

Thesis of Ph.D. Dissertation

## **Spreading of macromolecules at the air/water interface**

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## Introduction

Over the last century, the relation between bulk and surface properties of aqueous solutions of macromolecules has attracted growing attention in colloid science. Many previous studies have focused on polyelectrolyte solutions and mixtures with oppositely-charged surfactants. The increasing interest has many sources of motivation. The first is to develop a clearer understanding of the structure and the formation mechanism of macromolecular layers at fluid interfaces. The second concerns the widespread applications of these materials in everyday products, water treatment, biomedical device coating, and biocompatible materials. Moreover, since many biologically-relevant macromolecules are either polyelectrolytes (e.g., proteins, nucleic acids...) or surfactants (e.g., lipids), there is scope to extend the knowledge of the behaviour of synthetic P/S systems to fields including the delivery of functional molecules (e.g., DNA, protein/peptides and drugs) and even the fabrication of organic photovoltaic devices. In addition, since all of these materials are processible in aqueous media, a better understanding of the properties of P/S mixtures could lead to industrial applications with reduced environmental impact and better cost-effectiveness.

The bulk and surface properties of oppositely-charged polyelectrolyte/surfactant systems (P/S) systems have typically been treated from the thermodynamic equilibrium perspective. In the bulk, the binding of surfactant molecules to the polyelectrolyte backbone can be described as a cooperative process. This means that the binding occurs only after the concentration of surfactant reaches a given threshold. This concentration is known as *critical aggregation concentration* (cac), which is below the *critical micelle concentration* (cmc) of the surfactant, and it indicates the onset of surfactant binding. After, the bound surfactant amount increases sharply until the cmc is reached, where free micelles start to form. In a thermodynamic perspective, all the species present in the bulk must be at equilibrium. This description follows that of uncharged polymer/surfactant systems. In oppositely-charged P/S systems, however, the strong electrostatic interaction leads to non-equilibrium bulk association. At low surfactant concentrations, if the concentration of polyelectrolyte is kept constant, the mixtures are equilibrium one-phase systems containing swollen *complexes*, formed by micelle-like supramolecular surfactant aggregates wrapped by single polyelectrolyte chains, in equilibrium with the free surfactant molecules in solution. Aggregation of the complexes themselves is prevented since they have high charge density provided by the excess of polyelectrolyte segments. Besides, swollen *aggregates* with high surface charge, provided by the

polyelectrolyte excess, can be formed due to concentration gradients present during mixing. These aggregates can persist in solution even for months in a kinetically-trapped state.

As charge neutrality is approached, the diminishing charge on the complexes reduces the electrostatic repulsion between complexes, which in turn leads to their compaction. The complexes lose colloidal stability and P/S aggregates involving many polyelectrolyte chains are formed. Over time, precipitation (sedimentation or creaming) occurs, and the system evolves to an equilibrium two-phase state (associative phase separation).

At higher bulk surfactant concentrations, the free surfactant molecules in solution adsorb onto the surface of the compact aggregates, thus providing electrostatic stability and suppressing further aggregation. In this region, P/S mixtures are electrostatically stabilized non-equilibrium colloid dispersions.

In addition, steady state bulk properties can be affected by the sample history, e.g. changing the mixing order or the mixing protocol.

Although it has been well established that the non-equilibrium features of P/S systems play a crucial role with regards to their properties, their interfacial behavior has until recently been treated in terms of equilibrium adsorption of the complexes. However, over the last decade, there has been progress made on the influence of non-equilibrium effects at the air/water interface. For example, it has been systematically demonstrated that depletion of material at the interface can occur as a result of slow bulk aggregation, while enhancement can result from the interaction of P/S aggregates at the interface.

Significant attention has been paid over the years to the mechanism of P/S film formation at the air/water interface, and recently work has focused on the interaction of P/S aggregates from the bulk dispersions to their interface. Two main steps of interfacial layer formation were resolved for refined PEI/SDS aggregates: their adsorption followed by their dissociation and spreading. It was also discovered that on the continually-expanding surface of an overflowing cylinder, the majority of the material at the interface can originate from the dissociation of aggregates and the spreading of material, as opposed to complex adsorption. Furthermore, it was shown that highly charged aggregates of poly(amidoimine) dendrimers and SDS remained embedded intact at the air/water interface, while those with a lower charge were not detected.

From these studies, it can be inferred that the properties of P/S mixtures at fluid interfaces are determined by the interaction and, in some cases, the dissociation of P/S aggregates.

The relationship between the bulk and surface properties of aqueous P/S mixtures has, however, been established only in moderate concentration regimes. To date, there has been no evidence that non-equilibrium effects persist in P/S mixtures in very dilute conditions and several questions remain open. In particular, since the surface properties are governed by the interaction of pre-formed P/S aggregates in the bulk mixtures, is it possible to exploit the direct interaction of these aggregates with the surface of a pure aqueous subphase to form stable P/S films out-of-equilibrium conditions, both in terms of surface excess and surface mechanical features? Is the interaction dependent on the charge/structure of the aggregates or does it depend on the total amount of material available? Can the nature of P/S spread films be tuned by varying the ionic strength of the subphase?

The aim of this Ph.D. project is to provide insight into the delivery of macromolecules at the air/water interface by exploiting non-equilibrium effects, occurring both in the bulk of the solution as well as at its interface with air. For this purpose, I have carried out a systematic investigation of the sample history, by comparing macromolecular films formed with different methodologies at the air/water interface, i.e. by spreading or by adsorption from bulk solutions. Adsorbed layers and spread films were prepared at equivalent total final bulk concentration so that only the pathway was changed.

In a preliminary series of experiments, I investigated the mechanism of formation, the stability, the morphology and durability of films of defatted human serum albumin (DF-HSA) spread from droplets of concentrated aqueous solutions, following their contact with the interface of a standard phosphate buffer (SPB) solution. The motivations for this investigation are twofold. First, the physical properties, as well as the non-equilibrium nature, of the DF-HSA film spread by Marangoni flow are known since almost one century, therefore this particular system is an excellent candidate to practice on, in order to learn about the experimental procedures (sample preparation, surface cleaning and instrumental techniques) that would need to be applied systematically, later, for the cases of spread films from P/S aggregates dispersions. Second, this preliminary investigation offered an opportunity to shed more light on an old problem in the field of protein films at the air/water interface, i.e. the effects of the sample handling on the resulting physical properties of kinetically-trapped, surface loaded protein films.

In the main part of the thesis, the possibility of forming surface loaded films from self-assembled aggregates composed of oppositely-charged polyelectrolytes and surfactants, by exploiting the same Marangoni flow mechanism, is investigated and the results are compared

to adsorbed layers from pre-diluted solutions of the two components, in order to shed light on the mechanism of interaction of P/S aggregates with the bare air/water interface. The investigation is focused on the surface excess, the stability and the morphology of films spread from oppositely-charged P/S aggregate dispersions composed of poly(sodium styrene sulfonate)/dodecyltrimethylammonium bromide (NaPSS/DTAB), at a fixed bulk polyelectrolyte concentration (100 ppm) while varying the bulk surfactant concentration (from 0.43 to 25 mM) to generate aggregates with different charge/structure in the spreading aliquots.

In a first experiment, films spread from neutral NaPSS/DTAB aggregate dispersions are the focus, as previous studies indicated that uncharged aggregates may be good candidates for the preparation of spread, surface loaded P/S films. The characterization of the spread films was carried out using ellipsometry both under static and dynamic conditions, while NR was used in situ during compression/expansion cycles of the interface in surface pressure vs. area experiments.

This work was then extended to an investigation of the effects of the charge/structure of P/S aggregates on the surface loading mechanism as well as on the properties of P/S spread films at the air/water interface. A thorough investigation of the effects of the sample history on the film properties and stoichiometry has also been carried out. This work was then extended to an investigation of NaPSS/DTAB films spread on a subphase with elevated ionic strength.

Finally, the effects of the molecular architecture of the polyelectrolyte backbone, as well as its charge density, on the physical properties of the resulting spread films were investigated. For this purpose, the results on NaPSS/DTAB spread films are compared with two other systems: poly(diallyldimethylammonium chloride)/sodium dodecyl sulfate (Pdadmac/SDS) and poly(ethyleneimine)/SDS (PEI/SDS). Pdadmac is a strong linear polyelectrolyte, like NaPSS, while PEI is a hyperbranched polyelectrolyte whose charge density is pH-dependent.

## Methods

Surface loaded DF-HSA films were prepared by spreading several (1-10) droplets of protein solutions (0.01–0.1 mg/ml) onto the bare air/water interface, while the adsorbed layers were prepared by pre-dilution of the same amount of protein.

The P/S mixtures used for the preparation of spread films were prepared by fast-mixing, under stirring for three seconds, equal volumes of polyelectrolyte and surfactant stock solutions at double their final concentration in the spreading aliquots. This method was adopted to minimize concentration gradient during mixing in order to improve the reproducibility. After, an aliquot was spread onto the bare air/water interface. Adsorbed P/S layers were formed by mixing pre-diluted solutions of the two components. The total final bulk concentration was not varied, thus only the experimental pathways for film preparation was changed. The investigation of the properties of macromolecular spread films has been carried out through the combination of several surface sensitive techniques, namely: dynamic ellipsometry, surface tensiometry, Brewster angle microscopy (BAM) and neutron reflectometry (NR). In particular, NR was used to derive *in situ* the surface excess of the individual components. Complementary information on the bulk phase behaviour of the investigated P/S systems have been obtained by optical density measurements at a wavelength of 400 nm (O. D.400) and electrophoretic mobility measurements.

## New Scientific results

1. Spread films of DF-HSA can be trapped at the air/water interface out-of-equilibrium conditions. The dominant contribution to the surface loading mechanism is the Marangoni spreading of protein from the bulk of concentrated droplets rather than the direct transfer of material from their surface films. DF-HSA films formed by spreading are stable over time and can be successively compressed and expanded without any loss of material. In addition, the surface pressure hysteresis during the compression/expansion cycles can be explained in terms of morphological change in the surface film, rather than thermodynamic parameters (chemical potential);

2. The same Marangoni spreading can be exploited to form surface loaded films in oppositely-charged P/S systems by spreading small aliquots of P/S aggregates dispersions onto a pure water subphase. In this case, the polyelectrolyte alone is not surface active and it is only encapsulated in liquid crystalline aggregates, the dissociation of which at the interface leads to the P/S film formation;
3. Films spread from P/S aggregates on pure water are trapped in a metastable state, which can persist for a very long time after spreading, regardless of the charge/structure of the aggregates. These films are highly elastic and they behave as perfectly insoluble membranes. This was confirmed by surface pressure vs. area measurements performed during 5 consecutive compression/expansion cycles of the interface that converged to a limiting cycle, and by monitoring *in situ* the surface excess of the two components with NR.
4. The charge/structure of the aggregates has a strong impact on both the surface excesses of the two components as well as on the mechanical properties and lateral morphology of the resulting spread films. In particular, I have shown that the spreading of negatively charged/swollen NaPSS/DTAB aggregates results in the formation of a homogeneous monolayer at the air/water interface, whose surface excess never exceeds that of a saturated monolayer, either under static conditions by successive additions or under dynamic conditions by surface compression. The picture changes when positively charged/compact aggregates are used, as they have been shown to nucleate the formation of extended structures that ultimately act as reservoirs of surface active material, as confirmed by a range of surface sensitive techniques, performed simultaneously, under both static and dynamic conditions;
5. Polyelectrolyte segments and surfactant molecules bind at the air/water interface in the spread films prepared on pure water in a 1:1 molar charge ratio (measured directly for the first time using neutron reflection), independently of the charge/structure of the aggregates used for spread film preparation;
6. The presence of an inert electrolyte in the subphase (high ionic strength) promotes the thermodynamic equilibrium between the surface and the bulk of the mixtures. Equilibrium is reached quickly excluding the possibility of overloading the interface with aggregates. The entropically less favorable counterion release into the bulk promotes the formation of polyelectrolyte loops at the interface, when the surface is compressed, as opposite to the formation of extended P/S structures under dynamic conditions;



## Publications on the subject of the dissertation

- **Tummino, A.**, Toscano, J., Sebastiani, F., Noskov, B. A, Varga, I., Campbell, R. A., *Effects of Aggregate Charge and Subphase Ionic Strength on the Properties of Spread Polyelectrolyte/Surfactant Films at the Air/Water Interface under Static and Dynamic Conditions*, *Langmuir*, **2018**, *34*, 2312–2323.
- Campbell, R. A., **Tummino, A.**, Noskov, B. A, Varga, I., *Polyelectrolyte/Surfactant Films Spread from Neutral Aggregates*, *Soft Matter*, **2016**, *12*, 5304-5312.
- Campbell, R. A., Ang, J. C., Sebastiani, F., **Tummino, A.**, White, J. W., *Spread Films of Human Serum Albumin at the Air–Water Interface: Optimization, Morphology, and Durability*, *Langmuir*, **2015**, *31*, 13535–13542.

## Conference presentations on the subject of the dissertation

- **Tummino, A.**, Toscano, J., Sebastiani, F., Noskov, B. A, Varga, I., Campbell, R. A., *Spread polyelectrolyte/surfactant films at the air-water interface: effects of aggregate charge and subphase ionic strength*, 31<sup>st</sup> Conference of the European Colloids and Interface Society (ECIS 2017), 3–8 September 2017, Madrid, Spain, *Keynote speaker*;
- **Tummino, A.**, Campbell, R. A., Noskov, B. A, Varga, I., *Polyelectrolyte/Surfactant Films Spread from Neutral Aggregates*, 4th International Soft Matter Conference (ISMC), 12 –16 September 2016, Grenoble, France, *Poster*
- **Tummino, A.**, Campbell, R. A., Ang, J. C., Sebastiani, F., White, J. W., *Spread Films of Human Serum Albumin at the Air–Water Interface: Optimization, Morphology, and Durability*, 4th International Soft Matter Conference (ISMC), 12 –16 September 2016, Grenoble, France, *Poster*
- **Tummino, A.**, Campbell, R. A., Noskov, B. A, Varga, I., *Polyelectrolyte/Surfactant Films Spread from Neutral Aggregates*,

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- **Tummino, A.**, Campbell, R. A., Noskov, B. A., Varga, I.,  
*Polyelectrolyte/Surfactant Films Spread from Neutral Aggregates*,  
32<sup>nd</sup> European Conference On Surface Science (ECOSS 32), Grenoble (France)  
28 August–3 September, *Oral presentation*

### Other publications

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