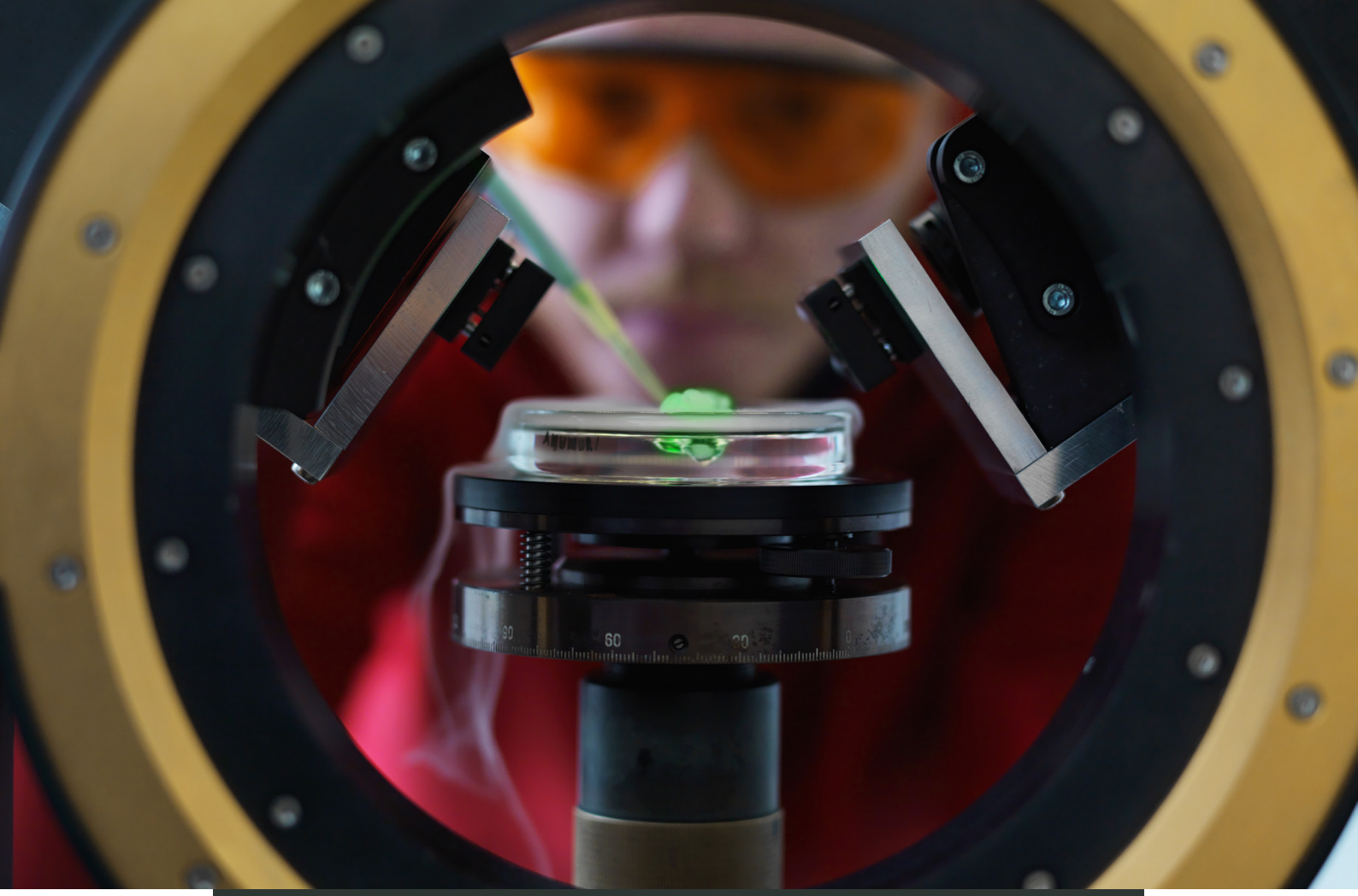

WHITE PAPER

Surface characterization and its usefulness to understand polymer – in fact any macromolecule – and surfactant interactions with the world

Adsorbing macromolecules can *be used to stabilise colloidal dispersions and to modify surfaces, thus supplementing surfactants in several formulations. However, polymer adsorption can also cause issues in processing where polymers adsorb irreversibly onto tubes and linings... Polymer and surfactant interactions can lead to complexes in bulk that deposit on surfaces, which is sometimes desired (hair conditioners) and sometimes not. Proper understanding of the driving forces can guide a product developer through important decisions, and there are tools that can be used to build this understanding. This paper aims to introduce the reader to these tools, the information they can give and what to think of before planning such a study. This paper also aims to introduce the reader to the driving forces behind adsorption since so many decisions in process- and product design can be done better considering these fundamentals.*

:CR: ADDING VALUE THROUGH SCIENCE

The driving forces behind adsorption

The adsorption and accumulation of molecules at an interface is driven and controlled by a series of forces and factors, most notably the hydrofobic effect (i.e. the propensity for hydrophobic moitifs to minimize their contact whith water molecules), electrostatic interactions, and entropy.

Adsorption of surfactants is mainly governed by the hydrophobic interactions, where the amphiphilic nature of the surfactants makes them self-assemble at the interface, in order to minimize the contact between water and the hydrophobic tail of the surfactant. In this case, the entropic loss from self-assembly at the interface is more than compensated for by the enthalpy gained as the water-water and tail-tail contact is increased. On hydrophilic (high energy) solid substrates, this leads to formation of a bilayer in the form of surface micelles, in which the hydrophilic head groups are both oriented towards the hydrophilic substrate and out towards the bulk water. On a hydrophobic (low-energy) surface, on the other hand, a monolayer with the hydrophobic tails directed towards the substrate is formed (Figure 1).

Electrostatic interactions will also play a role, especially for charged substrates and surfactants. For example, negatively charged SDS will not adsorb to negatively charged substrates such as silica due to the electrostatic repulsion from the overlapping Debye-layers of counter-ions. On the other hand, if the substrate is oppositely charged compared to the surfactant, a long-range attraction will lead to quick and strong adsorption at the interface, which will also be favored by the entropic gain from the release of counter-ions (both from the surfactant and the substrate). The attraction as well as the entropic gain will be reduced at higher ionic strengths, where the charges are effectively screened, but this will also reduce repulsions between headgroups of same charge, and thus affect the packing density on the surface.

In other words, the amphiphilic nature of the surfactants and the hydrophobic interactions they cause are the main driving forces for surfactant adsorption, but the exact end-result is a result of *all* factors at play in the system.

The loss in entropy for a molecule caused by adsorption will decrease with the size of the molecule and thus the propensity for adsorption normally increases with the molecular weight. This means that for large molecules, such as polymers and proteins, the penalty in entropic loss when adsorbing at an interface is of less significance. Therefore, amphiphilicity or charge is not required for adsorption to occur, see Figure 1. Moreover, if the large molecule is charged, the entropic gain of the system as a whole will be huge when adsorption takes place at an oppositely charged interface, due to the release of small counter-ions from both the polymer and the substrate. Additionally, polymers and especially proteins often contain hydrophobic domains, which will further increase the propensity for adsorption as well as cause a stronger attachment. For proteins, adsorption often leads to denaturation as the protein changes its conformation (mainly the tertiary structure) exposing its hydrophobic domains to the substrate. The final conformation of a polymer or protein adsorbed will be governed by all factors at play in the system; interactions between polymer segments, interactions between polymer and interface, as well as interaction between polymer and solvent (solvency).

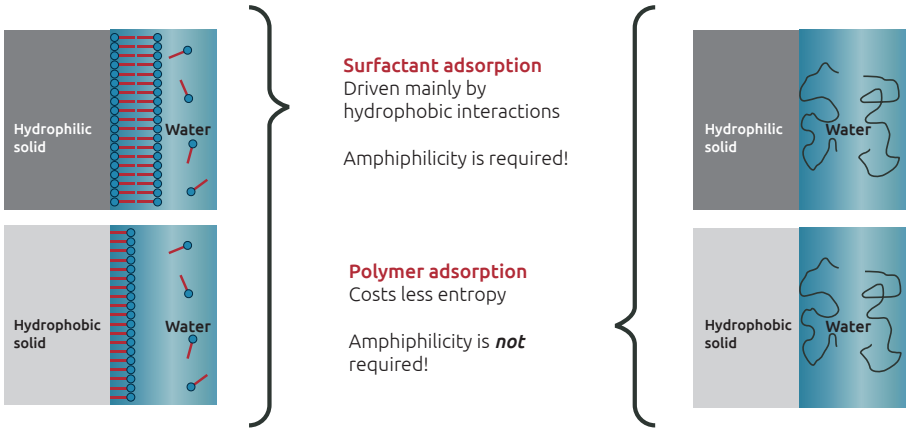

Figure 1. *Surfactant vs. polymer adsorption at solid surfaces in water*

The kinetics of adsorption and rearrangement

Adsorption of small molecules such as surfactants are in general fast, reaching steady state within minutes. Adsorption of larger molecules such as polymers and proteins is slower, due to the more restricted diffusion. Additionally, large molecules often undergo structural rearrangement on the surface in order to adopt a more favorable conformation, which means that it can take hours to reach a steady state.

Polymers and proteins are large and have multiple attachment points to the surface. This means that for such a large molecule to be removed, all anchoring points need to be detached simultaneously, which is statistically disfavored. From a practical point of view, this means that most polymers and proteins are irreversibly attached to the substrate and that they cannot be rinsed off. Furthermore, the sluggish behavior of large molecules adsorbed on the surface means that the initial conformation adopted is often a kinetically trapped conformation, and consequently that the most favorable conformation may never be reached.

"From a practical point of view, this means that most polymers and proteins are irreversibly attached to the substrate and that they cannot be rinsed off."

Surfactants, on the other hand, often only have one contact point with the substrate, and consequently they are normally easier to remove from the surface. Thus, a bilayer of non-ionic surfactants adsorbed on a hydrophilic surface is completely removed by rinsing with pure medium. A monolayer of surfactants adsorbed on a hydrophobic substrate is often more strongly attached, but is, at least, partially removed by rinsing. The difficulty to achieve complete removal in this case, is due to the fact that once most of the surfactants has been removed, the remaining surfactants will lay down on the hydrophobic substrate to minimize the tail-water contact. Surfactants with long hydrophobic tails will thus form multiple contact points with the substrate at low concentrations, thereby making them difficult to remove.

Techniques to study these phenomena

The number of molecules adsorbed to a surface is in most cases negligible compared to the number of molecules that remain in bulk, and therefore measurements of depletion from bulk is not very accurate. This means that to study and accurately measure adsorption we need to use techniques that are able to detect molecularly thin films on macroscopic surfaces. Two examples of such techniques are Ellipsometry and Quartz Crystal Microbalance with Dissipation (QCM-D). Both techniques can be used to follow adsorption as well as desorption (as when rinsing) in-situ at planar surfaces.

Ellipsometry

Ellipsometry is an optical technique that measures the change in elliptical polarization when polarized light is reflected at an interface. The interface needs to be planar and reflecting, but in principal solid/liquid, as well as solid/air and liquid/liquid interfaces can be investigated (although the scope of this paper is focused on solid/liquid interface). The technique is extremely sensitive, and if aligned and calibrated correctly a change of 0.01° in the polarization angles Ψ and Δ can be detected and interpreted in terms of phase shift and amplitude change caused by adsorption/desorption at the interface, corresponding to less than a monolayer of molecuels.[1] If the optical properties of the substrate and the ambient media are known, the mean optical thickness (d_f) and refractive index (n_f) of the adsorbed film can be determined numerically with a resolution of a few Ångströms in thickness and approximately 0.1 mg/m^2 in adsorbed mass.[2] However, optical contrast is needed to get accurate values for thickness and refractive index, meaning that a diffuse adsorbed layer will be detected, but the film needs to be more dense for calculations to be correct ($n_f > n_{bulk}$). On the other hand, errors in the thickness and refractive index will always be co-variant, meaning that an overestimation in the thickness will give an underestimation in the refractive index. This leads to that the product of the thickness and refractive index (often referred to

"The technique is extremely sensitive, and if aligned and calibrated correctly a adsorption corresponding to less than a monolayer can be detected"

as the optical mass) will be more stable and accurate. The optical mass, in turn, can be used to calculate the adsorbed mass, Γ (mg/m^2), using the de Feijter formula.[3]

$$\Gamma = d_f \frac{(n_f - n_{media})}{dn/dc} \qquad (1)$$

This equation is based on the approximation that the increments in refraction increase linearly with concentration up to the concentration found at the surface.

QCM-D

QCM-D is a micro-gravimetric technique, in which the heart of the instrument is the substrate itself. The substrate is a piezoelectric quartz crystal cut in such a way that it will oscillate in shear mode when an AC-current is applied across the surface. The resonance frequency is directly proportional to the mass of the crystal, and is so sensitive that even slight changes in the mass due to adsorption onto the crystal surface can be detected.[4] Consequently, an increase in mass is detected as a decrease in resonance frequency. The adsorbed mass determined by the instrument is the total mass associated with the film, including trapped water. Changes in the resonance frequency are continuously followed for the fundamental oscillation, as well as for several overtones, for the crystal. The instrument also continuously measures the dissipation of the oscillation; that is how quickly the oscillation is dampened as the driving amplitude is turned off. For thin and rigid films, measurements where the changes in frequency and dissipation are low, the Sauerbrey model can be used to estimate the total mass adsorbed:[5]

$$\Delta m = -\frac{\Delta f}{nC} \qquad (2)$$

where Δm is the change in mass, Δf is the change in frequency, C is the mass-sensitivity constant (5.72 m^2 Hz mg^{-1} at f0 = 5 MHz) and n is the overtone number.

The dissipation is related to visco-elastic properties of the adsorbed film, and by measuring both the frequency and the dissipation for all overtones several entities such as total mass, thickness, shear, and viscosity can be modeled for the adsorbed film using the more complex Voight model.[4] One can also model and calculate bulk properties such as viscosity and density.

Adsorption measurements – case studies

Comparison between Ellipsometry and QCM-D

To demonstrate the ability and the different, often supplementary, information given by these two techniques, the sequential adsorption of mucin and lactoperoxidase, will be presented in this section.

Mucins are a diverse group of proteins that consists of a large polypeptide backbone which contains one or more heavily glycosylated domains (68-81% in carbohydrate weight). These glycosylated domains are separated by short “naked” non-glycosylated patches. Because of the high concentration of oligosaccharides, the glycosylated domains are hydrophilic; they are also negatively charged due to the presence of sialic acid residues and sometimes also due to the presence of sulfated sugars. The “naked” patches and the end terminals on the other hand contain a normal distribution of amino acid residues, and are mostly hydrophobic. Furthermore, cystein residues are located in the end terminal, which provides for intra- and intermolecular disulfide bonds. Mucins do not have an ordered tertiary structure and can be seen as a rather stiff random coil polymer. Its function lies in its ability to bind water and form viscous lubricating hydrated layers (such as in the human mucus in saliva). One of the reasons to study mucin adsorption is the relevance for studies related to oral health.

When exposing a hydrophilic negatively charged silica surface to mucin at a concentration of 0.2 mg/ml ellipsometry indicates that a thick film (150 Å) is formed. The refractive index on the other hand is very low (1.35) indicating a low density of the film, and as a consequence the adsorbed mass is low. This means that mucin has adsorbed in a thick, highly hydrated but not very dense layer, with lots of loops and tails extending out to the bulk (see Figure 2). In the QCM-D data the mucin adsorption is seen as a substantial response in frequency and a huge increase in dissipation, indicating a thick viscous and "fluffy" layer (see Figure 2). The total mass calculated from the QCM-D data using the Voight model is several times higher than the "dry" mass calculated from ellipsometry, further indicating that the layer is highly hydrated (up to 90% water calculated by the relative difference in mass).

"This means that mucin has adsorbed in a thick, highly hydrated but not very dense layer, with lots of loops and tails extending out to the bulk."

Since both the protein and the substrate are negatively charged, the driving force for adsorption is not strong, and it is likely that only a few anchoring points are established and probably mainly with hydrophobic domains where there is no electrostatic repulsion. The weak attachment is further manifested in the fact that part of the mucin is readily removed when rinsed with pure buffer.

In the next step of the experiment, the surface with adsorbed mucin is exposed to lactoperoxidase. Lactoperoxidase (LP) is a cationic, globular and stable antimicrobial enzyme natively present in human saliva. It has an isoelectric point of 8.3 and a net charge of +4 eq/molecule at pH 7.0. The polypeptide backbone consists of a single polypeptide chain of 612 amino acids with a molecular mass of 78.5 kDa and a carbohydrate content of about 10%.

Since LP has a net positive charge it has a strong long-range attraction to both the silica substrate, as well as the pre-adsorbed mucin at the surface, which are both oppositely charged to LP. This enzyme can therefore adsorb both to vacant spots on the silica substrate as well as to the mucin and thereby cross-link and collapse the pre-adsorbed mucin on the surface (see illustration in Figure 2). In the ellipsometry results this is seen as a dramatic drop in the thickness and a substantial increase in the refractive index, indicating that a thinner more dense film has formed. The adsorbed mass also indicates that a large amount of LP has adsorbed, and stays attached when rinsing due to the strong attraction and multiple attachment points. Similarly QCM data shows a dramatic decrease in the dissipation, clearly indicating that the film is now more rigid, and the decrease in resonance frequency indicates that the mass has increased, which is also reflected in the total mass calculated. On the other hand, the relative mass (comparing dry and wet) indicates that the water content has decreased to 40% for the collapsed and more compact film, i.e. water has been excluded.

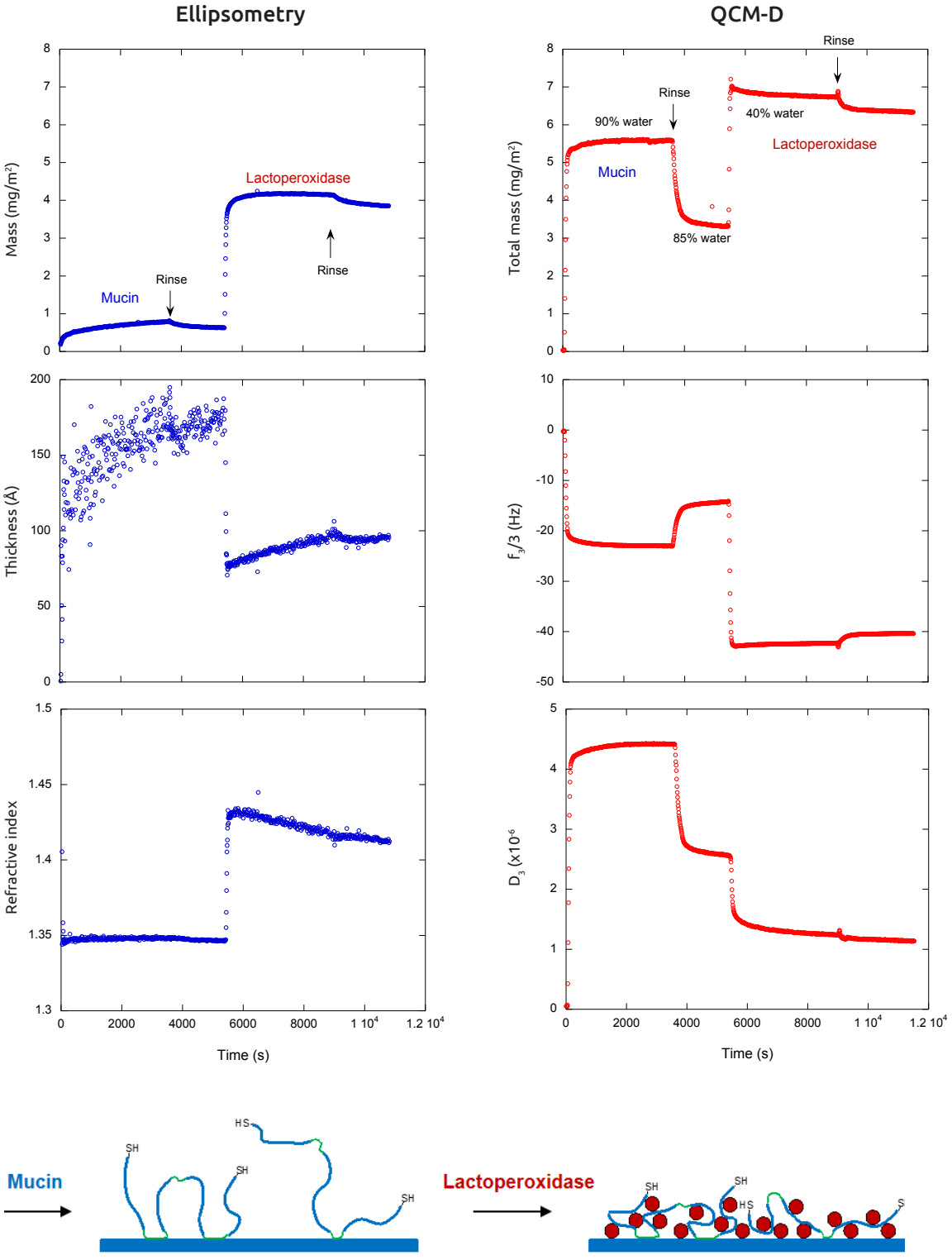

Figure 2. *Sequential adsorption of mucin and lactoperoxidase as studied by in-situ ellipsometry (top left) and QCM-D (top right), and schematic illustration of the molecular conformation on the surface (bottom). Adsorbed mass is calculated using the de Feijter formula (ellipsometry) and the Voight model (QCM-D).*

Comparison between substrates

Now that we know a bit more about how the two techniques work, and how to interpret the data we record, we will study the adsorption of mucin on two additional substrates.

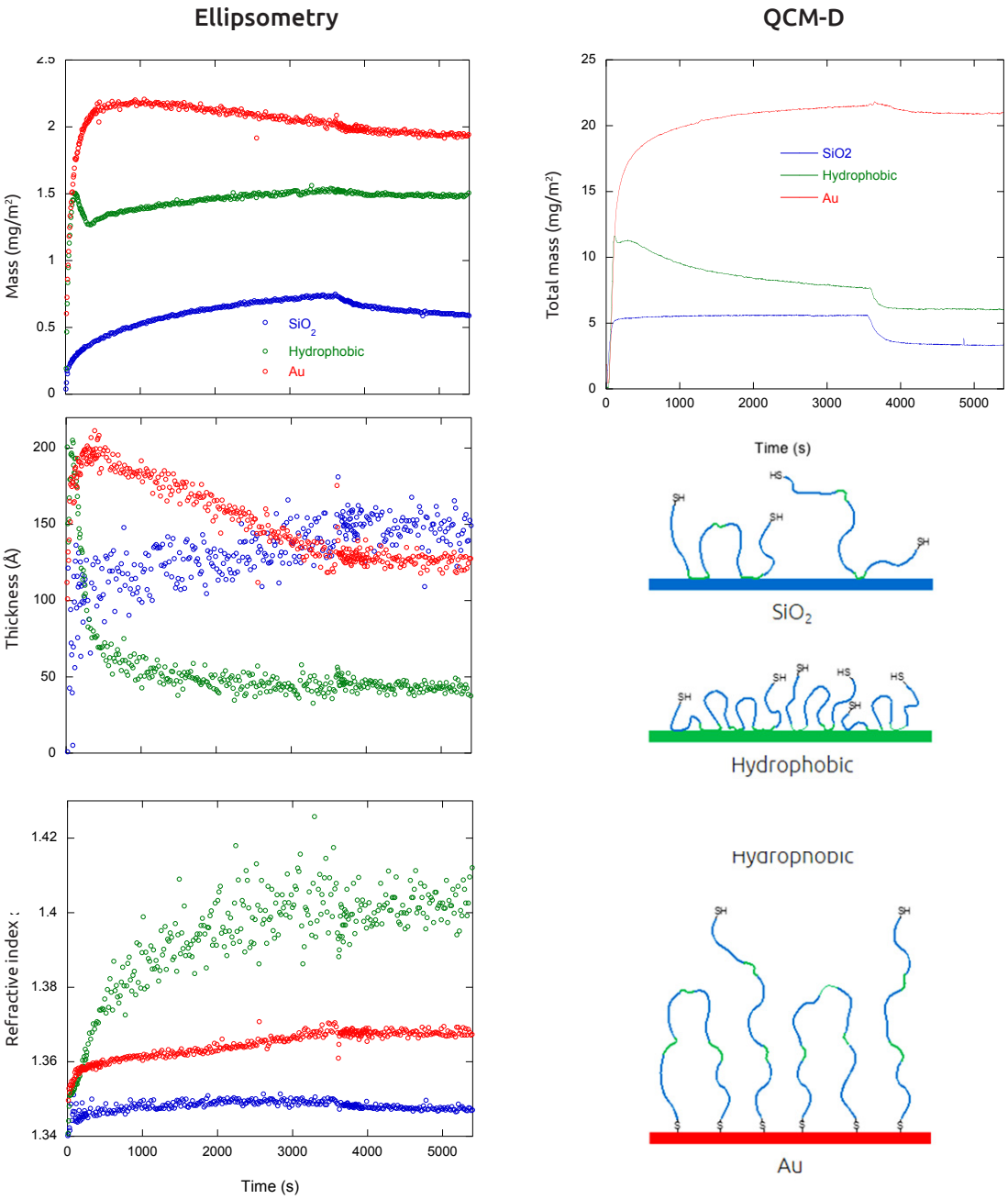

Figure 3. Adsorption of mucin to three different substrates (hydrophilic silica, hydrophobic C8-modified silica, and gold), as measured by ellipsometry (left) and QCM-D (top right). A schematic illustration of the mucin conformation on the three different substrates is provided in the bottom right cartoon.

As previously mentioned silica is hydrophilic and negatively charged and the net negatively charged mucin was found to have low affinity for silica, adsorbing in thick, highly hydrated low-density films. In comparison, the adsorption to a silica hydrophobized with an octylsilane (hydrophobic C8) substrate is much stronger, with considerably higher mass adsorbed. The film thickness is initially high, but quickly decreases to below 50 Å. This observation, in combination with the much higher refractive index, clearly indicates that mucin is adsorbed in a more compact and dense film on the hydrophobic substrate (see Figure 3). This is most likely due to the hydrophobic interaction between the hydrophobic surface and the hydrophobic non-glycosylated patches on the mucin protein. As mentioned before, the hydrophobic interaction is strong, but the long bulky, and rather stiff mucin protein needs to rearrange on the surface to facilitate contact with the hydrophobic patches. This re-arrangement process takes time, which is why the total mass recorded in the QCM is gradually decreasing as the protein folds back on the surface and expels water. The process is also observed in ellipsometry as a gradually increasing refractive index. Since the hydrophobic interaction is strong, and due to that the protein now has more attachment points, the protein is, from a practical point of view, irreversibly attached and nothing is removed during rinsing according to ellipsometry.

Finally, the highest adsorbed amount is reached for mucin adsorption onto gold surfaces. This time, the high mass is combined with high thickness and intermediate refractive index (see Figure 3). The high mass tells us that a larger number of molecules have attached, but this time as a thick film extending far out in the bulk, with lower density compared to the hydrophobic substrate, but higher density compared to the silica surface. Considering the nature of the mucin protein it is possible that the mucin now assumes a conformation where the thiol end-groups are oriented towards the gold substrate, since thiol is known to have specific affinity for gold, forming covalent bonds. The gradual decrease in thickness and increase in refractive index would in that case be due to compaction as parts of the mucin molecules bend to attach with both thiol end-groups to the gold surface (see schematic illustration in Figure 3). The covalent bonds formed are strong and irreversible, and thus no sign of detachment can be seen when the substrate is rinsed.

In summary, the combination of ellipsometry and QCM-D has for this example not only been used to demonstrate how the adsorption is affected by entropy, hydrophobic and electrostatic interactions, but also the strength of the interaction during rinsing, and even the effect of covalent bonds formed at the surface. We have also illustrated how films swell or compact due to outer stress or interactions with other adsorbing entities, and how this affects the water content in the adsorbed film.

Applications for adsorption measurements

In this paper the adsorption of mucin and lactoperoxidase was used as an example, but the adsorption and desorption at interfaces is of outmost importance in many processes, for instance, *detergency, fouling, and surface modification*. Both ellipsometry and QCM-D are therefore vital instruments in understanding the actual surface process, and can give crucial information, insight into, and deeper understanding of how products and processes can be designed, improved and tuned.

An example of how ellipsometry has been used for product development and deeper understanding is when studying adsorption/deposition of polyelectrolyte-surfactant complexes (so called coacervates) to both hydrophilic and hydrophobic substrates.[6] This is essentially the conditioning effect known from hair care products such as conditioners, and by studying the coacervate deposition at different compositions, the shampoo formulation can be tuned for best performance, and it is easier to understand why some polyelectrolytes are more effective than others. Using this approach ellipsometry has also been used to demonstrate the ability of the formed complexes to co-deposit with silicon oil,[7] which is one more important aspect for a conditioner.

At CR the combination of ellipsometry and QCM-D has successfully been used for: Investigating blocking agents and how they can be used to stop protein adsorption; protein and peptide immobilization at implant surfaces; deposition of protective coatings for stainless steel; optimization of shampoo formulations and how they are affected by water quality; detergency and removal of triglycerides; saliva deposition and how it is affected by dental products… just to mention a few examples.

Reasons to be cautious!

Measurements are often made on model surfaces, and thus the results are not always directly transferable to the actual system of interest, at least not in terms of absolute numbers. However, the general trends observed on model surfaces are normally found to be directly relevant for the understanding of the real system, provided that the model is selected with care and treated appropriately.

Mathematical models are used for transferring raw data signals into understandable numbers such as thickness and mass. For ellipsometry these numbers have been found to be in good agreement with data received from neutron reflection experiments for some systems, such as adsorption of C12E5 on silica, and ellipsometry is generally viewed as giving true values (especially for adsorbed mass). QCM-D, on the other hand, will always give the total mass adsorbed (including hydration water), and calculation models are much more sensitive and less accurate. This is especially true for “fluffy” viscous films, whereas better quality data is obtained for flat and rigid layers.

One more parameter to consider is the fact that during adsorption the bulk properties may also affect the data recorded. For ellipsometry this is caused by an increased bulk refractive index at high concentrations, and can easily be compensated for by inserting the true refractive index in the calculations. QCM-D, on the other hand, is extremely sensitive to even small changes in the bulk viscosity, and therefore a change in the signal when rinsing (due to a decrease in the bulk viscosity) is often miss-interpreted as a desorption from the surface. This might, in fact, be part of the explanation to why mucin appears to desorb to a higher extent in the QCM results, where no desorption is seen using the ellipsometer.

The value, however, of using these techniques with care has been shown not only in academic papers but also for several industrial problem statements.

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