Design, Synthesis and Fabrication of Phenolic Thin Films

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ABSTRACT

Design, Synthesis and Fabrication of Phenolic Thin Films

Arison Rajasingam

We present the synthesis and characterization of novel phenolic surfactants 12,12'-*disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate) and 12-mercaptododecyl 4-hydroxybenzoate. These molecules combine the phenolic functionality of tannins found in nature with self-assembly and organizational properties of surfactants. The surfactants consist of a* (C_{12}) *hydrophobic, ω-thiol or disulphide functionality: both commonly used anchors in self-assembly onto gold surfaces. Our modular synthetic route addressed the purification challenges often encountered by using acetyl protecting. Our synthetic strategy unraveled sodium thiomethoxide's ability of unmasking the thiol moiety and simultaneously deprotecting acetyl protecting groups in the presence of an internal ester. This provides an efficient procedure that can be used to synthesize similar surfactants. The synthesized surfactants were studied at the air-water interface by plotting isotherms that showed the thiol and disulfide group had a significant effect at air-water interface. Lower collapse pressure was obtained for thiolated or disulfide surfactant in comparison with the surfactant to its non-thiolated equivalent. We present Langmuir-Schaefer deposition and mixed monolayer as to produce films with varied density by depositing at different pressure. Controlling monolayer density and optimizing the spacing requirement for the interaction of phenol head group with for example proline rich proteins.*

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Chapter 1. Introduction

1.1. Self-assembly

Self-assembly is the spontaneous organization of molecules. In 1946, Zisman was the first to use self-assembly in monolayer formation.[1] Zisman's work details the deposition of amphiphilic molecules (eicosyl alcohol) from a non-polar solvent (n-hexadecane) onto substrates such as platinum and pyrex to form monolayers. His work concluded molecules can spontaneously organize onto a solid substrate to form monolayers given the following three conditions. Firstly, molecules must be able to adsorb onto surfaces to form tightly packed monolayers. Secondly, amphiphilc molecules are required, i.e. with the hydrophilic moiety and hydrophobic moieties located at the opposite extremities. Lastly, the molecules must be adsorbed on the surface ensuring the functionality is derived from the adsorbing molecules, but not from the surface on which the adsorption occurs.

Following Zisman's initial studies, the spontaneous organization of molecules to form films of one molecule thickness adopted the terminology: self-assembled monolayers (SAMs). The extremities of the molecules constituting the monolayer are referred to the head and anchor. The head provides the functionality of the surface. The anchor is responsible for the adsorption on and/or chemisorption to the substrate.

Self-assembly has proven to be a facile and cost effective way to produce functionalized films with a variety of molecules. The resulting (SAMs) have found uses as coatings[2], biosensors[3], and analytical separations[4] to name a few. This varied applicability of SAMs has led to an explosion in the research into SAMs, with variations in elements such as the type of molecules, depositions techniques, and conditions used to self-assemble the monolayers.

The following sections detail the role each element mentioned above and Langmuir and self-assembly deposition techniques.

1.2. Type of molecules

The functionality of a surface is derived from the exposed chemical groups of the surface coating (monolayer) can yield the desired chemical, optical, magnetic and targeting properties.[5] The following section gives an overview of functionalized films formed using polymers and in particular, surfactants.

Polymers are defined as macromolecules consisting of repeating units of monomers[6] and can be classified by structural units into an homopolymer[7], copolymer[8], linear[7], branched[9] and dendrimers with various architectures.[10] Increased use of polymers in self-assembly can be attributed to the ease of preparation and the ability to form layers independent of the size and curvature of substrate.[11] Monolayer and multilayers can form on planar and curved substrates such as spheres, rods, and tubes. The molecular organization of the films depends on interactions such as physisorption, chemisorption, electrostatic, hydrogen bonding, molecular recognition, and charge transfer interaction.[12] The resulting films can be useful for drug delivery scaffolds,[13] permeable membranes[14], sensors[15], and coating on medical devices.[16] A commonly used technique in polymer self-assembly is layer by layer (LBL) deposition. This technique employs a substrate that can be chemically modulated to adsorb to the species of interest. The classic example is the absorption of positive charged ions in solution to a negatively charged template. This technique provides a facile way to create layers on substrates. LBL deposition is further described in the deposition techniques section.

A natural surfactant is extracted from nature without the use of synthesis. Sources of natural surfactants include plants and microorganisms. Although natural surfactants are appealing to create functional films, the difficulty in extraction and availability in small quantity from nature serve as a limitation.[17] An alternative is the synthesis of surfactants that can be tailored to mimic binding sites, electrical properties, and chemical reactivity of surfactants found in nature. The design of surfactants must also take into account aggregation behaviour. This can be predicted using the empirical model outlined by Israelachvili that calculates a packing parameter.[18] The packing parameter $P = v/l \cdot a$ where,

(v) : volume occupied by the hydrophobic portion

(l) : length of the hydrophobic part

(a) : cross-sectional area of hydrophilic head group at the water interface

These define the shape of the aggregate formed in aqueous solution (Fig 1.1).

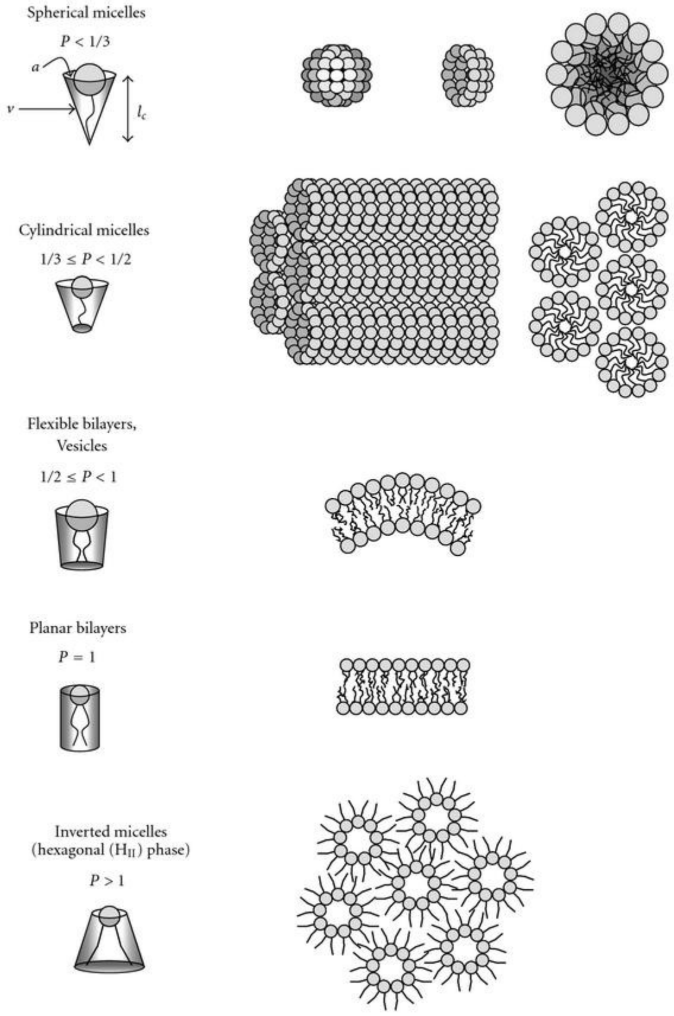


Figure 1.1: Determination of packing parameter [19]

Other factors such as concentration, temperature and solvent play an important role in the aggregation behaviour as well. These parameters also govern the type of film formed at an air-water interface or liquid solid surface (SAM). Our focus will be on the use of surfactants to create functionalized surfaces through self-assembly.

1.3. Deposition techniques

The type of deposition technique used is dependent on the design of the molecules, required lateral density within a monolayer, type of molecule-substrate interaction required and the interface at which deposition is to be conducted. The following paragraphs outline techniques such as layer-by-layer assembly, self-assembly, Langmuir-Blodgett, and Langmuir-Schaefer and their applications.

1) Layer by layer (LBL) assembly techniques were developed by Decher in 1991.[20] It is a versatile approach to create functionalized nanostructures in which properties can be modulated through substrate/template, adsorbing species, and mode of interaction driving assembly. The deposition process commences when a clean substrate serving as a template is immersed in a solution containing the species of interest. In the initial work by Decher[20], a negatively charged template is placed in a solution of positively charged polyelectrolytes. (Fig 1.2)

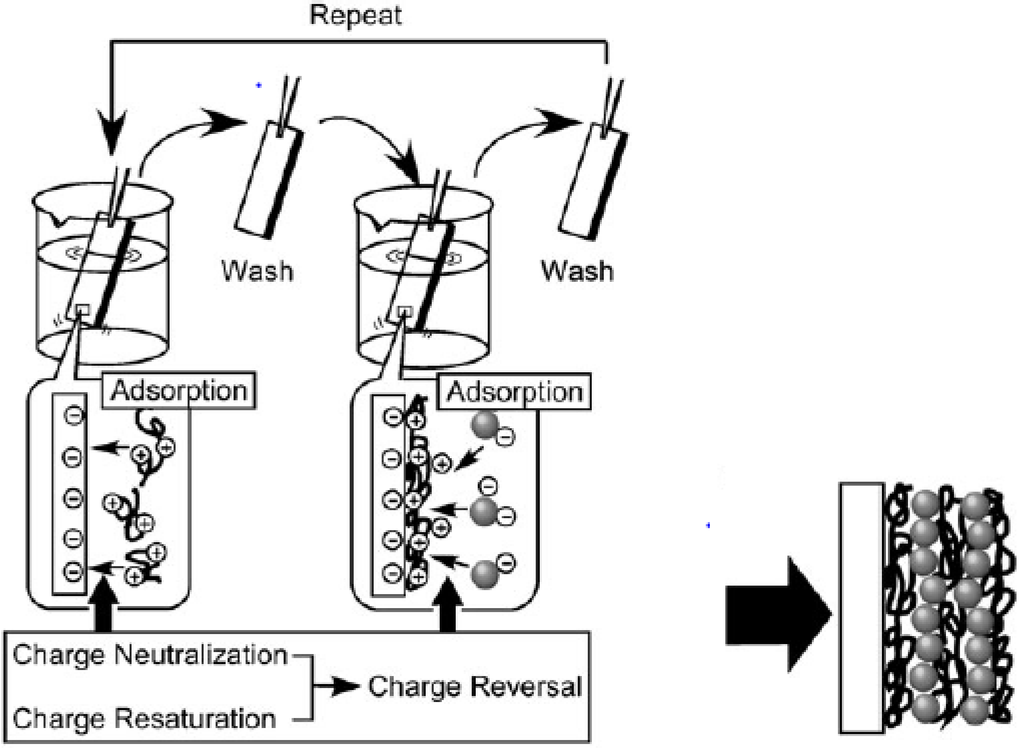


Figure 1.2: Layer by layer deposition overview [21]

Electrostatic interaction acts as the driving force and a layer of positively charged species adhere to the negatively charged template. An excess amount of positively charged species is present in solution in comparison to the species required to interact with the negatively charged template. The process of polymer adsorption with loops and trains leads to overcompensation, namely the absorption of an excess, oppositely charge species resulting in a net positive outer layer. The effect of this charge reversal is that once charge overcompensation is reached, no further adsorption occurs and so the process is self-limiting. Transfer of the positively charged substrate into solution containing negatively charged molecules would again lead to adsorption followed by overcompensation. This process can be repeated to form additional layers. The advantage of this technique is the ease of preparation and ability to prepare multiple uniform layers.[12] The template is a crucial component of LBL assembly. LBL is independent of the structure and size of the template but affected by the chemical composition that determines the mode of interactions and type of species that can absorb. Geometries include planar and colloidal that can be further specified to be non porous or porous.[22] Species can include but are not limited to polymers, lipids, proteins, DNA and nanoparticles. Although initially focused on electrostatic-driven adsorption, depending on the composition of the template, species can also adsorb though interactions such as hydrogen bonding, charge transfer and molecular recognition.[12] LBL assembly has found applications in areas such as enzyme delivery,[13] where enzymes are immobilized on mesoporous silica and then encapsulated by multilayers of a polyelectrolyte using layer by layer assembly. The results demonstrated that enzyme activity was prolonged when encapsulated.[13] LBL presents a facile method to coat implantation device with polysaccharides that have demonstrated anti-adhesion and antibacterial properties[23]

2) Small molecule self-assembly at the liquid-solid interface involves a substrate, molecules of interest, and SAM-favorable conditions. The substrate is immersed in solution for a set period of time required for monolayer formation. A large range of substrates have been used in self-assembly and the choice is dependent on the molecules that will adsorb to form the monolayer. A variety of organosulfur compounds have been surveyed in the formation of SAMs, the most prominent are adsorption of alkanethiols, diaklyl disulfides, diakyl sulfide, alkyl xanthate, and dialkylthiocarbamate. The most popular of the organosulfur compounds is the alkanethiol and their adsorption onto gold substrates.[24]

> Other substrates for alkanethiolates include silver[25], copper[26] and this is not an exhaustive list. The popularity of gold can be attributed the inertness of the gold and the possible chemisorption between gold and thiol. The alkanethiol-gold yields robust, uniform films that are easy to prepare. The alkanethiols initially studied provided robust and thermally stable films. Studies done show films are stable at temperatures at even as high as 200 °C. The robust monolayer can be attributed to the chemisorption of the thiolate onto the gold. The nature of the Au-S bond has not been deduced but the general consensus is the thiol is oxidized at the gold surface to form the corresponding thiolate.[24]

The adsorption of the most commonly used organosulfur compound, an alkanethiol, producing the corresponding alkanethiolate is thought to follow the following (reaction scheme 1.1)

$$\text{R-SH} + \text{Au}^{0}_{n} \rightarrow \text{R-S}^{-}\,\text{Au}^{+} \cdot \text{Au}^{0}_{n} + {}^{1}/_{2}\,\text{H}_2$$

Scheme 1.1: Thiol adsorption on gold

The organization of alkanethiolates on the gold is affected the hydrophobic chains, substrate, strength of interaction between alkanethiolates-gold surface and presence of defects. Hydrocarbon chains position maximizing the van der Waals interactions (VDW). This is more favorable in the absence of a bulky head group that can increase the distance between molecules. Also longer hydrocarbon chain provides more points of contacts for effective interaction.

The organization of the atoms in the substrate affects the organization of the self-assembled monolayer. Gold (111) atoms adopt a hexagonal conformation and the corresponding alkanethiolates SAMs display this conformation whereby the sulfur atom occupies the three-fold hollow site on the gold surface. The distance between such sites on the gold (111) surface is commensurate with the area required for a close-packed alkyl chain.

The strength of interaction plays a role in the organization of SAMs. Thiolates adsorb onto gold creating robust film but the adsorption is labile enough for the diffusion on the alkanethiolate at the gold surface. This allows the alkanethiolates to reorganize following the initial adsorption to fully cover the gold substrate. Alkanethiolates are able to cover the entire surface of the gold in comparison to other substrates.

The organization of alkanethiolates on gold is affected by defects such as grain boundaries (i.e. the misalignment of the crystalline grains). The adsorption onto grain boundaries reduces the van der Waals interaction with neighbouring chains, leads to not as closely packed films.[27]

In addition to the above parameters, the effect of substituting alkanethiol with dialkyl disulfide was investigated by Whiteside's group.[28] These compounds produced identical SAMs with respect to film thickness and quality. Similar to free thiols, diakyl disulfides adsorbed as alkanethiolates at the gold surface. The reaction observed for diakyl disulfide is given below

$$\text{R-S-S-R} + \text{Au}^0_n \rightarrow \text{R-S}^- \, \text{Au}^+ \cdot \text{Au}^0_n$$

Scheme 1.2: Disulfide adsorption on gold

The main distinctions were the rate of adsorption and susceptibility to oxidation. The rate of adsorption is faster for alkanethiolates in comparison to disulfides but disulfides are less likely to be oxidized that allows for longer storage of the molecules prior to use. Solution self-assembly presents an effective and easy way to create robust novel films.

Another route to produce alkanethiol SAMs is by in-situ deprotection of thioacetyl terminated adsorbates.[29] Thioacetyl terminated adsorbates can be deprotected in-situ using a base to form free thiols and the corresponding SAMs. The SAMs resulting from thioacetyl terminal group were shown to be equivalent to SAMs formed by the corresponding thiols.[29] An alternative approach to solution self-assembly is the deposition of a pre-organized film. The first reports of creating organized thin films deposited form the air-water to air-solid interface was by Langmuir and Blodgett in 1930 and now commonly referred to as Langmuir-Blodgett deposition.[30] The molecules at the air-water interface are compressed to a specific area corresponding to the desired structural organization or molecular density. Langmuir-Blodgettry (Fig 1.3) is the vertical deposition of a monolayer onto a

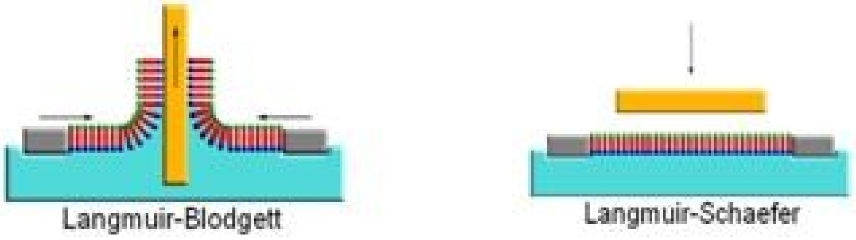


Figure 1.3: Deposition techniques used at the air-water interface

substrate where the direction, upstroke or down stroke deposition, depending on the nature of the substrate (hydrophilic or hydrophobic) whether head groups or tails are desired at the substrate-monolayer interface.[30] Langmuir-Blodgettry provides a simple means to create and control the composition and packing density within a monolayer. Recent interest in this field has been revived with the ability to pattern the deposited organic film on the nanometer scale. Langmuir-Blodgett technique allows controlling size, shape and orientation of patterns by varying monolayer composition, transfer velocity, substrate, and subphase temperature. An example is work done by Chi et al[31], altering transfer speeds created patterns of stripes and channels with different monolayer composition. The stripes corresponded to liquid condensed phase and channels to liquid expanded phase.[31] This patterning ability of Langmuir-Blodgett was employed in the fabrication of silver nanowire with pentagonal cross section and 50nm in length. Using a Langmuir trough, they were able to deposit films of silver nanowires at the air-water interface and compressed. At 0 mNm^{-1} pressures, the silver nanowires are not aligned. Compression at the air-water interface led to alignment of nanowire.[32]

Langmuir-Schaefer (LS) is the horizontal transfer (Fig 1.3) of the monolayer onto a substrate at the air-water interface at constant pressure. As with Langmuir-Blodgettry (LB), an insoluble monolayer is pre-organized through compression and then the substrate is placed horizontally onto the air-water interface, and adsorption of molecules occurs depending on the affinity to the substrate and exposed functional group.

Alternatively, the horizontal substrate can be placed in the subphase and the film lowered until it is in contact with the film, termed reverse LS deposition. For our purposes these methods can be employed with an organosulfur compound spread at the air-water interface to capitalize on the ease and strength of the gold-sulfur interaction[33]. To further understand the principle of deposition at the Langmuir-Blodgett and Schaefer techniques, the molecular organization at the air-water interface must be examined.

1.4. Monolayers at air-water interface

Insoluble surfactants can be spread at air-water interface to study the structural organization and the underlying forces involved before conducting depositions at the air-water interface. A Langmuir film balance, coined in 1917[34] , is still used today and also referred to as a Langmuir trough. The Langmuir trough consists of Teflon well with two Teflon barriers at opposite ends used for compression of a film. The surface pressure is detected using a Wilhelmy plate. The compound of interest is dissolved in a spreading solution, whereby the spreading solvent should have a high spreading coefficient on water and readily evaporate. Chloroform is the most commonly used solvent and up to 20% methanol can be tolerated. The solvent evaporation leaves behind a film (monolayer) of the insoluble surfactant. The molecules can then be compressed using the barriers and the surface pressure measured as a function of molecular area. The plot of surface pressure versus molecular area is referred to as a surface pressure-area isotherm (Fig 1.4). The isotherm provides an indication of the organization of molecular films, which then determines the properties of films transferred onto a solid substrate.

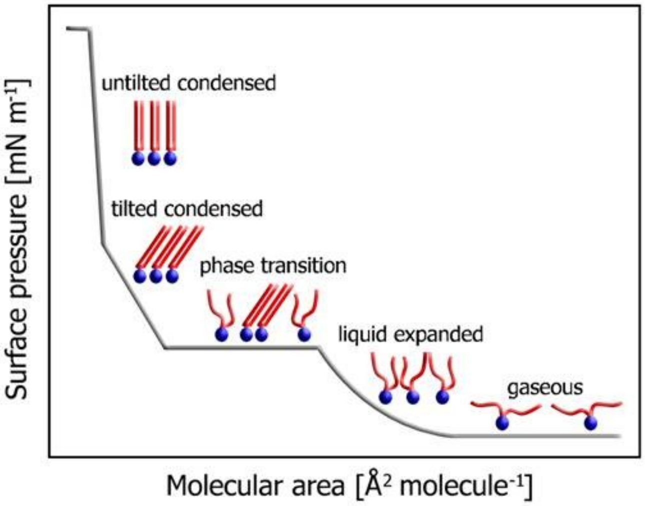


Figure 1.4: Schematic isotherm: plot of surface pressure versus molecular area with schematic depiction of phases formed

Monolayer phase transitions at the air-water interface can be deduced by analyzing the compressibility of the curve and the corresponding area. At 0 mNm^{-1} and high molecular areas the molecules are considered to have little or no interaction with each other. They orient themselves with the hydrophilic group submerged in the subphase and the hydrophobic tails lying on the surface. This phase is called the gaseous phase. With further compression, beyond a critical area, there is a phase transition from gaseous to liquid expanded phase, where the hydrophobic tails begin to interact with each other. This phase, analogous with a 3-dimensional liquid, has high compressibility and low orientational order. Further compression can lead to an organized monolayer, referred to as a condensed phase. In general, these phases are characterized by the lowest compressibility that is the greatest change in surface pressure in regards to a decrease in area. There are actually many sub-classifications based on orientation of the hydrocarbon chain. The orientation can be tilted to the water surface or perpendicular but this discussion is beyond the scope of this thesis. In some cases, the transition between condensed phases is evident from a slope change in the isotherm, for example the transition from a tilted to upright transition. Finally, when the film at the interface can no longer remain a single monolayer under compression due to space and conformational restrictions, the film buckles (termed collapse). The organization at the collapse is difficult to identify and can comprise a buckling of the film, loss of material to the sub-phase, multilayer formation and/or amorphous aggregation. The phase transitions shown in the schematic of the isotherm are not present for all molecules and all conditions. Monolayer formation can also be affected by parameters such as the temperature, concentration and the type of molecule used. In addition isotherms are used to determine the pressure at which deposition are viable, with a general strategy to chose deposition areas 5 $\mathrm{mNm^{-1}}$away from the gaseous phase and collapse pressure.

1.5. Our strategy and LS deposition of thiol examples

Usually, depositions of molecules from the air-water interface do not yield robust films as the molecules physisorb onto substrate. On the other hand, films resulting from solution self-assembly do not always provide complete coverage of substrate.[35] The following problems were addressed with our approach of using monolayer deposition technique such as LS that allows the control of monolayer density and the ability to pre-organize films. By designing surfactants that contain a thiol anchor, chemisorption between thiol and gold can be exploited to form robust films. The following strategy is seldom adopted as solution self-assembly provides a time and cost-efficient way to create films of thiolated compounds on gold coated substrate. However, self-assembly does not enable good control over the lateral spacing of molecules within monolayer that is an essential variable for our long-term goal of studying protein interactions with the film. The necessity to control the lateral spacing will be addressed in the section pertaining to tannins. A question concerning LS depositions with thiolated compounds is the orientation of molecules at the air-water interface. It is expected that the hydrophilic moiety of the surfactant to be submerged in the subphase (water) and more hydrophobic thiol moiety away from the subphase, although before deposition the molecular organization and orientation must be determined. The Metzger group created monolayers of 3 types of thioacetylalkylquinolinium tricyanoquinodimethanide $CH_3C(O)\text{-}SC_{11}H_{22}Q^+ - 3CNQ^-$, $CH_3C(O)\text{-}SC_{14}H_{28}Q^+–3CNQ^-$ and $CH_3C(O)\text{-}SC_{16}H_{22}Q^+–3CNQ^-$ using solution self-assembly, LS and LB. The group demonstrated that solution self-assembly did not yield the uniform films required for the above molecules to be used as unimolecular rectifiers for devices to convert AC current to DC current. Langmuir-Schaefer was used to create molecular electronic devices such as rectifiers. [35] Combining self-assembly and LS has also been used in creating biosensors to study antigen-antibody complex formation. Ihalainen's groups deposited mixed monolayers consisting of lipids with disulfide anchors (DSPPC-1-palmitoyl-2-(16-*S*-methyldithio) hexadecanoyl)-*sn*-glycero-3-phosphocholine) and alkyl terminated lipids with disulfide in the head moiety (DPPGL- 1, 2-dipalmitoyl-*sn*-glycerol-3-phosphoglycolipoate). DSPPC was used to adsorb onto gold substrates using Langmuir-Schaefer depositions. Anti-bodies were attached to DPPGL using the disulfide moiety in the head group. The deposited lipids and presented robust system that can be used to study antigen-antibody complex formation. The resulting antigen-antibody complexes were confirmed by measuring the height using scanning probe microscopy.[36]

1.6. Tannin head group

The functional group of our interest derives from a class of polyphenolic compounds referred to as tannins. The defining characteristics of this subclass of polyphenolic molecules include water solubility, molecular weight, phenolic character, structure and ability to interact with and in some cases precipitate proteins. In terms of weight requirement, 500 – 4000 kDA is characteristic of these phenolic metabolites. The phenolic character corresponds to the number of aromatics groups and hydroxyl substituents present. The standard is 12-16 phenolic groups and 5-7 aromatics per 1000 molecular weight.[37] An example of a hydrolyzable tannin is shown (Fig 1.5); such tannins often contain galloyl moieties comprising a phenyl ring substituted with three hydroxyl groups followed by an ester linkage.

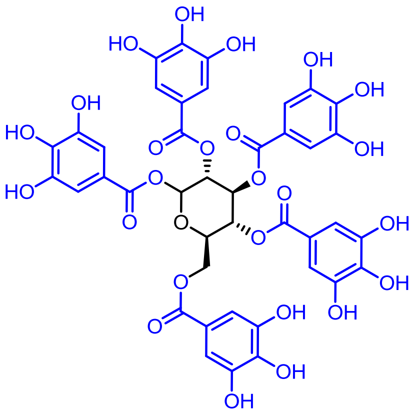


Figure 1.5: Chemical structure of 1,2,3,4,6-penta-O-galloyl-D-glucopyranose showing the galloyl moiety of tannins (highlighted in blue)

Tannins are commonly found in plants and fruits[38] and alcoholic beverages.[39] Some of the reported benefits of high tannin content in food include the apoptosis of cancerous cells[40] and the prevention of cardiovascular disease.[41] Their ability to avoid or reduce such problems has been linked to three attributes. First is their ability to chelate metals such as iron. Lactoferrin, belonging to a subclass of tannin is able to provide antimicrobial activity against E-coli by chelating iron present.[37] Tannins have also shown ability to form complexes with metals such as copper, aluminum, magnesium, and calcium.[42] The second is their radical scavenging capabilities. In vivo, a common route to radical production is the release of an electron from mitochondria of an aerobic cell that can lead to the production of superoxide O_2^-. The superoxide radical can be decomposed by superoxide dismutase to hydrogen peroxide or can be protonated to form hydroxyl ($HO\cdot$) and hydroperoxl ($H_2O\cdot$) radicals**.** Hydroxyl radicals are reactive radicals and play an active role in lipid peroxidation. Lipid peroxidation is a radical mediation chain reaction commenced by hydroxyl radical removing hydrogen from an unsaturated lipid. Radicals have been linked to cardiovascular disease, arthritis and cancer resulting from lipid peroxidation induced by oxygenated radicals. Tannins capable of anti-oxidant activity may prove instrumental in disease prevention and the intake of anti-oxidants such as vitamin C and E has shown amelioration and benefits against cardiovascular disease.[37] The third attribute is the ability of tannins to form complexes with proteins. The most common complexes formed by tannins are with histidine rich proteins (HRPs)[43] and proline rich proteins (PRP).[44] Tannin-PRP interaction involves the stacking of the galloyl ring onto the pyrolidine phase of proline residues. The binding of proline residues is enhanced with an increase in the size of the polyphenol: an increase in the number of aromatic rings has shown tannins to bind proline residues more effectively. The driving force is the hydrophobic effect and hydrogen bonding plays a secondary role in interaction between tannins and proline residues.[45] Similarly, tannin-HRP is suspected to be hydrophobically driven.[46] The interaction of the imidazole ring of the histidine residues with the aromatic ring of the tannins is suspected as the mode of interaction.[47] The tannin-protein complex has specific space requirements for the stacking interactions as will be discussed in Chapter 3. Our concept is to create a tannin mimetic surface with control over lateral spacing to which would confer these useful properties to the substrate. An example, application of such films would be the use of tannins in determining glucose level in saliva. Patients with high glucose level have shown to secrete elevated amounts of salivary proline rich proteins, therefore detecting a tannin-protein complex could be used to monitor patient with elevated glucose content.[3] Another potential application that would exploit the protein binding capacity is as a surface coating for immobilized phases in column chromatography[48] or as a biocompatible coating for nanoparticles.

1.7. Thesis overview

This thesis focuses on the concept of combining the structural and organizational properties of surfactants with the phenolic properties of tannins. The route needs to be modular to enable the synthesis of molecules with variation in anchor, chain length and head group. Both thiol and disulfide anchors are employed as they form robust films on gold coated solid supports. The hydrophobic spacer provides the surface activity and surface organization and needs to be appropriate to both self-assembly and Langmuir monolayer depositions. The phenolic head group will provide the functionality of the film and for proof-of-principle; both mono and tri-hydroxy phenolic surfactants will be created.

Chapter 2 focuses on the development a modular route for synthesis of molecules and highlights the challenge of purification especially with molecules containing strongly adhesive functional groups. This was published as Rajasingam, A.; Schmidt, R.; Woo, S.; DeWolf, C.; Forgione, P. *Synthetic Communications* **2014**, 44, (8), 1066-107.

Chapter 3 demonstrates that the surfactants are able to form Langmuir monolayers at the air-water interfaces and that surface coatings can be generated by a variety of deposition methods including solution self-assembly and LB and LS depositions. Preliminary work on controlling the surface density within these deposited films is also shown. This chapter is written as a manuscript to be submitted to Langmuir.

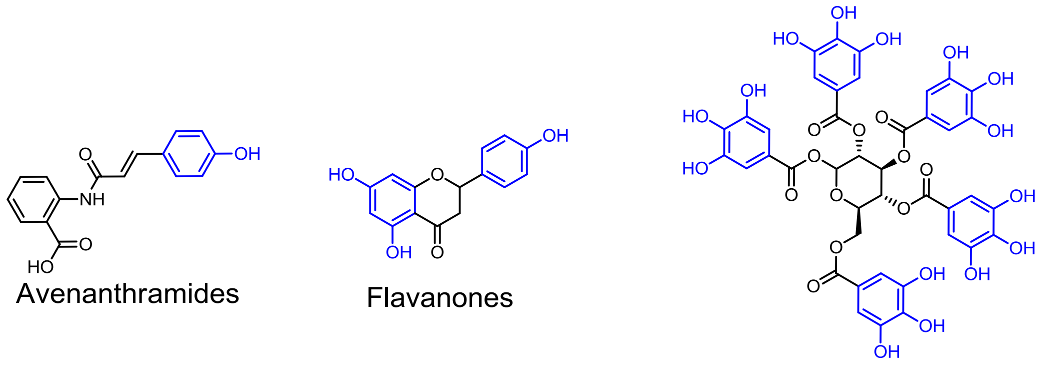
Chapter 4 will provide an overall discussion, final conclusions and future directions.

Chapter 2. Efficient preparation of novel phenolic surfactants for self-assembled monolayers

This chapter was published as a communication (Rajasingam, A.; Schmidt, R.; Woo, S.; DeWolf, C.; Forgione, P. Synthetic Communications 2014, 44, (8), 1066-107) and is included as this chapter as published with the following exceptions: the abstract and keywords can be found in Appendix A (included for completeness) and the order of text has been modified such that the experimental follows the introduction. I carried out all experimental work and wrote the first draft of the manuscript under the supervision of Drs. Pat Forgione and Christine DeWolf. Dr. Rolf Schmidt contributed to experimental design, discussion and editing of the manuscript. Dr. Simon Woo contributed to the editing of the manuscript.

2.1. Introduction

Surfactants are an important class of molecules that can be used for a variety of applications. Films formed using surfactants have found applications as protective coatings,[49] models to study protein adsorption on varied surfaces[50] and fabrication of microarrayed chips.[51] The anti-oxidant and protein binding abilities of tannins[37] (Fig. 2.1), when combined with the film forming capabilities of surfactants, present potential applications for these novel surfactants as biosensors[3], coatings[2] and immobilized phases in column chromatography.[4] Tannins are a class of polyphenol-derived compounds commonly found in plants and fruits[52] and alcoholic beverages.[53] Some of the reported benefits of high tannin content in food include the apoptosis of cancerous cells[54] and the prevention of cardiovascular disease. [55]



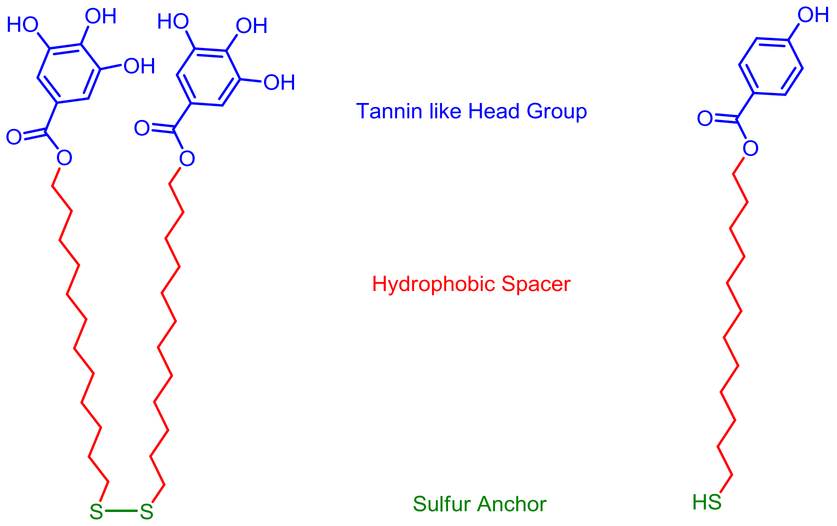
Avenanthramides

Flavanones

Pentagalloyl Glucose

Figure 2.1: Naturally occurring tannins

Our focus is to create tannin-like films that can confer biocompatibility and protein-binding abilities to a surface. We designed novel surfactants (Fig 2.2) combining tannin-based head groups and a hydrophobic spacer with either ω-thiols or terminal disulfides to produce monolayer films chemisorbed onto gold surfaces. These molecules exploit the ease of self-assembly and the strength of the Au-S bond. Both thiols and disulfides, although structurally different, have been shown to form alkyl thiolate self-assembled monolayers (SAMs) at the gold surface.[28] Furthermore, film properties such as film thickness, wettability and rate of formation were independent of the oxidation state of the surfactant.[28] A variety of functionalities, including different phenolic moieties, can be introduced at the head group to control the exposed surface of the monolayer.[24]



1A **1B**

Figure 2.2: Novel phenolic surfactants

C$_{16}$ aliphatic surfactants with mono-, di- and tri-hydroxyl substituted aromatic rings have been synthesized exclusively without ω- thiol functionality *via* direct esterification.[56] The synthesis of related surfactants has also been accomplished through DCC activated esterification of an aliphatic alcohol with the corresponding carboxylic acid. [57,58,59] Other synthetic routes involve the use of catalytic Pd[60] or acidic functional ionic liquids. [61] Once the long hydrocarbon chain of the alcohol is incorporated into the surfactant molecule, the chromatographic behavior of the alcohol and the esterification product is often similar. In cases where the esterification is incomplete, purification of the product can be challenging due to co-elution of the esterification product with any unreacted aliphatic alcohol during chromatography. As a result, alternative routes were investigated that would overcome these limitations, namely a strategy where the galloyl moiety would be protected to facilitate purification of the intermediates. This approach still required a method to introduce the ω- thiol group, and needed to consider the chemoselectivity of reactions subsequent to the esterification reaction in order to maintain the integrity of the internal ester linkage. The hydroxyl groups of the gallic acid moiety are often protected using acetyl[62] and benzoyl groups.[63] This modification is used to accommodate multi-step synthesis where the hydroxyl groups may interfere with the transformations required during the synthesis. The polarity of the gallic acid moiety is also reduced, facilitating purification by normal phase column chromatography.

The other consideration is the ω-functionalization for gold-thiol chemistry and chemisorbed films. Organic thiosulfates have found wide use in self-assembly, as an Au-S bond forms as a result of the cleavage of an $S\text{-}SO_3$ bond at the gold surface. Preparation of the thiosulfate salt is required for this route that may add unnecessary complication to the synthesis.[64] Syntheses of thiol functionalized amphiphiles have been accomplished through nucleophilic substitution with hexamethyldisilathianes in the presence of tetrabutylammonium fluoride.[65] A general route to introduce ω - thiol groups involves the deprotection of thioacetates. This method appeared to be more suited to our needs since the thioacetate group could be introduced into our surfactant molecule through an S_N2 displacement reaction on an appropriate intermediate.[66]

Conditions for the removal of the protecting groups, once they have served their purpose, also needed to be considered. Previously used modes of acetyl deprotection include costly metals such as palladium.[63] Another, more cost-effective route was described using ammonium acetate providing selective deprotection under neutral conditions. However, it has not been demonstrated in the presence of thioacetates.[67] Thioacetates have been deprotected using sodium thiomethoxide,[67] and using tertbutylammonium cyanide[68]. The procedure using tertbutylammonium cyanide allows for the deprotection of thioacetates in the presence of an acetate group but is not exclusively selective for the deprotection of thioacetates, while sodium thiomethoxide is reported to have better selectivity. Thus, the timing of and the conditions used for the deprotection step(s) in the sequence would have to be considered carefully in order to avoid potential issues with a lack of chemoselectivity. Possible oxidation of the deprotected thiol to the corresponding disulphide was only a minor concern, since, as mentioned previously, both species can be used to produce gold-thiolate SAMs. Above all, however, the deprotection steps must not cleave the internal ester. The resulting novel surfactants will be further analyzed as self-assembled monolayers for possible tannin-like properties, and these studies will be reported elsewhere in due course.

2.2. Results and discussion

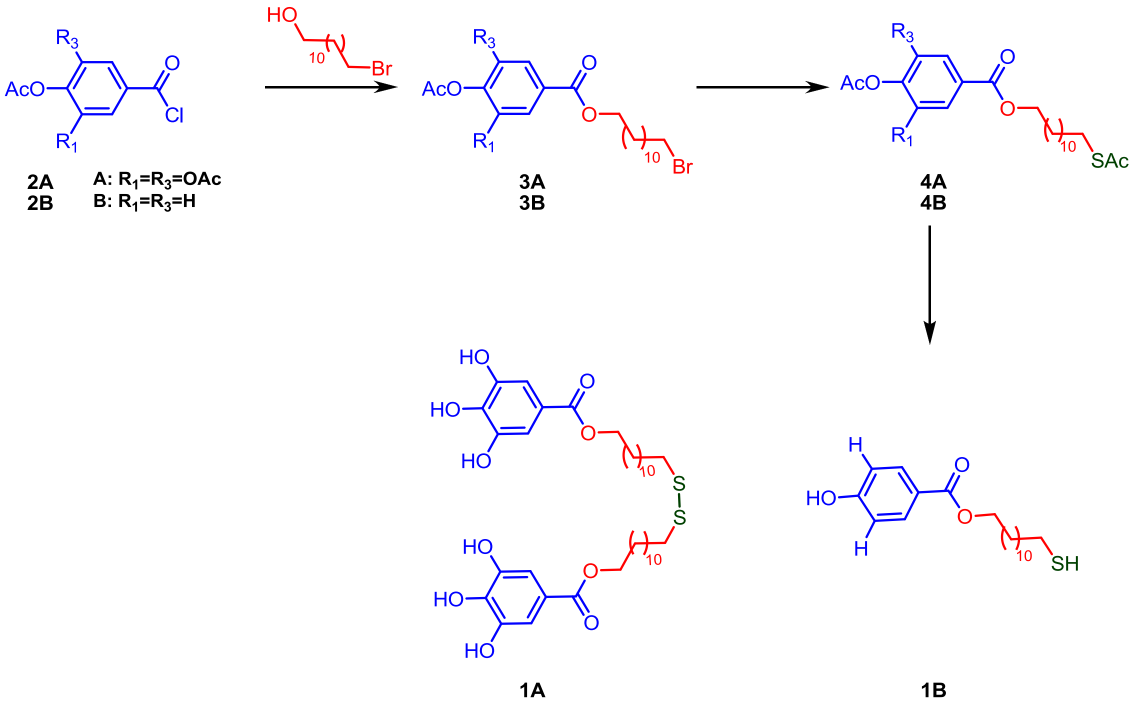
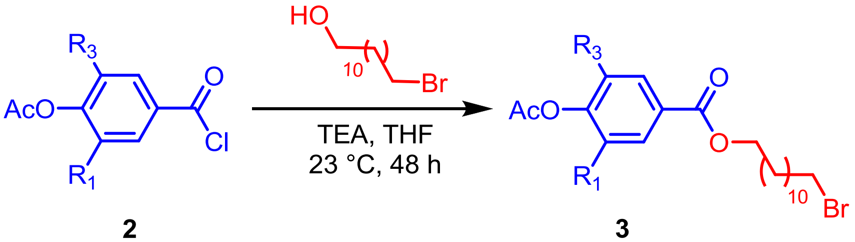


Figure 2.3: Efficient preparation of amphiphilic surfactant

The initial esterification of the proposed modular route was accomplished *via* an acid chloride (2A) prepared from the corresponding carboxylic acid[69] (Table 2.1). Initial results employing a 1:1 ratio of acid chloride (2A): alcohol provided the corresponding bromide product (3A) in 56 % yield. However, a significant amount of unreacted alcohol remained that rendered the subsequent purification challenging (entry 1). Increasing the amount of the acid chloride (2A) employed to 1.25 equivalents both increased the yield to 72% and simplified the purification process (entry 2). These conditions were then also applied for the preparation of the protected mono-hydroxy bromide (3B, entry 3) from the corresponding acid chloride (2B).



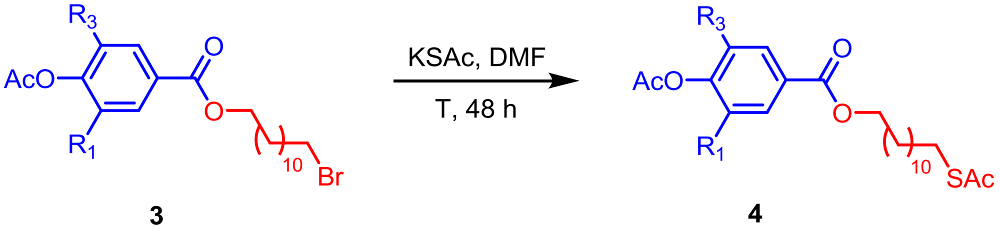
2 **3**

A: R_1=R_3=OAc B: R_1=R_3=H

Entry	Compound	Equivalents Acid Chloride	Equivalents Bromododecanol	Yield (%)
1	3A	1.0	1.0	56
2	3A	1.25	1.0	72
3	3B	1.25	1.0	63

Table 2.1 Effect of equivalents of acid chloride on esterification

The subsequent S_N2 substitution of thioacetate on the alkyl bromide (3A) was then investigated[66] (Table 2.2). Initial attempts employing a 1:1 ratio of the brominated starting material (3A) and potassium thioacetate at room temperature proved unsuccessful yielding a complex mixture (entry 1). When the reaction was conducted starting at a lower temperature, however, the corresponding thioacetate product was obtained in a 78% yield for the triacetylated compound (4A, entry 2) and 69% yield for the monoacetylated analog (4B, entry 3).

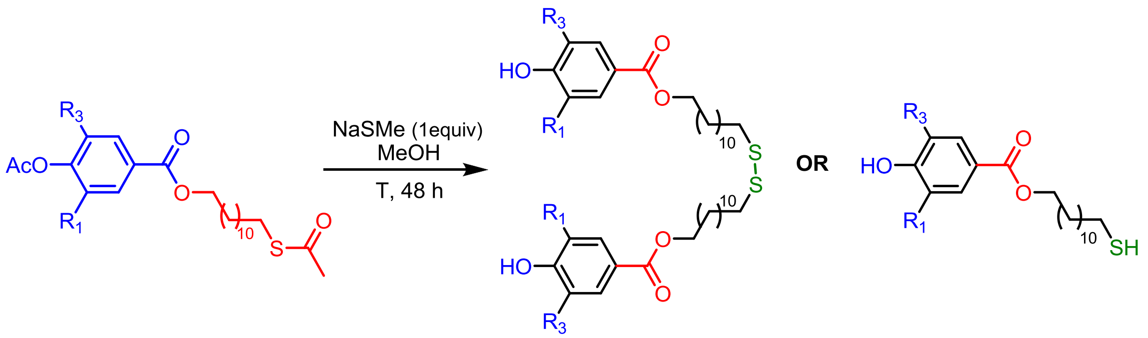


A: R_1=R_3=OAc B: R_1=R_3=H

Entry	Compound	T (°C)	Yield (%)
1	4A	23	----
2	4A	-78-23	78
3	4B	-78-23	69

Table 2.2: Synthesis of thioesters from bromide

The key deprotection of the thioacetate (4) to produce the corresponding thiol or disulfide (1) in the presence of the internal ester was subsequently examined[67] (Table 2.3). Initial attempts employing a 1:1 ratio of sodium thiomethoxide to thioester at 23 °C proved unsuccessful (entry 1), producing a mixture of unidentified products. In contrast, when the starting reaction temperature was lowered, the sodium thiomethoxide deprotected the thioacetate to form the corresponding thiol but also displayed a unique ability to simultaneously deprotect the phenolic acetates (Table 2.3). The transformation provided yields of 53% for the trihydroxy (1A, entry 2) and 36% for the monohydroxy (1B, entry 3) products. The cleavage of the phenolic acetates, while welcome, was somewhat surprising since Wallace and Springer had reported that esters were stable to these reaction conditions, and had even reported an example of the cleavage of a thioacetate in the presence of acetates of primary and secondary alcohols.[67] The fact that the internal ester remained intact is in accord with their observations, and suggests that the cleavage of the phenolic acetates may be a reflection of the higher stability of a phenol as a leaving group compared to an aliphatic alcohol. Cleavage of phenolic acetates has been previously observed in mildly basic methanol,[70,71] which may indicate that the deprotection of the phenolic acetates in our reaction is a consequence of the mildly basic methanolic reaction conditions produced by the sodium thiomethoxide. Regardless, this double deprotection employing sodium thiomethoxide presented an ideal route, as the thiol and hydroxyl groups on the aromatic ring were unmasked while a key internal ester linkage was maintained that facilitated the synthesis of these surfactants. Interestingly, while the monohydroxy analog was isolated as the free thiol, the trihydroxy analog was more susceptible to air oxidation and was isolated as the disulfide. The reason for this difference is not clear, but is inconsequential for our purposes as both can be used to create the desired thiolate-gold surfaces.



4 **1A** **1B**

A: R_1=R_3=OAc B: R_1=R_3=H

Entry	Compound	T (°C)	Yield (%)
1	1A	23	----
2	1A	-78-23	53
3	1B	-78-23	36

Table 2.3: Simultaneous deprotection of acetyl protecting groups and thioacetate of amphiphilic surfactant

2.3. Conclusions

In summary a novel di-deprotection strategy has been employed in order to synthesize a novel class of surfactants. The most commonly faced challenges in the preparation of surfactants involve the polarity of the molecules and the ability to remove impurities that tend to co-elute with the desired product, both of which were surmounted in this route by the judicious use of protecting groups. By delaying the deprotection steps until the end of the synthesis, the chemoselectivity of thioacetate over acetate cleavage was overcome by employing a single reagent to simultaneously remove both these protecting groups to produce the desired surfactants in good yield. The use of sodium thiomethoxide in a di-deprotection strategy as demonstrated herein could be applied to a range of related surfactants. The novel surfactants synthesized above have been further analyzed as self-assembled monolayers for possible tannin-like properties, and these results will be presented in due course.

Supporting Information

Full experimental detail, 1H and 13C NMR spectra can be found in the Appendix.

Chapter 3. Mimicking a Tannin: Creating a Phenolic Surface Using Self-assembly

This chapter has been prepared as a manuscript for submission to Langmuir with the following authors: A. Rajasingam, R.Schmidt, P. Forgione and C. DeWolf. I carried out all experimental work and wrote the first draft of the manuscript under the supervision of Drs. Pat Forgione and Christine DeWolf. Dr. Rolf Schmidt contributed to experimental design and discussion.

3.1. Introduction

Polyphenols, a class of molecules comprising aromatic rings and hydroxyl groups, have found uses in avenues such as nutrition[72] and health.[52, 73] The functional properties of this class of compounds include metal chelation, anti-oxidant activity and the ability to interact with specific proteins.[37] Surfaces modified using phenolic compounds have found applications as coatings on electrodes[74] and biosensors.[75] Such films can be self-assembled as surfactant or polymeric films. Self-assembled monolayers of surfactant have been reported for ω-thiolated long chain phenolic surfactant by Horton et al[76], to emulate the interaction of phosphate pollutants occurring in water systems resulting from soil fertilization. The phosphate species are known for its interaction with natural organic components such as phenolic compounds in water. They used solution self-assembly to create thiolated phenol films on gold coated atomic force microscopy tips to study the effect of ordering and placement (*ortho-* versus *meta-*) of phenolic diols on their interaction with phosphate species. Using adhesion forces as a function of pH, they determined that film ordering and intermolecular hydrogen bonding determined the hydroxyl availability and solvation that both impacted the film pKa and interaction with the phosphate. Moreover, the self-assembled films of 4-(12-mercaptododecyl)benzene-1,2-diol and 5-(12-mer captododecyl)benzene-1,3-diol were determined by ATR to be low density and disordered, attributed to the bulky terminal phenol group.

Self assembled films of polyphenols such as tannin acid have been shown by Caruso et al. to chelate with metals,[77] especially with iron.[77, 78] The films form as a result of a complex formed between the polyphenols and metals and self-assembles on a variety of substrates, including adsorption onto a capsule that can then be dissolved to yield a polyphenol-metal framework with proposed applications in drug delivery. The work done by Caruso et al[77] and Horton et al[76] demonstrates the ability of polyphenol (tannin) films to confer the properties of the polyphenol to the substrate.

The functional group of our interest is the galloyl moiety (Fig 3.1) that is found in the subclass of polyphenols referred to as tannins, with applications including metal adsorption,[79] decontamination of crude oil contaminated soils[80], corrosion prevention[81] and their interaction with proteins[3]. Tannins have been shown to precipitate proteins, specifically proline rich proteins that can be found in human saliva[44] and it has been proposed that monolayers and bilayers of gallic acid-derived lipids could be used to tether cells[82].

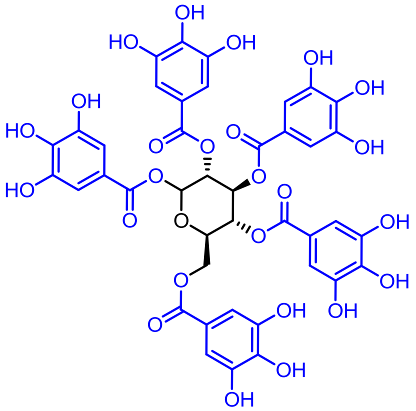


Figure 3.1: 1,2,3,4,6-penta-O-galloyl-D-glucopyranose

Tannin-proteins interactions involve hydrophilic induced stacking of galloyl rings of tannins to the pyrrolidine face of proline and are favoured when there is a sequence of multiple proline residues in the peptide sequence. (Fig 3.2) This suggests that for a surface film to be effective in binding proteins, thereby providing a biocompatible surface for proteins and cells, the control of lateral spacing between the phenolic head groups is crucial. As noted above the space around the hydroxyl can play an important role in the strength of interaction with other species.[45] The number of phenols may also be an important parameter both for regulating the protein interaction and for films properties. The latter derives from our group's previous work[56,83] demonstrating that phenolic lipid films shows extreme cohesiveness and a tendency to form a hydrogen-bond network between the head groups. In this study, we report on the film forming capabilities of two phenolic surfactants, 12-mercaptododecyl 4-hydroxybenzoate and 12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate). Control over lateral phenol density in films deposited onto solid surfaces was approached by) controlling the number of hydroxyl groups on the phenyl ring and varying the intermolecular distance between the head groups. The former will be utilized to determine the optimal number of hydroxyl groups for protein binding and the latter will control ring spacing.

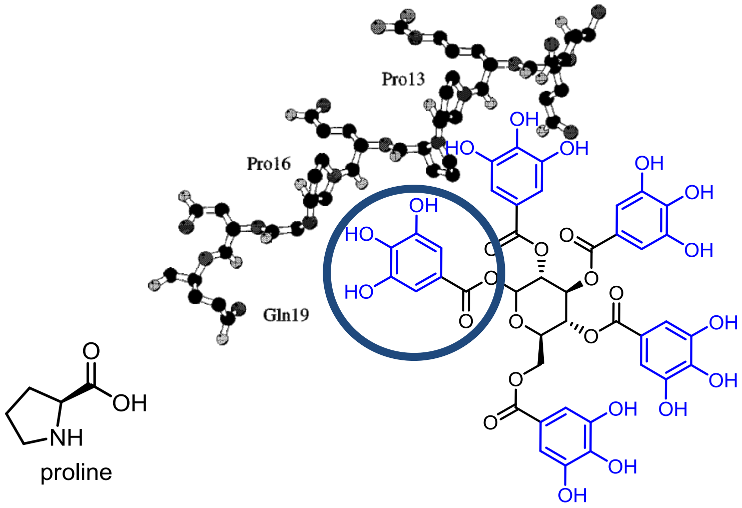
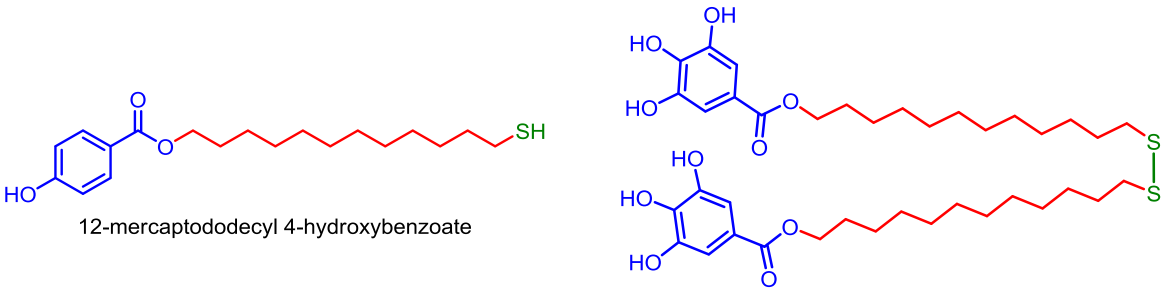


Figure 3.2: Tannin-proteins interactions involve hydrophilic induced stacking of galloyl rings of tannins to the pyrrolidine face of proline

We have previously reported the synthesis of ω-thiolated and disulphide phenolic surfactants[84] (Fig. 3.3). A key aspect in the selection of this class of polyphenol is the presence of the carboxylic acid group which can be easily be converted to esters, amides, and acyl chlorides; this approach was used to create a modular synthetic route to control the number of hydroxyl substituent on the head groups and create surfactants that can be used to functionalize surfaces.



12-mercaptododecyl 4-hydroxybenzoate

12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate)

Figure 3.3: Novel thiolated phenolic surfactant

The design was created with the intention of employing chemisorption between thiol to gold to create robust films. For reasons outlined below, control over lateral density was sought by 1) the deposition from the air-water interface at different molecular areas/surface pressures, 2) the use of disulfides as an alternative to thiolated surfactant as these molecules have different spatial requirements, and 3) deposition of mixed monolayers that include a non-thiolated spacer. Lateral density can be controlled by varying the parameters used for solution self-assembly; however this method can yield non-uniform surfaces and island formations[35]. This may be especially problematic for phenolic surfactants where strong intermolecular interactions have been reported.[56] This led us to attempt to control the lateral density by pre-organization of a film at the air-water interface and deposition by Langmuir techniques to the solid surface. The strength of intermolecular interactions can also be manipulated by variation of the number of hydroxyl groups on the head group. Similarly the anchoring group can be varied to manipulate film organization. Disulfides for example, will have different area requirements at the air-water interface that would lead to altered organization and densities. Whitesides et al[28] has previously shown that kinetically the adsorption of disulfides is slower, both disulfides and thiols can yield similar quality SAMs. Moreover, the use of a disulphide may prove advantageous for film fabrication as it may provide better stability for the storage of the compounds prior to use. Finally, the deposition of mixtures of thiolated and non-thiolated surfactants with subsequent removal of the physisorbed component. The physisorbed material can be removed after deposition and the space may be backfilled by self-assembly with a shorter, non-functional spacer that will retain the spacing and ensure accessibility for the protein to the phenolic head group. Vogel et al[85] had previously demonstrated this approach using a mixture of thiolipids and palmitic acid deposited from the air-water interface onto gold using Langmuir-Blodgett deposition. The physisorbed palmitic acid is washed off, only leaving the chemisorbed thiolipid. Fluorescence microscopy images taken following the washing of the film showed the disappearance of domains corresponding to palmitc acid. It was demonstrated that self-assembly of a second of thiolated compound can fill the voids created by removed palmitic acid. This approach using mixed monolayers may provide an alternative route to manipulating the surface density. In this paper, we report the behaviour of two phenolic compounds (12-mercaptododecyl 4-hydroxybenzoate and 12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate)) at the air-water interface and compare films formed by LB, LS and self-assembly depositions.

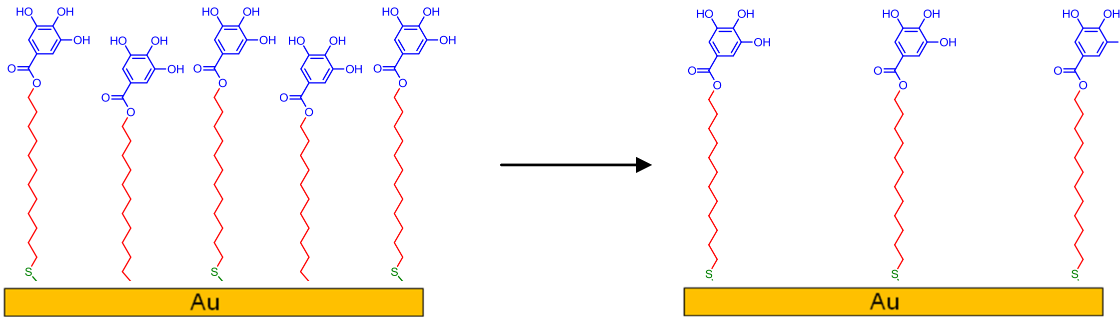


Figure 3.4: Mixed Monolayer to control monolayer density

3.2. .Experimental

3.2.1. Materials

Gold, titanium, mica sheets, chloroform (HPLC grade), methanol and ethanol were purchased from Sigma-Aldrich. Nanopure water with 18.2 MΩ resistivity was obtained from a Barnstead filtration system. 12-mercaptododecyl 4-hydroxybenzoate and 12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate) were synthesized as reported previously.[84] The synthesis of dodecyl 3,4,5-trihydroxybenzoate and dodecyl 4-hydroxybenzoate were conducted according to published procedure.[84, 86]

3.2.2. Substrates preparation

Gold coated substrates were prepared using mica sheets (3.5 × 2cm) that were cleaved using a fine blade revealing newly exposed facet. First, 5 nm titanium was evaporated onto the mica to serve as an adhesive agent between the gold and mica followed by the evaporation of 100 nm gold. Both titanium and gold were evaporated using (name of evaporator), at a rate of 0.1 nm/min. The layer thickness was constantly measured throughout the evaporation process using a quartz crystal. The gold substrates were stored in vials under argon and cleaned with the ozone cleaner for 5min immediately prior to use.

3.2.3. Solution self-assembly

After cleaning, the gold coated substrates were placed in a 1mM solution of the desired thiol in $CHCl_3$:MeOH (97:3%) for 24 h at 23° C. The resulting substrate was rinsed with ethanol and dried under a stream of nitrogen.

3.2.4. Surface pressure-area isotherms

Surfactant monolayers were spread from a 1 mM chloroform/methanol (97:3) solution on an ultrapure water subphase at room temperature (22-23°C) in a Langmuir film balance (Nima Technology, Coventry, UK). At least 2 minutes was allowed for evaporation of the chloroform. Compression isotherms at a speed of 5.0 cm^2/min from a maximum area 80 $Å^2$/molecule to minimum 20 $Å^2$/molecule.

3.2.5. Langmuir-Schaefer deposition

The surfactant film was compressed to the desired target pressure and allowed to stabilize for 30 min. The gold was lowered horizontally until it touched the air-water interface and made contact with the exposed functional group. The substrate remained in contact with the film at the air-water interface for 30 min to allow for reaction of the surfactant with the gold surface. The substrate was then raised from the surfaces and dried with nitrogen gas.

3.2.6. Langmuir-Blodgett deposition

The substrate was immersed in the aqueous subphase after which the surfactant film was spread and compressed to the target pressure and allowed to stabilize for 30 min. The film was deposited as the substrate was removed, i.e. on the upstroke with the head group facing the gold substrate. After removal, the substrate was dried with nitrogen gas.

3.2.7. Contact angle measurements

Static contact angles were measured using ultrapure water. The size of the droplet was controlled using a graduated 3cc syringe. The droplet was allowed to come into contact with the surface and the syringe is slowly raised. The image of the drop is captured using a camera with a 10x magnification lens using image pro-plus software for the image processing and analysis.

3.3. Results and discussion

Starting with 12-mercaptododecyl 4-hydroxybenzoate, the first approach was to deposit the film from the air-water interface using Langmuir deposition methods. The surface pressure-area isotherm obtained for these compounds is shown in (Fig 3.5).

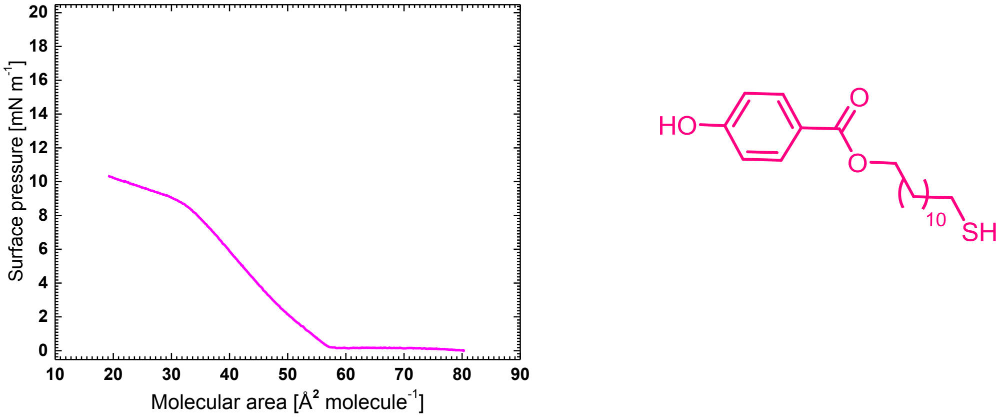


Figure 3.5: Isotherm of 12-mercaptododecyl 4-hydroxybenzoate

The isotherm displays a transition from gaseous phase to a liquid expanded phase (critical area, A_{crit}) at 57 Å^2/molecule. The film remains in the liquid expanded phase until approximately 30 Å^2/molecule at which point the film collapses at a surface pressure of 8 mNm^{-1}. The relatively low collapse pressure brought into question the molecular orientation. On one hand, the low collapse could be due to increased solubility, due to the ω-thiolation, with the surfactant oriented with the phenol submerged in the subphase. Alternatively, long-chain thiols (with the thiol as the head group) have been shown to have low collapse pressures. For example, octadecanethiol has a collapse at as low as 14 mNm^{-1}.[87] Therefore one cannot exclude the possibility of either the thiol serving as the head group tethered to the subphase or, more likely, mixed orientations. Although the phenol head group should provide sufficient polarity for this group to be submerged in the subphase, the orientation of the phenol and thiol groups were determined by characterizing and comparing films made by solution self-assembly, Langmuir-Blodgett and Langmuir-Schaefer. In solution self-assembly, thiol moiety should chemisorb onto the gold surface leaving the phenolic moiety exposed at the surface, especially with long adsorption times where the chemisorption should dominate over physisorption. For such films, the contact angle obtained was 53±1 ° (Table 3.2) which is in good agreement with the advancing (58°) and receding (44°) contact angle measurements for 11-(4-methoxyphenyl)undecyltrichlorosilane SAMs on silicon wafers[88] and static contact angle measurements on thiophenol SAMs on gold (57 °).[89] It should be noted though that the reported contact angles for longer chain phenol and hydroxyl terminated SAMs on gold vary significantly and are strongly dependent on sample preparation and film organization.[88-90] When the film is deposited on the up stroke via Langmuir-Blodgettry, the submerged group will contact the subphase. If the thiol were submerged in the subphase, the contact angle should closely match the contact angle of the film produced through solution self-assembly. Alternatively, with a Langmuir-Schaefer deposition, the exposed group will make contact with the substrate and the submerged head group will be exposed after deposition. If the phenol is submerged in the subphase, the exposed thiol should chemisorb on the gold substrate and the resultant contact angle in this case should correspond to that obtained through solution self -assembly. The films produced by Langmuir-Schaefer and Langmuir-Blodgett depositions at 5 mNm^{-1} gave contact angles of 50±6 ° and 61±3 ° respectively (see Table 3.2). The former is in good agreement with the value for the SAMs while the higher contact angle of the latter correlates well with the higher contact angles reported for a lower polarity thiol group.[91] Based on these contact angle measurements, the phenol moiety was determined to be towards the subphase and the thiol moiety is facing away from the subphase at the air-water interface, therefore a downward Langmuir-Schaefer deposition was chosen for all subsequent experiments.

To demonstrate the effect of the thiol moiety on the organization and stability of the film at the air-water interface, surface pressure-area isotherms were obtained for the comparable length, non-thiolated surfactant, dodecyl-4-hydroxybenzoate.

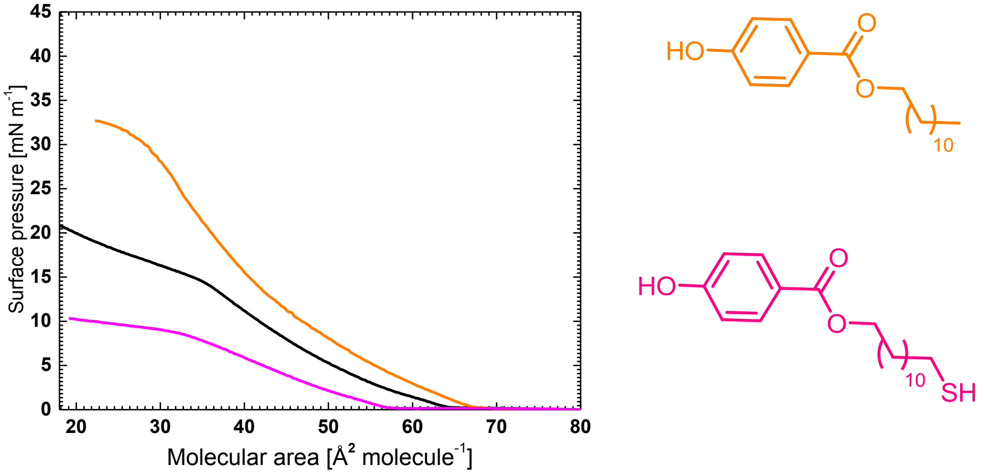


Figure 3.6: Surface pressure-area isotherm for dodecyl-4-hydroxybenzoate (orange), 12-mercaptododecyl-4-hydroxybenzoate (magenta) and a 1:1 mixture of these compounds (black) on a water subphase at room temperature (22 – 23 ºC).

The dodecyl-4-hydroxybenzoate isotherm (Fig 3.6)) displays a transition from gaseous phase to a liquid expanded phase at 68 Å^2/molecule. The film remains in the liquid expanded phase until approximately 30 Å^2/molecule at which point the film collapses (at a surface pressure of 32mN/m). The replacement of the thiol with a methyl group translated to an increase in collapse pressure from 8 to 32 mNm^{-1}. This correlates well with the work by Ihalainen et al.[36] who compared isotherms of DPPC (1,2-dihexadecanoyl-sn-glycero-3-phosphocholine) and DSDPPC (1-palmitoyl-2-(16-(S-methyldithio)-hexadecanoyl)-*sn*-glycero-3-phosphocholine, the latter with a chain terminal methyldisulfide group used to anchor lipid on gold substrate. The lipid with a terminal disulphide on one chain collapsed at lower pressures ($\Delta\pi \approx 12$ mNm^{-1}) than the corresponding non-ω-functionalized lipid. Although the terminal disulphide enhanced condensation of the condensed phase monolayer, it also lowered the stability, attributed to the disulphide creating unequal chain lengths within the lipid. In our case, the lipid forms a liquid expanded phase and the equivalency of the chain lengths is not expected to be a major contributor to the stability and collapse pressure.

Mixed monolayers may provide a means to increase the pressure to which the ω-thiolated surfactant can be compressed and in turn yield a greater range of areas where depositions can be made from the air-water interface. The increase in collapse pressure for mixed monolayers involving one lipid with a terminal disulphide in the chain has been reported by Ihalainen.[36]

A 1:1 mixture of the thiolated and non-thiolated surfactant yielded an isotherm (black line in Fig 3.6) intermediate to both single component monolayers: a liquid expanded only phase which has a critical area at 63 Å^2/molecule. The film collapses at 35 Å^2/molecule at a surface pressure of 15 mNm^{-1} which is significantly higher than that of the corresponding thiolated surfactant. The collapse areas for all three monolayers are comparable, suggesting that thiol does not provide strong enough tethering to the subphase to enable significant orientational changes as have been observed with bola-amphiphiles. The 1:1 mixture increases the range of areas accessible for deposition of the chemisorbing film (longer isotherm) and, as discussed earlier, may provide access to greater lateral spacing by subsequent removal of the thiolated.

The second route to control lateral density of films involved using the molecular structure to vary the intermolecular distance. Linking two surfactants by a disulfide (Fig 3.7) yielded a bolaform (two-headed) surfactant. The conformational freedom of the chains is restricted and Langmuir monolayers of bolaform surfactants are known to adopt different conformations at the air-water interface; such molecules would be expected to have different space requirements (molecular areas) at the interface. Moreover, one could envision the disulfide bolaform surfactant requiring more space to approach the gold surface in solution self-assembly than a single-chain thiolated surfactant. Once again, before depositing the films, the monolayer organization at the air-water interface was determined (Fig 3.7). 12,12'-Disulfanediylbis(dodecane-12,1-diyl)-bis(3,4,5-trihydroxybenzoate) was synthesized to mimic the galloyl moiety of tannins in nature. For comparison, the isotherm of dodecyl 3,4,5-trihydroxybenzoate is also shown in (Fig 3.7) as is the isotherm for a 2:1 molar mixture of 12,12'-disulfanediylbis(dodecane-12,1-diyl)-bis(3,4,5-trihydroxybenzoate): dodecyl 3,4,5-trihydroxybenzoate (keeping in mind that the disulfanediylbis(dodecane-12,1-diyl)-bis(3,4,5-trihydroxybenzoate) comprises two head groups and the mixture is therefore 1:1 in term of head groups, i.e. for every two traditional surfactants there is 1 bolaform surfactant)

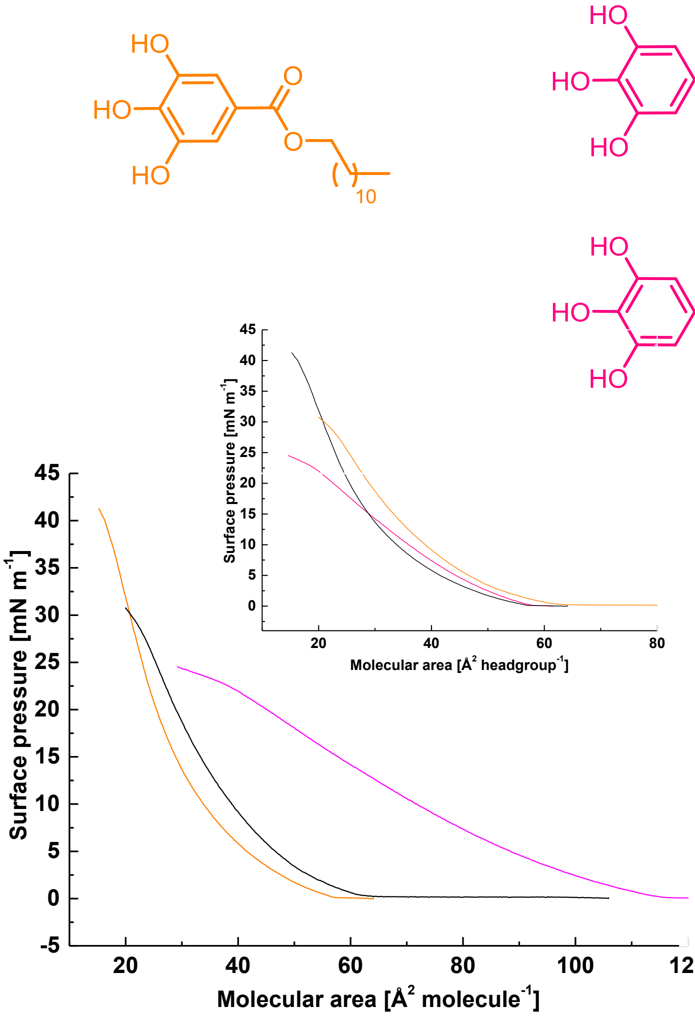


Figure 3.7 : Surface pressure-area isotherm for dodecyl 3,4,5-trihydroxybenzoate (orange), 12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate) (magenta) and a 2:1 mixture of these compounds (black) on a water subphase at room temperature (22 – 23 ºC).

The isotherm for 12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate) (Fig 3.7) displays a transition from gaseous phase to a liquid expanded phase at 120 Å^2/molecule. It is anticipated that in this phase, that the molecule adopts a horseshoe orientation with both galloyl moieties attached to the surface as has been reported for bolaform surfactants.[92] The film remains in the liquid expanded phase until approximately 40 Å^2/molecule at which point the film collapses at a surface pressure of 23mN/m. The inset to (Fig 3.7) shows the area per head group, highlighting the lower compressibility for the disulphide compared to the non-bolaform surfactant (the slope is lower and the isotherm flatter than a traditional liquid expanded phase), attributed to the lower conformational freedom with the chains connected and two head groups contacting the subphase. (Table 3.1) summarized the key parameters for the isotherms, namely the critical area (A_{crit}), collapse area (A_{coll}) and collapse pressure (π_{coll}). The smaller molecular areas (A_{crit} and A_{coll}) for dodecyl 3,4,5-trihydroxybenzoate compared to dodecyl-4-hydroxybenzoate are likely due to differences in head group orientation to optimize intermolecular hydrogen bonding as was reported for the corresponding phenolic lipid monolayers.[83] The greater capacity of the dodecyl 3,4,5-trihydroxybenzoate hydrogen bonding may also account for the slightly higher collapse pressure.

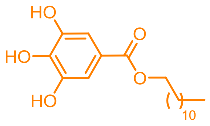
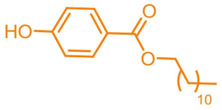
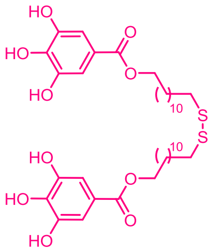
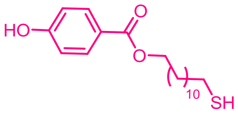
	Compounds	A_{crit} (Å^{2}headgroup)	A_{coll} (Å^{2}headgroup)	π_{coll} (mNm^{-1})
A		61	23	37
B		68	30	32
C		120	40	23
D		57	31	8
A+C 2:1 Mixture (1:1 in headgroups)		63	24	28
B+D 1:1 Mixture		64	33	15

Table 3.1: Isotherm data compilation of key parameters for the isotherms: critical area (A_{crit}), collapse area (A_{coll}) and collapse pressure (π_{coll}).

The impact of the functionalization becomes evident when one considers the deviation from ideal mixing. Figure 3.8 shows a plot of ΔA (taken to be the actual area per molecule for the mixture minus the expected area for an ideal mixture).Positive deviations from ideality imply repulsive interactions or less efficient packing whereas negative deviations imply attractive interactions or more efficient packing. In both cases positive deviations are observed, although these are much more significant for the disulphide bolaform surfactant where the conformational freedom of the chains is highly restricted. Thus there is a significant disruption of the film packing even in the liquid expanded phase.

In all cases the non-ω-functionalized compounds exhibit higher collapse pressures; both ω-functionalization and linking the chains, hinders the film compression. In particular the addition of a terminal polar thiol which reduces conformational freedom of the chains and increases solubility yields a significantly lowered collapse pressure. The addition of traditional (non-functionalized) surfactant to either compound increased the film stability enabling compression to higher pressures likely due to increase van der Waals interactions holding these molecules at the air-water interface. The improved stability and presumed horseshoe conformation of the bolaform disulphide surfactant may prove advantageous in terms of ensuring exposed sulphur for reaction with the gold substrate.

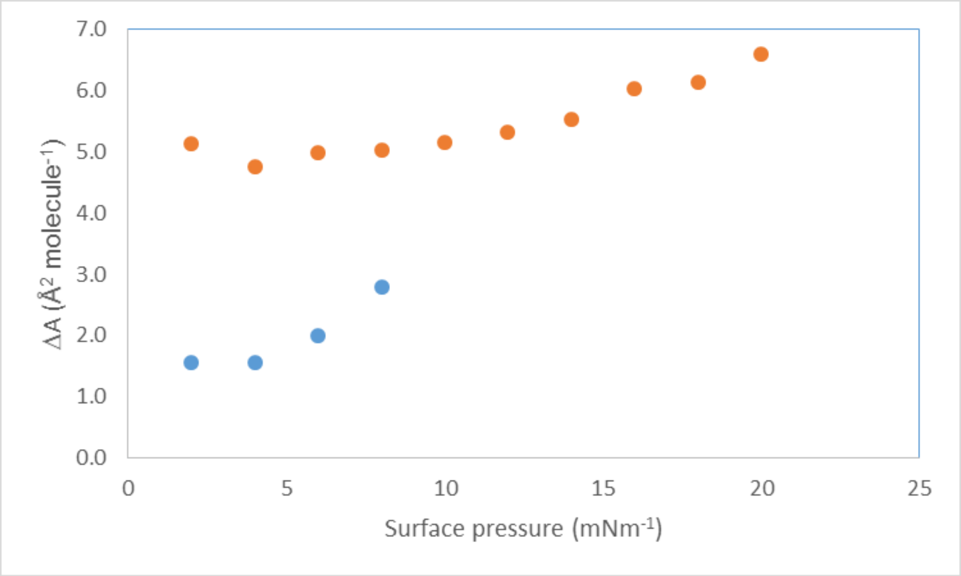
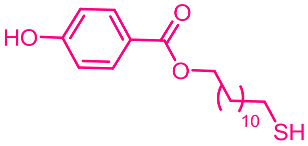


Figure 3.8: Plot of ΔA (taken to be the actual area per molecule for the mixture minus the expected area for an ideal mixture

Langmuir-Schaefer depositions for ω-thiol functionalized surfactants are less common, as solution self–assembly provides an easy way to functionalize surfaces. For our purposes, the ability to form chemisorbed films with varied film density was required to accommodate protein-tannin interaction. Isotherms were used to determine the structural organization. At the gaseous phase, molecules are far apart from each other and cannot be transferred as an organized film onto a substrate. Similarly the collapse pressure must be avoided during deposition as the organization is neither monolayer nor well-defined and molecules can adopt various conformations. The concept was to compress to different pressures within the liquid-expanded phase which can be associated to different film densities. Based on these considerations, depositions were made at 5 and 10 mNm^{-1} for the monohydroxy and 5, 10 and 18 mNm^{-1} for trihydroxy. With an increase in pressure, the average intermolecular distance between the polar head groups is expected to decrease.

Langmuir-Schaefer and solution self-assembly techniques were both used for depositions of the thiolated surfactant for 12-mercaptododecyl 4-hydroxybenzoate (henceforward referred to as *monohydroxy thiol*) and disulfide bola amphiphile, 12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate) (henceforward referred to as *trihydroxy disulfide*). The corresponding films were compared using contact angle measurements.



Deposition	**Surface Pressure mNm^{-1}**	**Contact Angle$^{\circ}$**
Self-Assembly	--	53 ± 1
Langmuir-Schaefer	5	50 ± 6
Langmuir-Schaefer	10	53 ± 1

Table 3.2: Monohydroxy Thiol Contact angle measurement from solution self-assembly and LS at 5mNm^{-1}

Contact angle measurement was used to determine the wettability of the films deposited onto gold coated substrates. A hydrophilic surface would prefer interaction with the water surface, causing water to spread and consequently a lower contact angle than a hydrophobic surface. SAMs formed by the monohydroxy thiol yielded a static contact angle value of 53°±1. As noted above, this lies within the range of advancing and receding angles of 44° and 58°, respectively that were reported by Foster et al.[88] SAMs formed of molecule comprising an 11 carbon polymethylene spacer, methyl protected phenol and trichlorosilane terminal group which anchor molecules onto the silicon wafers. Following self-assembly, the methyl group removed in-situ to obtain phenol moiety. Despite the difference in the anchoring group, substrate and number of carbons in hydrophobic chains, our film gives a comparable contact angle as the functionality of the films is determined in large by the chemical composition head group, namely the phenol. Films deposited by Langmuir-Schaefer technique at 5 and 10mNm^{-1} produced films with contact angles of 50°±6 and 53°±3, respectively, and correlated well the SAM contact angle. The limited range of pressures for deposition was insufficient to induce large changes in the film and changes to lateral spacing were therefore not reflected in the phenol exposure and corresponding contact angle. On the other hand, this also implies that all three films (SAM and LS) showed surface good coverage and phenol exposure. (Table 3.4) shows the static contact angles measured for films of the trihydroxy disulfide compound deposited by solution self-assembly and LS deposition. The contact angle measurements obtained for solution self-assembly was 72°±1, which is significantly higher than the value obtained for the monohydroxybenzoate SAM in this work and for dihydroxybenzoate SAMs thiol compounds reported in the literature (Table 3.3); there are no reports for trihydroxybenzoate SAMs. Moreover, it should be noted that the range of static contact angles reported for these systems, prepared under reportedly similar condition, is large whereby head group orientation[76] and sample humidity[93] play an important role. The head group orientation and film wettability are affected by intermolecular hydrogen bonding[76], film disordering[89] and hydroxyl placement.[76, 89]

As the number of hydroxyl group increase, the hydrophilicity of the film would be expected to increase. In contrast we observed higher contact angles indicating higher hydrophobicity. It may be that the head group organization to accommodate extensive intermolecular hydrogen bonding for monolayers gallic acid-derived lipids,[73, 82] may induce a head group orientation with the aromatic ring exposed. Alternatively, the self-assembly process for the disulfide may be hindered due to the large bulky molecule. This could reduce the surface coverage yielding a film with significantly more alkyl chain exposed. Additionally, the gallic acid head group had been shown to be strongly self-adhering[82] and the strong physisorption of additional layers upon solution self-assembly cannot be excluded.

The contact angles for films formed by LS deposition were lower (Table 3.4) than self-assembly but still higher than obtained for the monohydroxybenzoate films. With respect the SAM formed by the trihydroxybenxoate molecule, the LS depositions seem to have better wettability suggesting better phenol exposure. Compression of the film may induce an uprighting of the chain and improve the exposure of the disulfide for reaction with the gold substrate.

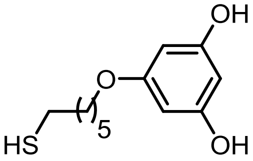
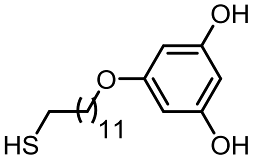
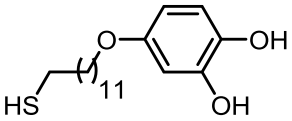
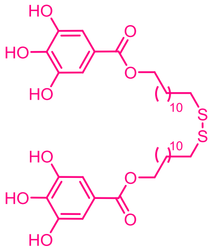
Compounds	Static Contact Angles (SAMs)	Reference
	50° 59° (after drying)	K. V. Gothelf, A. G. Larsen. *J. of Colloid and Interface Science* 2002, 255, 356-362
	42°	A. A. Azmi, et al. *J. of Colloid and Interface Science* 2013 , 393, 352-360
	31°	A. A. Azmi, et al. *J. of Colloid and Interface Science* 2013 , 393, 352-360

Table 3.3: Literature survey of contact angle measurements of phenolic thiolated surfactant



Entry	Deposition	Pressure mNm^{-1}	Contact Angle
1	Self-Assembly	--	72 ± 1
2	Langmuir-Schaefer	5	63 ± 4
3	Langmuir-Schaefer	10	61 ± 4
4	Langmuir-Schaefer	18	66± 5

Table 3.4 Comparison of contact angle measurement of self-assembly and LS for 12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate)

3.4. Conclusion

We have demonstrated that phenolic surface coatings on gold substrate can be created using Langmuir-Schaefer deposition of organosulfur functionalized phenolic surfactants. Previous work has focused on the self-assembly of such films, however deposition of Langmuir monolayers should yield better control over lateral spacing between molecules. The Langmuir films formed by 12-mercaptododecyl 4-hydroxybenzoate and 12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate) (showed low collapse pressures although the stability of the films to higher surface pressures was achieved through the formation of mixed monolayers with the surfactants lacking the organosulfur functionalization. However, collapse areas (area per head group) were not significantly affected by the presence of the organosulfur. This is likely because the twelve carbon chain forms a liquid-expanded phase and a greater impact would be expected for long chain surfactants exhibiting condensed phase behaviour. For the surface functionalization studies, using the thiol surfactant yields films with better exposure of the phenol head group (needed for most potential applications) than using the disulfide, although the disulfide may still provide better long-term storage. Future work will be directed at determining lateral spacing and phenol orientations and their effect on protein adsorption.

Chapter 4. Conclusions and future work

Two novel phenolic surfactants were synthesized that emulate natural occurring tannins a surfactant with self-assembly capabilities. The polarity of the galloyl and co-elution of impurities proved difficult in the purification of the surfactant. Acetyl protecting groups reduced the polarity of the phenolic head group and facilitated purification on column chromatography.

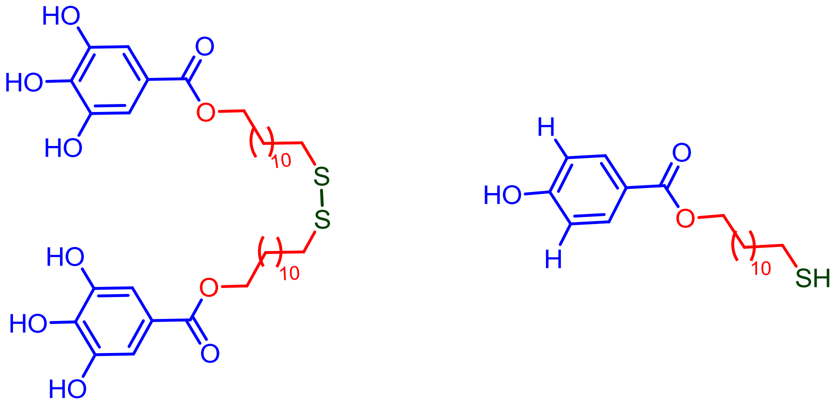


Figure 4.1: Novel Thiolated Phenolic Surfactant

In our synthetic scheme, sodium thiomethoxide was used to convert the thioacetate intermediate to the corresponding ω- thiol in the presence of an internal ester. In addition this works unveiled sodium thiomethoxide's ability to simultaneously deprotect the acetyl protecting groups. This enabled us to develop a novel strategy which can deprotect thioacetate and acetyl in a single step while maintaining the internal ester linkage in the surfactant. As future work, this modular route will be adapted, as needed, to synthesize additional phenolic surfactants varying the length of the hydrocarbon chains.

The isotherms of the phenol surfactants terminated with thiol and disulfide display a liquid expanded phase at all pressures up to collapse. A longer carbon chain, for example C16 or C18, may induce formation of a condensed phase. These surfactants can be used to obtain further insight on film organization and effect of thiol at the air-water interface.

Solution assembly has been the predominant technique for the adsorption of monolayers onto gold substrates. The advantages of thiolated SAMs on gold include a robust films, ease of preparation and ability to form closely packed monolayers. Phenolic surfactants with organosulfur functionalization were investigated with the intention to emulating tannins molecules in nature and by extension their properties such as protein interaction. A survey of literature revealed the spacing between the adsorbed molecule within a monolayer or monolayer density is critical to facilitate the interaction of the galloyl moiety and proteins such as proline rich protein. We adopted the seldom used strategy for sulfur terminated surfactants, Langmuir Schafer depositions to create phenolic films. Our approach used LS rather than solution self-assembly to form phenolic surface coatings as it provides the means to pre-organize the film to a desired molecular area and corresponding monolayer density.

The monohydroxy thiol and trihydroxy disulfide films differed from previous work in terms of structure, behaviour at the air-water interface and deposition method (Langmuir-Schaefer instead of solution self-assembly). In contrast to previous phenolic surfactants reported, these surfactants consist of an ester linkage which connects the phenol to hydrocarbon chain designed to better mimic the galloyl moiety of tannins. In the synthetic route presented, the carboxylic was converted to an ester but it can also be converted to other functional groups such amides and acyl chlorides. This provides a versatile synthetic route to create surfactants with different head groups.

Isotherms of monohydroxy thiol-terminated surfactants revealed a lower collapse for the thiolated or disulfide surfactant in comparison to its non-functionalized counterpart. In particular, the thiol moiety has a significant effect on the collapse pressure such that the orientation of this ω-functionalized surfactant at the interface was questioned. Using contact angles of deposited films, it was determined that the phenol moiety is oriented towards and the thiol away from the subphase. The stability of the monolayer under compression (i.e. collapse pressure), could be improved by forming mixed monolayers with the corresponding non-functionalized surfactants. Both mixtures showed positive deviation from ideality indicating the disruptive nature of the ω-functionalization. In the future, the trihydroxy disulfide should be reduced to the thiol to determine the relative impacts of the thiol and disulfides.

Once organization of the surfactant was determined at the air-water interface, deposition was conducted using Langmuir-Schaefer technique for both monohydroxy thiol and trihydroxy disulfide surfactants. Our contact angle results for depositions of the monohydroxy thiols using Langmuir-Schaefer at 5mNm^{-1} and 10mNm^{-1} was in agreement with results observed of solution self–assembly. The lack of change wettability at different pressures can be attributed to the small range of pressures where deposition can be made. The use of mixed monolayer of monohydroxy thiolated: monohydroxy non thiolated increased displayed a collapse at a higher pressure therefore a greater range of pressures where depositions can be made.

The contact angle measurements of the trihydroxy disulfide SAMs for self-assembly were higher than expected in comparison to similar head group such mono and dihydroxyl substituted aromatic rings. We suspect that the intermolecular hydrogen bonding results in an exposed surface exposed comprising more the hydrophobic aromatic rings than the hydroxyl groups. In addition, the disulfide compounds presents a bulkier compound in comparison to the monohydroxy thiol leading to films which may yield less surface coverage and with more alkyl chain exposed increasing the hydrophobicity. LS depositions were made at 5, 10, 18 mNm^{-1} which provide lower contact angle and increase hydrophilicity in comparison to films obtained from solution self-assembly indicative of greater exposure of phenols at the surface.

In extension to the results with mixed monolayer, our future work will focus the deposition of mixed monolayer onto gold substrates. Ideally films transferred at the air-water interface will resemble as the schematic shown in (Fig 4.2). As the non functionalized surfactant will physisorb and thiolated surfactant will chemisorb onto the gold coated substrate, removal of the physisorbed surfactant will yield a surface with only self-assembled molecules. Backfilling with an appropriate chemisorbing surfactant can be used to ensure full extension of the chains and accessibility of the phenol head groups. The percentage of the thiolated to non thiolated surfactant can also be varied to optimize interaction of the phenolic surface group in produced films.

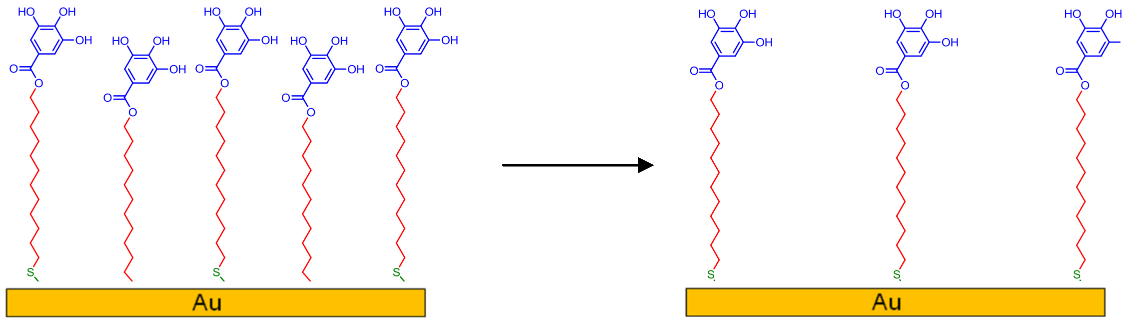


Figure 4.2: Schematic of mixed monolayers

In addition to characterization of films produced using contact angle measurement, reductive desorption can be used to quantitatively to determine the number of alkanethiolates adsorbed onto the gold surface. A charging current can be applied and desorption will be indicated by the reduction in current. Following desorption, the charging current will gradually return to its initial value. The desorption process will result in a peak when plotted on a voltammograms. The area under the peaks can be used to calculate the number of adsorbed alkanethiolates.

Reductive adsorption will also be beneficial in determining the ratio of physisorbed to chemisorbed molecules in our mixed monolayer experiment. Reductive adsorptions have been shown to be able to differentiate between physisorbed and chemisorbed molecules. The difference will be marked by different peaks on the voltammograms and current utilized in the desorption process.

A long term goal for this project will be to study the possible interaction between the functionalized surfaces with proline rich proteins. Further adjustments to parameters such monolayer layer density, functional head groups, length of hydrophobic spacer can be modified to optimize protein interaction and functionalized surface.

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Chapter 6. Appendix

6.1. Abstract-Efficient preparation of novel phenolic surfactants for self-assembled monolayers

Abstract: *Novel molecules have been synthesized combining the phenolic nature of tannins and self-assembling properties of surfactants. These single chain (C12) surfactants with potential biocompatibility have been synthesized with an ω-thiol or disulphide functionality, both commonly used anchors in self-assembly onto gold surfaces, using a modular route. Protecting groups for the phenol and thiol moieties played a key role for overcoming the challenges often associated with the purification of surfactants. The tasks of unmasking the thiol moiety and simultaneously deprotecting the acetyl protecting groups of the phenols were accomplished using sodium thiomethoxide. This modular route can be extended to synthesize other surfactants with the potential ability to form robust layers with biocompatible properties.*

Keywords: Tannins, Surfactants, Sodium thiomethoxide, Deprotection, Thiols, Disulfides

6.2. Abstract-Mimicking a Tannin: Creating a Phenolic Surface Using Self-assembly

Abstract: *The following paper discusses the structural organization of thiolated phenolic surfactants at the air-water interface. Deviating from the traditional preparation of self-assembled monolayers for thiolated surfactants, Langmuir depositions of ω-thiolated surfactants from the air-water interfaces are used as a means to control monolayer density. The films are characterized using contact angle measurements and compared to films produced from solution self-assembly which is expected to form packed monolayer. Isotherms show that the presence of a thiol or disulfide moiety leads to decrease in the pressure at which collapse occurs and with the thiol yielding a greater destabilization. Contact angle measurement of the 12-mercaptododecyl 4-hydroxybenzoate revealed similar wettabilty for films produced both by solution self-assembly and Langmuir-Schaefer deposition. Mixed monolayers comprising surfactants with an organosulfur functionalization and the corresponding non-functionalized surfactant were studies as a means to stabilize the films and increase the range of molecular areas available for depositions. The mixed monolayers were stable to higher pressures but did exhibit positive deviations form idealist. Langmuir-Schaefer depositions of both thiolated and disulfide surfactants were demonstrated to be viable methods to produce films with control over molecular area and exposure of a phenolic functionality with potential for bioapplications.*

Keywords: Tannins, Surfactants, Isotherm, Self-Assembly, Langmuir-Schaefer

Chapter 7. Supporting information

7.1.1. General Procedure for the synthesis of acetylated compounds

Acetic anhydride (5.8 equiv) was added to hydroxyl substituted benzoic acid (1.0 equiv) followed by the addition of sulfuric acid (0.058 equiv) drop wise.[62] The solution was stirred for 1 h. Water (0.19 equiv) was then added to the reaction mixture and allowed to stir for 3h. The contents were then transferred to a separatory funnel and extracted with ethyl acetate (10 × 10 mL). The combined organic layers were dried with Na_2SO_4 and filtered through cotton. The resulting mixture was concentrated under reduced pressure to provide the corresponding product.

7.1.1.1. 3,4,5-triacetoxybenzoic acid

White solid, yield 1.50 g (84%), limiting reagent: gallic acid: 6 mmol; ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm 7.84 (s, 2H, ArH), 2.30 (s, 9H, OAc)

Characterization details previously reported.[94] CAS#: 6635-24-1

7.1.2. General Procedure for the Synthesis of Compounds (2A-2B)

Thionyl chloride (3 equiv) was added to acetylated reactant (1 equiv) in benzene (0.5 M), and the reaction mixture was then heated at reflux for 2 h.[69] The resulting mixture was concentrated under reduced pressure.

7.1.2.1. 3,4,5-tris(acetyloxy)-benzoyl chloride (2A) (CAS#: 70475-59-1) [95]

Yellowish white solid, crude product used, limiting reagent: 3,4,5-triacetoxybenzoic acid: 1 mol; ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm 7.84 (s, 2H, ArH), 2.30 (s, 9H, OAc)

7.1.3. General Procedure for the Synthesis of Compounds (3A-3B)

12-Bromododecanol (1 equiv) was added to a solution of **2** (1.25 equiv) in THF (0.1 M), followed by triethylamine (1.25 equiv), and the reaction mixture was stirred at room temperature for 48h. Ethyl acetate was used to transfer the contents to a separatory funnel. The organic layer was washed with ammonium chloride (0.1 M) (5 × 10 mL). The combined aqueous layers were extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried with Na_2SO_4 and filtered through cotton. The resulting mixture was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography eluting with mixtures of hexane and ethyl acetate.

7.1.3.1. 5-((12-bromododecyloxy)carbonyl)benzene-1,2,3-triyl triacetate (3A)

White solid, yield 1.91 g (72%), limiting reagent:12- bromododecanol 4.8mmol; m.p 68-69 °C; IR: 2921, 2850, 1776, 1723, 1594, 1493, 1470, 1427, 1374, 1326, 1244, 1186, 1056, 1017, 903, 849, 782, 766, 721, 642, 430cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm 7.78 (s, 2 H, ArH), 4.29 (t, 2 H, J = 7.0 Hz, CO_2CH_2), 3.40 (t, 2 H, J = 7.0 Hz, Br-CH_2), 2.29 (s, 9 H, OAc), 1.84 (q, 2 H, J = 7.0 Hz, CH_2), 1.71 (q, 2 H, J = 7.5 Hz, CH_2), 1.30 (m, 16 H, CH_2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ ppm 167.6, 166.4, 164.4, 143.3, 138.5, 128.7, 122.1, 65.7, 34.0, 32.8, 29.4, 29.2, 28.1, 25.8, 20.5, 20.1; ESI MS: calcd. $C_{25}H_{35}BrO_8$ $(M + H)^+$ *m/z*: 543.1594; found: $(M + H)^+$ *m/z*: 543.1617

7.1.3.2. 12-bromododecyl 4-acetoxybenzoate (3B)

Yellow oil, yield 1.08 g (63%), limiting reagent: 12-bromododecanol 4.0 mmol; IR: 2926, 2854, 1762, 1720, 1604, 1504, 1465, 1413, 1368, 1274, 1196, 1160, 1115, 1100, 913, 763, 702cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm 8.00 (d, 2 H, *J* = 8.5 Hz, ArH), 7.10 (d, 2 H, *J* = 8.5 Hz, ArH), 4.24 (t, 2 H, *J* = 7.0 Hz, CO_2CH_2), 3.30 (t, 2 H, *J* = 7.0 Hz, Br-CH_2), 2.25 (s, 3 H, OAc), 1.78 (q, 2 H, *J* = 7.0 Hz, CH_2), 1.68(q, 2 H, *J* = 7.0 Hz, CH_2), 1.30 (m, 16 H, CH_2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ ppm 168.8, 165.8, 154.1, 131.1, 128.1, 121.5, 65.7, 34.0, 32.8, 29.4, 29.2, 28.7, 26.0, 25.8, 21.1; ESI MS: calcd. $C_{21}H_{31}BrO_4$ $(M + H)^+$ *m/z*: 427.1484; found: $(M + H)^+$ *m/z*: 427.1476

7.1.4. General Procedure for the Synthesis of Compounds (4A-4B)

The following procedure was an adaptation from Zheng et al.[66] A solution of potassium thioacetate (1.2 equiv) in DMF (1 mL) was added to compound **3** (1 equiv) in DMF (0.1 M) at -78 °C and the reaction mixture was warmed to 23 °C and stirred for 48 h. Ethyl acetate was used to transfer the contents to a separatory funnel. The resulting mixture was then washed with water (5 × 10 mL). The organic layer was then washed with $Na_2CO_{3\ (sat)}$ (5 × 10 mL). The combined aqueous layers were extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried with Na_2SO_4 and filtered through cotton. The resulting mixture was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting with mixtures of hexane and ethyl acetate.

7.1.4.1. 5-((12-(acetylthio)dodecyloxy)carbonyl)benzene-1,2,3-triyl triacetate (4A)

White solid, yield 1.55 g (78%), limiting reagent: 5-((12-bromododecyloxy)carbonyl)benzene-1,2,3-triyl triacetate(3A) 3.68 mmol; m.p (52-53 °C); IR: 2921, 2849, 1773, 1719, 1695, 1471, 1404, 1428, 1374, 1248, 1215, 1191, 1059, 1017,902, 848, 783, 764, 430cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 7.71 (s, 2 H, ArH), 4.21 (t, 2 H, *J* = 7.0 Hz, CO_2CH_2), 2.79 (t, 2 H, *J* = 7.5 Hz, COS-CH_2), 2.23 (s, 3 H, SAc), 2.24 (s, 9 H, OAc), 1.66 (q, 2 H, *J* = 7.5 Hz, CH_2), 1.48 (q, 2 H, *J* = 7.5 Hz, CH_2), 1.19 (m, 16 H, CH_2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ ppm 196.0, 167.6, 166.4, 164.4, 143.3, 138.5, 128.7, 122.1, 65.7, 30.6, 29.5, 29.4(3C), 29.2, 29.15, 29.10, 28.8, 25.9, 20.5, 20.1; ESI MS: calcd. $C_{27}H_{38}O_9S$ $(M + H)^+$ *m/z*: 539.2315; found: $(M + H)^+$ *m/z*: 539.2320

7.1.4.2. 12-(acetylthio)dodecyl 4-acetoxybenzoate (4B)

White solid, yield 0.68 g (69%), limiting reagent: 12-bromododecyl 4-acetoxybenzoate(3B) 2.81 mmol; m.p (46-47 °C); IR; 2922, 2853, 1759, 1722, 1607, 1510, 1468, 1410, 1365, 1270, 1191, 1123, 1109, 1105, 1113, 919, 756, 710, 600, 550cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm 8.06 (d, 2 H, *J* = 9.0 Hz, ArH), 7.15 (d, 2 H, *J* = 9.0 Hz, ArH), 4.29 (t, 2 H, *J* = 7.0 Hz, CO_2CH_2), 2.84 (t, 2 H, *J* = 7.0 Hz, COS-CH_2), 2.30 (s, 6 H, SAc), 1.72 (q, 2 H, *J* = 7.0 Hz, CH_2), 1.53 (q, 2 H, *J* = 7.5 Hz, CH_2), 1.35 (m, 16 H, CH_2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ ppm 195.9, 168.7, 165.7, 154.1, 131.0, 128.0, 121.5, 65.2, 30.6, 29.4, 29.1, 29.2, 28.7, 25.9, 21.1; ESI MS: calcd. $C_{23}H_{34}O_5S$ $(M + H)^+$ *m/z*: 423.2205; found: $(M + H)^+$ *m/z*: 423.2205

7.1.5. General Procedure for the Synthesis of Compounds (1A-1B)

The following procedure was a modified procedure of that previously reported by Wallace et al.[67] Sodium thiomethoxide (1equiv) in MeOH (1 M) was added to a stirred solution of **4** (1.11equiv) in MeOH (0.1 M) at -78 °C and the reaction mixture was warmed to 23 °C and stirred for 48 h. Ethyl acetate was used to transfer the contents to a separatory funnel. The resulting mixture was then washed with water (5 × 10 mL). The organic layer was then washed with $Na_2CO_{3(sat)}$ (5 × 10 mL). The combined aqueous layers were extracted with ethyl acetate (2 × 20 mL). The combined organic layer was dried with Na_2SO_4 and filtered through cotton. The resulting mixture was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting with mixtures of hexane and ethyl acetate.

7.1.5.1. 12,12'-disulfanediylbis(dodecane-12,1-diyl) bis(3,4,5-trihydroxybenzoate)(1A)

White solid, yield 0.33 g (53%), limiting reagent: Sodium thiomethoxide (1.67 mmol); m.p (87-88 °C); IR: 2916, 2848, 2316, 2337, 1716, 1456, 1036, 667, 413, 402cm^{-1} . ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm 9.22 (s, 2 H, ArH), 8.90 (s, 1 H, ArH), 6.93 (s, 2 H, ArH), 4.13 (t, 2 H, J = 6.5 Hz, CO_2CH_2), 1.75 (t, 2 H, J = 6.5 Hz, COS-CH_2), 1.59 (m, 2 H, J = 6.5 Hz, CH_2), 1.27 (m, 16 H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ ppm 166.2, 145.9, 138.7, 120.0, 108.9, 64.3, 39.9, 39.8, 39.6, 39.4, 38.4, 29.4, 29.0, 28.7, 28.1, 25.9. ESI MS: calcd. $C_{38}H_{58}O_{10}S_2$ $(M + H)^+$ m/z: 738.3471; found: $(M + H)^+$ m/z: 738.3473

7.1.5.2. 12-mercaptododecyl 4-hydroxybenzoate (1B)

White solid, yield 0.13 g (36%), limiting reagent: Sodium thiomethoxide (1.065 mmol); m.p (64-65 °C); IR: 2922, 2851, 1686, 1608, 1467, 1279, 1056, 772, 418, 410cm-1 . ^{1}H NMR (DMSO-d6, 500 MHz) δ ppm 7.94 (d, 2 H, J = 9.0 Hz, ArH), 6.87 (d, 2 H, J = 9.0 Hz, ArH), 6.12 (s, 1 H, ArH), 4.28 (t, 2 H, J = 6.5 Hz, CO_2CH_2), 2.67 (t, 2 H, J = 6.5 Hz, COS-CH_2), 1.74 (q, 2 H, J = 7.5, CH_2), 1.64(q, 2 H, J = 7.0 Hz, CH_2), 1.36(m, 18 H, CH_2); ^{13}C NMR (DMSO, 125 MHz) δ ppm 166.8, 160.0, 131.8, 122.8, 115.2, 65.0,39.2, 29.6, 29.4, 29.2, 28.7, 28.4, 26.0; ESI MS: calcd. $C_{19}H_{30}O_3S$ (M + H)+ m/z: 339.1994; found: (M + H)+ m/z: 339.2003

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