse and less organized compared to the air-water interface. This is thought to be because of the solvation effect by the oil molecules. Van der Waals interactions can establish between the oil molecules and the surfactants’ tail groups, so that tail-tail interactions are much reduced. This is particularly the case in the study of non-ionic dodecanol ethoxylate surfactants discussed in Chapter 6.

In this Chapter the structural study of a series of non-ionic dodecanol ethoxylate surfactants at the oil-water interface was discussed. In order to assess the role of the head group size on the surfactant conformation, the structure of the adsorbed layer was determined as a function of the increasing number of ethoxylate groups using neutron reflectivity. The fitted data suggested that the interface can be divided broadly into two regions: a rough monolayer in contact with the oil phase and a diffuse region, which extends towards the aqueous phase. The head group, as opposed to the globular conformation adopted at the air-water interface, assumes an almost fully extended conformation. This change in conformation can be attributed to the presence of oil molecules in the head group region. The presence of oil molecules associated with the head group region seems to be responsible for the existence of the diffuse underlying structure. It is shown how the interaction between the primary monolayer and the diffuse region increases in strength as the size of the head group decreases. This is not surprising as the surfactant molecules become progressively more hydrophobic and surface active.

The structure of a lipid monolayer at the oil-water interface is reported in Chapter 7. The investigation aimed to explore the possibility of using a lipid monolayer at the oil-water interface as a model for a biological membrane. At low lipid spread amount a rough,
diffuse monolayer was observed at the interface. An increase in the amount of spread lipid results in the formation of a monolayer in addition to micelle-like aggregates at the interface. It is believed that the formation of these micelle-like aggregates must be attributed to spontaneous emulsification processes occurring at the interface. As a result, this first experiment led to a rather low surface coverage and such systems cannot yet be used as suitable membrane models. A possible alternative methodology to deposit a densely packed monolayer and limit the extent of spontaneous emulsification is discussed at the end of the chapter.

Chapter 8 reports the co-adsorption of fatty acid and alkylated azacrown ether both at the air-water and oil-water interface. This mixed system is used for metal ion extraction processes at the liquid-liquid interface. The fatty acid chain length was found to affect the retention of azacrown ether at the air-water interface: the optimum conditions were achieved by matching the azacrown ether chain length with that of the fatty acid (palmitic acid). At the oil-water interface, mixed azacrown ether-palmitic acid monolayers showed a remarkable uniformity in composition with increasing spread amount. Interestingly, saturation was already achieved at the lowest spread amount. In presence of azacrown ether, palmitic acid seems to readily desorb from the interface and dissolve in the bulk oil phase, where it plays an important role in equilibrating the interfacial concentration of azacrown ether. The structure of a mixed layer is thicker and rougher compared to pure azacrown ether layers, suggesting the instauration of a dynamic exchange between the bulk phase and the interface. Such exchange ensures a continuous turnover which ultimately results in more metal ions transported through the interface.