SELF-ASSEMBLY OF PULLULAN ABIETATE ON CELLULOSE SURFACES

Sheila Elizabeth Gradwell

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

in

Chemistry

Alan R. Esker (Chair)
James M. Tanko
T. Daniel Crawford

August 19, 2004
Blacksburg, VA

Keywords: self-assembly, surface plasmon resonance, SPR, cellulose surface modification, pullulan abietate, Langmuir-Blodgett thin films, adsorption
SELF-ASSEMBLY OF PULLULAN ABIETATE ON CELLULOSE SURFACES

Sheila Elizabeth Gradwell

Abstract

Wood is a complex biocomposite that exhibits a high work of fracture, making it an ideal model for multiphase man-made materials. Typically, man-made composites demonstrate interfacial fracture at failure due to abrupt transitions between neighboring phases. This phenomenon does not occur in wood because gradual phase transitions exist between regions of cellulose, hemicellulose, and lignin and therefore adhesion between adjacent phases is increased. The formation of interphases occurs as a consequence of the self-assembly process which governs the formation of wood. If this process was understood more thoroughly, perhaps tougher man-made, biobased composites could be prepared. To study self-assembly phenomena in wood, a system composed of a model copolymer (pullulan abietate, DS=0.027) representing the lignin-carbohydrate complex (LCC) and a model surface for cellulose fibers was used. The self-assembly of the polysaccharide pullulan abietate (DS=0.027) onto a regenerated cellulose surface prepared using the Langmuir-Blodgett (LB) technique was studied via surface plasmon resonance (SPR). Rapid, spontaneous, and desorption-resistant cellulose surface modification resulted when exposed to the model LCC. Adsorption was quantified using the de Feijter equation revealing that between 9-10 anhydroglucose units (AGUs) adsorb per nm$^2$ of cellulose surface area when cellulose is exposed to pullulan abietate (DS=0.027) compared to the adsorption of 6.6 AGUs per nm$^2$ of cellulose surface area when cellulose is exposed to unsubstituted pullulan.
To my mom, Debra Klinger, who taught me to love people, think wisely, and follow my dreams. Her incredible strength, wisdom, courage, and determination have taught me about the kind of person that I want to be.
ACKNOWLEDGEMENTS

A great many people have helped me through my graduate school years here at Virginia Tech and I count my lucky stars for each and every one of them for they have each affected my life in a very special way. I’d first like to express my thanks to my advisor, Dr. Alan R. Esker, for his guidance during my pursuit of this degree and encouraging my growth as a scientist. I am thankful for our countless discussions whether about science or life in general, somehow he always made me see the bigger picture even when I got lost in all of the details. I would also like to extend my thanks to Dr. Wolfgang G. Glasser who has been extremely supportive and whose insight into the field of wood science has been a great asset to my work. I feel truly blessed to have had him as part of my committee. I’d also like to thank my other committee members, Dr. T. Daniel Crawford, Dr. James Tanko, and Dr. Herve Marand for providing their opinions and insight as to my research.

In addition, I’ve been blessed with the best lab mates a girl could ever ask for. Each one of them has a very special place in my heart. First and foremost is Jianjun Deng who has become like a brother to me. He is one of the smartest people that I have ever met and has helped me with countless problems that I’ve encountered in lab. I am also indebted to his wife, Hongyang Xue, for teaching me how to cook authentic Chinese food. I’d like to thank everyone in the Esker group (past and present) for all their help along the way, scientific or otherwise: Rituparna Paul, Ufuk Karabiyik, Abdulaziz Kaya, Woojin Lee, Wen Yin, Suolong Ni, Bingbing Li, Dr. Hyong-Jun Kim, Jenn Stockdill, John Hottie, Joe Polidan, Sabrina Segnere, Roderick Seals, Vidhya Sivakumaran, Kevin
Dawson, and Sarah Huffer. Scott Renneckar from Dr. Glasser’s lab has helped me immensely by sharing with me everything he knows about wood.

I would especially like to thank Jan McGincy, Patrick McGinty and the rest of the chemistry stockroom staff for making sure that all my packages were always taken care of. Thank you to wonderful men in the machine shop, Melvin Shaver, John Miller, and Scott Allen for always fixing my Wilhelmy plates. Thank you to Gary Scott for fixing our electrical problems in lab and always being cheerful. I am eternally grateful to Aysen Tulpar whose mastery of SPR allowed me to get up and running with my experiments and Dr. Ray Dessy who was very helpful when I encountered SPR problems. Mr. Mohammed Aziz Hussain, Dr. Tim Liebert, and Dr. Thomas Heinze were kind enough to supply the pullulan abietate used in my experiments and were extremely helpful in fielding all of my questions. Mr. Thomas Ryan, Reichert Analytical Instruments, was an essential part of the completion of this work by helping me to troubleshoot all of my SPR problems. Steve McCartney from the Materials Science department here at Virginia Tech was always ready to lend a hand when my AFM experiments went haywire. Thank you to the United States Department of Agriculture as part of its NRI program (contract # 9902352) and the National Science Foundation (#CHE-0239633) who have made this work possible by funding my research and allowing Dr. Esker to purchase an SPR for our lab.

Above all, I’d like to thank my family and friends for their unfaltering support and faith in me. Thanks for believing in me even when I didn’t. Stephanie Hooper and Mary Tam have been my family away from home. They have become like sisters to me and were always there for me whenever I needed them. They have become two of my
closest and dearest friends. Without them, I don’t know what I would have done. We have had so many great times together and leaving them will be one of the saddest days of my life. Stephanie was one of the best roommates that I’ve ever had, as were Jenn Stockdill and Alice Harper. All of these girls have provided me with many stories to tell and a lot of memories to enjoy. The most important people in my life deserve recognition for getting me to this point. My parents, Debra and Robert Klinger, Ronald Gradwell and Barbara Bechtel as well as my sister, Jenna Gradwell, have given me all of the love and support that I’ve needed. My grandparents, Gerald and Geraldine Flynn and Edith Klinger, have provided me with an abundance of love, support, and prayers to last me a lifetime. I’d also like to thank my grandparents John Pierce and Grace Gradwell who are no longer with me but will always be in my heart. Thank you to Kristen Beninsky and Candice Moser who have helped me to realize that I can accomplish anything that I set my mind to. Last, but surely not least, thank you to Robert Poltrok whose love and support over the last few months has been monumental.
# TABLE OF CONTENTS

| List of Figures | viii |
| List of Tables | xiii |

## 1. Introduction
1.1. Self-Assembly in Biological Systems .................................................. 1
1.2. Wood Structure and Properties ................................................................. 2
1.3. Wood-Based Composites ............................................................................ 5
1.4. Biomimetic Approach to Composite Development ........................................ 6
1.5. Model System ............................................................................................. 9
1.6. Langmuir-Blodgett (LB) Technique ............................................................. 12
1.7. Surface Plasmon Resonance (SPR) ............................................................. 16

## 2. Experimental Methods .............................................................................. 25
2.1. Cellulose Model Film Preparation ............................................................... 25
2.2. Pullulan Abietate Synthesis ........................................................................ 28
2.3. Surface Plasmon Resonance .................................................................... 28
2.4. Surface Tension Measurements .................................................................. 31
2.5. Atomic Force Microscopy .......................................................................... 32
2.6. Ultraviolet Spectroscopy .......................................................................... 33
2.7. Refractive Index Increment Determination ............................................... 33
2.8. Reflectivity Simulations using Winspall™ ................................................ 34
2.9. Reflectivity Profiles ................................................................................... 36
2.10. Reflectivity Profile Fitting ......................................................................... 37

## 3. Solution Characterization Results and Discussion .................................... 38
3.1. Critical Aggregation Concentration Determinations .................................. 38
3.2. Ultraviolet Spectroscopy Results ................................................................. 40

## 4. Surface Characterization Results and Discussion .................................... 43
4.1. Calibration of the Surface Plasmon Resonance Refractometer .................. 43
4.2. Determination of the Optical Constants for Gold and Cellulose ............... 48
4.3. The de Feijter Equation .............................................................................. 60
4.4. Pullulan Abietate Reflectivity Simulation .................................................. 60
4.5. Refractive Index Increments of Pullulan and Pullulan Abietate (DS=0.027) ... 61
4.6. Surface Plasmon Resonance Analysis ....................................................... 63
4.7. Adsorption of Pullulan and Pullulan Abietate (DS=0.027) onto Cellulose ..... 65
4.8. Adsorption Isotherms for Pullulan and Pullulan Abietate (DS=0.027) ......... 69
4.9. Atomic Force Microscopy Results ............................................................. 81

## 5. Conclusions ............................................................................................... 84

## 6. Suggestions for Future Work .................................................................... 86

## 7. References ................................................................................................. 88
LIST OF FIGURES

Figure 1.1. Gradual vs. abrupt transitions between two distinct phases. ....................... 2
Figure 1.2. The repeating structure of cellulose. ................................................................. 3
Figure 1.3. The hierarchical arrangement of wood. ............................................................. 4
Figure 1.4. Glass transition temperatures ($T_g$) of long-chain cellulose mono-, di-, and triesters (LCCE) with fatty acid substituents as a function of methylene content (in weight %) of the ester substituents. ................................................................. 7
Figure 1.5. Structure of inter-unit linkages in lignin-carbohydrate complexes. .............. 9
Figure 1.6. Structures of the repeating unit of pullulan and abietic acid ...................... 10
Figure 1.7. Desilylation of trimethylsilyl cellulose to regenerate cellulose ................. 11
Figure 1.8. Phase transitions in Langmuir monolayers. ................................................... 15
Figure 1.9. Langmuir-Blodgett deposition modes. ............................................................ 16
Figure 1.10. Kretschmann prism arrangement used in surface plasmon resonance. ...... 18
Figure 1.11. Schematic of Kretschmann prism configuration. ........................................ 18
Figure 1.12. Diagram of reflection and refraction at an interface when the incident angle is lower than the critical angle. ................................................................. 20
Figure 1.13. Diagram of reflection at an interface when the incident angle is greater than the critical angle, producing a refracted beam at 90° to the normal. ................. 20
Figure 1.14. Change in resonance angle caused by adsorption. .................................... 23
Figure 2.1. KSV 2000 Langmuir-Blodgett trough used for transfer of TMSC onto SPR sensor slides. ................................................................. 26
Figure 2.2. Components of the SPR setup. ................................................................. 29
Figure 2.3. Winpall™ layer system used in the determination of the refractive index of gold. .......................................................... 34

Figure 2.4. Winpall™ layer system containing regenerated cellulose................................. 35

Figure 2.5. Winpall™ layer system configuration, thickness, and refractive index values used to simulate pullulan and pullulan abietate adsorption onto a cellulose surface....... 35

Figure 3.1. Critical aggregation concentration curves for pullulan abietate and pullulan determined using the Wilhelmy plate technique at 22.5 °C. ........................................ 39

Figure 3.2. Structure of an anhydroglucose unit........................................................ 40

Figure 3.3. UV spectra of abietic acid and pullulan abietate in a mixed solvent system composed of 44% water and 56% methanol by volume................................................. 41

Figure 4.1. Theoretical SPR curve for an ethylene glycol standard obtained using Winpall™. ....................................................................................................................... 45

Figure 4.2. Pixel to angle relationship for ethylene glycol standards of known concentration............................................................................................................. 45

Figure 4.3. Ethylene glycol calibration SPR output..................................................... 47

Figure 4.4. Plot used in determination of the SPR coefficients. ....................................... 48

Figure 4.5. Reflectivity profiles for an SPR sensor slide with 20 layers of regenerated cellulose on its surface in terms of pixel number. ......................................................... 51

Figure 4.6. Reflectivity profiles for an SPR sensor slide with 20 layers of regenerated cellulose on its surface in terms of resonance angle...................................................... 52

Figure 4.7. The region fit with a third-order polynomial function to determine the SPR dip minimum for an SPR sensor slide coated with 20 layers of regenerated cellulose. ... 53
Figure 4.8. Theoretical data obtained (assuming \( n=1.523 \)) for un-swollen cellulose compared to experimental data obtained by determining the SPR minimum of each reflectivity profile. ............................................................... 54

Figure 4.9. Winspall™ layer system used to acquire theoretical data in Figure 4.8 for un-swollen cellulose........................................................................................................54

Figure 4.10. The effect of \( \kappa \) on the minimum angle and its reflected intensity assuming \( n=0.17 \) for the gold layer........................................................................................... 56

Figure 4.11. Relationship between the real part of the refractive index, \( n \), for gold and the reflected intensity of the SPR minimum for \( \kappa=5.83 \). .......................................................... 57

Figure 4.12. Determination of \( \frac{d\theta_{sp}}{dL} \) for pullulan abietate on 20 layers of cellulose using reflectivity simulation data. ........................................................................................................... 61

Figure 4.13. Determination of the refractive index increments for pullulan and pullulan abietate (DS=0.027) at 20 °C and a wavelength of \( \lambda = 780 \) nm........................................ 63

Figure 4.14. Raw SPR data for the adsorption of pullulan abietate onto 40 layers of regenerated cellulose at 20 °C. .............................................................................................................. 64

Figure 4.15. "Maximum" and corrected \( \Delta \theta_{sp} \) values for the adsorption of pullulan abietate (DS=0.027) onto 20 layers of regenerated cellulose at 20 °C............................. 66

Figure 4.16. "Maximum" and corrected \( \Delta \theta_{sp} \) values for the adsorption of pullulan abietate (DS=0.027) onto 30 layers of regenerated cellulose at 20 °C. ......................... 67

Figure 4.17. "Maximum" and corrected \( \Delta \theta_{sp} \) values for the adsorption of pullulan abietate (DS=0.027) onto 40 layers of regenerated cellulose at 20 °C. ......................... 68
Figure 4.18. "Maximum" and corrected Δθsp values for the adsorption of pullulan onto 20 layers of regenerated cellulose at 20 °C. ................................................................. 69

Figure 4.19. Adsorption isotherm of pullulan abietate (DS=0.027) on 20 layers of regenerated cellulose at 20 °C. ................................................................. 72

Figure 4.20. Adsorption isotherm of pullulan abietate (DS=0.027) on 30 layers of regenerated cellulose at 20 °C. ................................................................. 73

Figure 4.21. Adsorption isotherm of pullulan abietate (DS=0.027) on 40 layers of regenerated cellulose at 20 °C. ................................................................. 74

Figure 4.22. Adsorption isotherm of pullulan on 20 layers of regenerated cellulose at 20 °C. ................................................................. 75

Figure 4.23. Schematic displaying differences in the thickness of the adsorbed layers of pullulan and pullulan abietate on cellulose. ................................................................. 77

Figure 4.24. Adsorption of pullulan abietate (DS = 0.027) onto 20 layers of regenerated cellulose beginning with an initial exposure of the film to a solution below the cac at T=20 °C................................................................. 79

Figure 4.25. Adsorption of pullulan abietate (DS = 0.027) onto 20 layers of regenerated cellulose beginning with an initial exposure of the film to a solution above the cac at T=20 °C................................................................. 79

Figure 4.26. Schematic illustrating the adsorption of pullulan abietate on cellulose with initial adsorption occurring below the cac. ................................................................. 80

Figure 4.27. Schematic illustrating the adsorption of pullulan abietate on cellulose with initial adsorption occurring above the cac. ................................................................. 80
Figure 4.28. AFM height images for a) 20 layers of regenerated cellulose, b) pullulan abietate (c=22.8 mg•L⁻¹) exposed to 20 layers of cellulose, c) pullulan abietate (c=699 mg•L⁻¹) exposed to 20 layers of cellulose, d) pullulan (c=23 mg•L⁻¹) exposed to 20 layers of cellulose, e) pullulan (c=633 mg•L⁻¹) exposed to 20 layers of cellulose.
LIST OF TABLES

Table 4.1. Slopes of theoretical thickness vs. $\theta_{sp, theo}$ plots along with corresponding refractive index values assuming a thickness of 4.2 Å per layer for cellulose. .................. 58

Table 4.2. Slopes of experimental and theoretical thickness vs. $\theta_{sp}$ plots along with corresponding refractive index values for varying thicknesses per layer of cellulose. .... 59

Table 4.3. Data used in the calculation of $\Gamma$ values for pullulan and pullulan abietate.. 70

Table 4.4. Thickness of adsorbed pullulan and pullulan abietate on cellulose. ............... 77
1. Introduction

1.1. Self-Assembly in Biological Systems

Many materials found in nature such as tendon, bone, and wood exhibit a combination of properties that have yet to be documented for any synthetic material.\(^1\) The unique properties of some of nature’s materials stem from the way they are structurally self-assembled. In nature, molecular units or aggregates of molecules are embedded or intertwined within other phases, which in turn are similarly organized at increasing size scales.\(^2\) This gradual transition from one phase to the next (Figure 1.1) is responsible for the absence of interfacial delamination commonly seen in made-made materials, which have a more abrupt transition between phases.

The concept of creating man-made systems that mimic biological systems has generally been seen as an impossible task. In recent years, man has been able to gain a deeper understanding of these materials due to advances in probing biological systems at the molecular level.\(^3\) From this work, scientists have been able to create so-called “hierarchical structures” from metals, ceramics and polymers, however, there are still many systems in nature lacking a man-made analog.
Figure 1.1. Gradual vs. abrupt transitions between two distinct phases. The dotted line represents a gradual transition between adjacent phases inherent to natural composites. The solid line represents a sharp interfacial region characteristic of synthetic composites.

All biological structures have evolved through a process of trial and error equivalent to thousands of years of laboratory experiments. Therefore, it seems intuitive that nature’s materials have superior structural design to man-made materials, which have been in production for only a fraction of that time. Biological materials have the ability to sense and to repair localized damage to their structures. This ability is a particularly attractive feature and a desirable goal for man-made structural systems.\(^1\)\(^4\) Many scientists believe that biological materials can be used as models for the development of new classes of synthetic materials for use in architecture, engineering, aerospace, biomedicine, and telecommunications.\(^2\)\(^4\)

1.2. Wood Structure and Properties

Wood, in particular, is a biological composite material possessing an excellent combination of mechanical properties. On a mass basis, the stiffness and strength of
wood is comparable with materials such as aluminum, steel, and glass-fiber composites. Another attractive mechanical property of wood is the high resistance to the propagation of cracks upon the introduction of a stress. It has become a dream of many scientists to develop a synthetic composite product possessing either similar or superior properties compared to those of wood. The structure of the cell wall in wood can be used as a guide for the development of a multiphase composite that exhibits a gradual transition between two distinct phases potentially leading to the development of future synthetic wood composites. In order to do this, the interactions between the components of wood need to be more thoroughly understood.

Between 95 and 98% of the wood cell wall is made up of three polymeric materials: cellulose, hemicellulose, and lignin. Lignin is not present in very young cells, but is deposited in the wall in the later stages of cellular differentiation. The remaining 2-5% is made up of lower molecular weight materials collectively referred to as extractives. Some examples of extractives are terpenes, fatty acids, aromatic compounds and volatile oils.

The largest component of the cell wall is the polysaccharide cellulose. This polymer exists as linear chains composed of anhydro-D-glucopyranose units linked by β-(1-4) glucosidic bonds as shown in Figure 1.2. In plants, cellulose molecules are arranged into microfibrils (10-20 nm in diameter) containing crystalline and amorphous regions as shown in Figure 1.3. The composite structure of the cellulose microfibrils in the S2 layer

![Figure 1.2. Repeating unit of cellulose.](image-url)
of the cell wall is responsible for the stiffness and strength of plants. The toughness of wood is an anisotropic property, being approximately one hundred times greater when the crack propagates across the grain than when it travels with the grain. When wood is fractured along the grain, the crack travels through the middle lamella, which lacks a fibrous component. This mechanism leads to the disassociation of microfibrils rather than fracture of the cell wall itself. In contrast, when the fracture occurs perpendicular to the grain, the crack has to break the cell wall to propagate. Jeronimidis has shown that the cellulose fibers in the $S_2$ portion of the cell wall adopt a helicoidal orientation which is optimal for retaining both stiffness and toughness upon the induction of strain.

![Figure 1.3. The hierarchical arrangement of wood.](image)

The strong association and almost perfect parallel alignment of the cellulose molecules gives rise to extensive regions of crystallinity within the cellulose microfibril.
These highly ordered regions are interrupted approximately every 600 Å by amorphous regions. The microfibrils of cellulose form the open framework of the cell wall and are imbedded in a binding matrix of hemicelluloses and lignin. Spaces which are not filled with either cellulose, a hemicellulose, or lignin are often occupied by water.

Hemicelluloses are also polymeric materials composed of anhydrosugar units. The difference between cellulose and hemicellulose is that the latter can be composed of several different sugar units and typically contains a much smaller number of sugar units per polymer chain. The hemicelluloses are rather hygroscopic and are nearly saturated with water. Some examples of sugars that are used to make hemicelluloses in wood are D-glucose, D-galactose, D-xylose, D-mannose, and L-arabinose. The hemicelluloses along with lignin surround the crystalline regions of cellulose. Lignins are unlike the other two components of wood in that they are not carbohydrates. Instead, they are very complex, crosslinked, three-dimensional polymers formed from phenolic units. The absolute configuration of lignin varies in different morphological regions, different types of cells and different types of wood. Lignin is insoluble in virtually all simple solvents. If the wood is extensively milled or enzymatically degraded, the solubility of lignin increases drastically. Since milling conditions are often times extremely severe, it is likely that chemical bonds are broken leading to the increased solubility. This observation has lead to the belief that lignin forms a three-dimensional network.

1.3. Wood-Based Composites

Wood composites are a class of materials that generally consist of solid fragments of wood held together by some sort of adhesive. Plywood, fiber board, particleboard, oriented strand board (OSB), and wood plastic composites are all examples of composite
materials commonly found in today’s market. Over time, wood composites have been designed to utilize smaller and smaller wood fragments, progressing from plies to strands to fibers to fine “flour” measuring only microns in size. The latter represent the dimensions of wood fragments typically used for wood plastic composites produced by thermal extrusion processes. Wood composites are capturing ever-larger markets, partially in response to reductions in the supply of solid, large dimension timber.

Composites in general are materials that combine the high strength and stiffness characteristics of a fiber (or particle) with the ductility of a (continuous) matrix. In many man-made composites the fiber-matrix interface is the weakest point resulting in “fiber pull-out” and failure before the fiber reaches its true strength potential. The ideal wood-like composite would combine the features of a high-strength and high-stiffness (hollow) fiber embedded in a continuous matrix from which it never (under any condition of moisture or temperature) separates interfacially, and with which it produces a lightweight material. The National Academy of Sciences has recently recognized biological composites such as wood as ideal model structures for the development of future composite materials.

1.4. Biomimetic Approach to Composite Development

In order to adopt the principles of biomimetics to the process of composite formation from wood fibers, two crucial elements must be understood. First, how can a matrix material, i.e. a lignin-rich layer, be deposited on the surface of a cellulose fiber? Second, how can this layer of matrix material be consolidated to form an adhesive bond between fibers? Solutions to these problems may potentially be accomplished thermally or biologically, i.e., by biomimetics.
Thermoplastic cellulose composites may potentially be formed when cellulose fibers are surface-coated with melt-deformable copolymers. Such copolymers may consist of saccharidic amphiphiles containing waxy substituents. Cellulose and/or cellulose derivatives have served as both adsorbing surface substrates and adsorbable amphiphiles.\textsuperscript{18-27} Employing this established method of surface modification using amphiphilic copolymers with olefinic character, a thermoplastic coating may be produced on cellulose fiber surfaces. Cellulose mono- and di-esters with long chain fatty acids have recently been shown to represent thermoplastic entities with melting/softening points that decline in accordance with methylene group content (Figure 1.4).\textsuperscript{28, 29} Thus, melt-processable cellulose esters with low degrees of substitution (DS) may potentially open a route towards thermoplastic fiber composites by adsorption processes.

![Graph: Glass transition temperatures (T\textsubscript{g}) of long-chain cellulose mono-, di-, and tri-esters (LCCE) with fatty acid substituents as a function of methylene content (in weight %) of the ester substituents.](image)

**Figure 1.4.** Glass transition temperatures (T\textsubscript{g}) of long-chain cellulose mono-, di-, and tri-esters (LCCE) with fatty acid substituents as a function of methylene content (in weight %) of the ester substituents.
A biology-mimicking approach may involve the surface adsorption of molecules amenable to enzymatic crosslinking. During secondary wall formation in wood cells, cellulose is spun from rosette structures into an aqueous sol-like hemicellulose solution. There is a body of work describing how hemicelluloses regulate (bacterial) cellulose fibril diameter. These studies demonstrate that the adsorption of heteropolysaccharides plays a key role in establishing an interfacial region that prevents delamination and fiber pull-out in wood. This adsorption process is governed by self-assembly, a process driven by thermodynamics that results in the aggregation of bipolar molecules on the fiber surface. Self-assembly behavior has been observed with many other natural amphiphilic polymers including oligosaccharide-protein block copolymers, hydroxyethyl cellulose, fluorine-containing cellulose diblock structures, xylan-rich heteropolysaccharides and their derivatives, and lignin-carbohydrate complexes.

This project was motivated by a vision of biomimetics. Presuming that a cellulose surface enriched with lignin or lignin precursors becomes susceptible to the generation of phenoxy radicals by enzyme catalysis, free radicals would then be able to form network polymers (thermoset adhesives) by coupling. Felby et al. and Huettermann et al. have shown that wood fibers can be enzymatically activated in vitro with phenoloxidase and/or laccase, and this treatment can be used to produce wood composites with enhanced auto-adhesion between components. The lignin-coated fibers that are formed when a lignin-carbohydrate complex is adsorbed to a cellulose surface may possess the potential for enzymatic activation and the development of adhesive bonds that are similar to those in wood.
1.5. Model System

The intractability of cellulose makes it difficult to recreate wood’s structure from solvent or melt processes. In order to circumvent this problem, cellulose derivatives have been prepared to study the interactions between cellulose, hemicellulose and lignin indirectly.

Self-assembly behavior has been studied because it is known to cause the hierarchical organization of the wood composite. Amphiphilic block copolymers have been found to exhibit the formation of micelles even at low concentrations. Self-assembled structures have also been observed with natural amphiphilic polymers including oligosaccharide-protein block copolymers, hydroxylethyl cellulose, fluorine-containing cellulose diblock structures, and xylan-rich heteropolysaccharides and their derivatives. Lignin-carbohydrate complexes have also been found to form micellar structures in water. Lignin-carbohydrate complexes (LCCs) consist of sugar chains and small lignin fragments attached as pendant side chains. The lignin is believed to be attached to the sugar moieties via an ether linkage as shown in Figure 1.5. The exact nature of the interactions between these two materials is still unclear but it is known that they are covalently linked. The LCCs exhibit a tendency to form micelles in aqueous solution due

![Figure 1.5. Structure of inter-unit linkages in LCCs.](image)
to the hydrophobicity of the lignin portion of the complexes. The study of these materials becomes relevant due to the fact that in natural systems, self-assembly behavior is believed to be responsible for the formation of the wood composite matrix.\textsuperscript{56}

Polysaccharides themselves are hydrophilic, but if pendant side chains containing hydrophobic groups are attached, amphiphilic character can be established.\textsuperscript{57} Akiyoshi and coworkers have studied the self-assembly behavior of hydrophobized polysaccharides in water.\textsuperscript{21, 58} Pullulan (Figure 1.6) is an extracellular water-soluble polysaccharide produced by strains of \textit{Aureobasidium pullulans}.\textsuperscript{59, 60} It is an $\alpha$-D glucan consisting of $\alpha$-1,4- and $\alpha$-1,6- linkages which behaves as a flexible chain in aqueous solution.\textsuperscript{61} Akiyoshi found that cholesterol substituted pullulan derivatives were capable of forming hydrogel nano-particles by their self-assembly in water. By changing the degree of cholesterol substitution, the size, density, and colloidal stability of the nanoparticles could be controlled.\textsuperscript{21, 62} 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{Structures of the repeating unit of pullulan and abietic acid, respectively.}
\end{figure}

The aim of this project was to determine the adsorption properties of pullulan and a pullulan derivative to a cellulose surface. As stated above, pullulan derivatives have the ability to self-assemble in solution and therefore can aid in the study of lignin/hemicellulose/cellulose interactions. A pullulan derivative containing abietic acid
was studied to determine the effect of substitution with hydrophobic moieties on adsorption behavior. Abietic acid is a hydrophobic molecule extracted from tree resin shown in Figure 1.6. Pullulan abietate represents a model lignin-carbohydrate complex that may be susceptible to enzymatic activation and therefore may be capable of forming adhesive bonds similar to those in wood.

In order to study the adsorption properties of the model LCC onto a cellulose surface by surface plasmon resonance, a well-defined cellulose film must be prepared on a gold slide. The insolubility of cellulose in common solvents makes film preparation from native cellulose difficult. Schaub et al. were the first to determine that a hairy-rod polymer, trimethylsilylcellulose (TMSC) could be deposited onto a silicon substrate and be reconverted to cellulose in situ. TMSC is a cellulose derivative having pendant hydrophobic side chains that wrap around the cellulose backbone forming a hydrophobic shell. TMSC forms homogeneous, well-oriented monolayers that are able to be transferred to a substrate with a constant transfer ratio of 1.00. The TMS side groups can be cleaved by exposure of the film to aqueous acids as shown below, leaving a well-defined, ultrathin cellulose film (Figure 1.7).

\[ \text{TMSC} \rightarrow \text{Cellulose} \]

\[ R = H ; \text{Si(CH}_3)_3 \]

**Figure 1.7.** Desilylation of trimethylsilyl cellulose to regenerate cellulose.
TMSC transfers very well onto a hydrophobic silicon or glass substrate. The gold sensor slide used in surface plasmon resonance (SPR) actually consists of a glass slide coated with a layer of chromium and a second layer of gold. The chromium aids in the adhesion of the gold onto the glass. The glass side of the slide can be made hydrophobic with a 1, 1, 1, 3, 3, 3-hexamethyldisilazane treatment. However, in order to interpret the LB-transfer information, it is desirable that both sides of the substrate be hydrophobic so that both sides pick-up uniform monolayers on the up and downstroke. Therefore the gold must also be made hydrophobic. Hydrophobic gold surfaces can be obtained through the deposition of an alkanethiol self-assembled monolayer.

Self-assembled monolayers (SAMs) are monolayers formed through the spontaneous adsorption of molecules onto a solid surface from solution. Self-assembled monolayers are interesting because they have the potential of forming monolayers with a well-defined composition, structure, and thickness. SAMs are relevant in this work because they have the ability to make a gold surface hydrophobic, thereby allowing the formation of model cellulose surfaces for surface plasmon resonance studies.

1.6. **Langmuir-Blodgett (LB) Technique**

The Langmuir-Blodgett (LB) technique is used to transfer monomolecular films from the surface of a liquid onto the surface of a solid substrate. Water is typically used as the subphase (liquid upon which monolayer forms) although mercury, glycerol, and other materials have been used. In order for the monolayer to remain on the surface of the water, it must be amphiphilic. Most of the early work involving Langmuir monolayers was conducted using long-chain carboxylic acids such as stearic acid.
These molecules contain a polar head group which anchors them to water’s surface and a hydrophobic tail that prevents the molecule from dissolving.\textsuperscript{70}

Langmuir monolayers are produced on an apparatus traditionally referred to as a Langmuir trough. This trough itself is made of a hydrophobic material such as Teflon™ and is designed to contain the subphase. Movable barriers span the water’s surface and a device is attached to measure surface pressure.\textsuperscript{71} The measurement of the surface pressure (\(\Pi\)) as a function of the molecular area (\(A\)) at constant temperature is known as a \(\Pi\)-\(A\) isotherm. This plot is the two-dimensional analog of the three-dimensional pressure vs. volume diagram. The \(\Pi\)-\(A\) isotherm gives information about thermodynamic phase transitions within the monolayer. There are fundamentally two different methods of measuring surface pressure: the Langmuir balance and the Wilhelmy plate technique. For the Langmuir balance, a clean portion of the subphase surface is separated from the film-covered surface by a partition and the force on the partition in this region is measured by a float connected to a conventional balance.\textsuperscript{72} With the more common technique, the Wilhelmy plate technique, an absolute measurement is made by determining the surface tension by solving for a force on a plate that is suspended from an electrobalance or sensitive spring and is partially submerged in the subphase.\textsuperscript{70} A measurement is taken both for the film covered surface (\(\gamma\)) and the clean subphase (\(\gamma_0\)) and their difference is the surface pressure, \(\Pi=\gamma_0-\gamma\), the two-dimensional analog of a bulk or osmotic pressure.

In order to prepare an LB-film, a solution containing an amphiphile with known concentration is prepared in a volatile organic solvent such as chloroform. The solution is spread on the surface of the subphase and the solvent is allowed sufficient time to
evaporate. At this point, the amphiphilic molecules exist in a phase similar to a gas, where there is sufficient space between neighbors so there are no significant interactions between neighbors and the tail portions of the molecules have complete freedom of motion. The barriers of the LB-trough are then compressed in order to bring the molecules on the surface closer together. Continued compression of the barriers leads to the formation of a so-called liquid-expanded (LE) phase, where interactions begin to occur between neighbors, but the hydrophobic chains are still flexible. The existence of a LE phase is dependent on the length of the hydrophobic chains. An increase in the length of the hydrophobic chain leads to increased van der Waals’ attractions and enhanced cohesion. Therefore, in some cases, the LE phase cannot be observed, and there is a direct transition from the gas phase to a liquid condensed (LC) phase. Further compression causes the formation of the LC phase where the amphiphiles become closely packed and assume a vertical orientation relative to the subphase. The molecules in the LC phase can assume a tilted or untilted orientation as shown in Figure 1.8. Compression past the untilted LC phase causes collapse of the film into multilayers which minimize the increased energy imposed by close packing. In LB-film transfer, the monolayer must be deposited when the molecules are at their closest packing, just before collapse occurs. Usually the LC phase is desirable for LB-transfer, with the molecular orientation as a variable depending on the desired orientation of the resultant LB-film. This condition ensures that the close-packed molecules are oriented vertically, with minimal voids.
There are several transfer modes for the deposition of molecules onto a substrate, X, Y, and Z-type transfer, which are shown in Figure 1.9. If a hydrophobic substrate is used, the most commonly observed type of transfer is Y-type (Figure 1.9a). For Y-type deposition, a hydrophobic substrate is lowered into the subphase where the molecules orient their hydrophobic regions toward the substrate. On the upstroke, the polar headgroups of molecules on the surface are attracted to the outward facing headgroups already deposited on the substrate. The film is built up by a continuous series of upward and downward strokes until an even number of layers is achieved with the hydrophobic tails facing the air. For the less common types of transfer, X-type (Figure 1.9b) and Z-type (Figure 1.9c) deposition, molecules are only deposited on the downstroke and upstroke, respectively. In some cases, it would be ideal to manufacture films with the basic structure being a monolayer rather than a bilayer. In X- and Z-type transfer, the
molecules in every layer would theoretically have the same orientation rather than the alternating orientation of molecules seen in Y-type transfer. X-ray diffraction studies of multilayers prepared via X-type transfer show that a bilayer structure often exists despite the predicted stacked monolayer structure shown in Figure 1.9b. This observation implies that molecules rearrange during or after transfer to form a more energetically favorable conformation. Overall, LB-films generally exist with the hydrophobic tails of the final layer oriented toward air.

Figure 1.9. Langmuir-Blodgett deposition modes.

1.7. Surface Plasmon Resonance (SPR)

Surface plasmon resonance (SPR) is a unique optical, surface-sensitive technique with numerous applications in the fields of chemistry and biochemistry. SPR is an
attractive technique in the biosensing field because it is capable of measuring the kinetics of biomolecular interactions in real time for a variety of functionalized surfaces.\textsuperscript{77-79} These interactions take place in a fluid medium which can be tailored to mimic conditions encountered \textit{in vivo}\textsuperscript{80} such as DNA-DNA,\textsuperscript{81-83} antibody-antigen,\textsuperscript{84, 85} and DNA-protein\textsuperscript{86, 87} interactions. SPR is even capable of monitoring the adsorption of weakly bound unlabeled analyte molecules to the surface of a substrate.\textsuperscript{77}

The most frequently used SPR prism setup is the Kretschmann prism arrangement (Figure 1.10), which operates on the principle of total internal reflection.\textsuperscript{88} The Kretschmann configuration consists of a glass substrate coated by a thin solid film of either gold or silver. In the case of gold, a chromium layer must first be deposited to ensure adhesion of gold to the glass surface. Other metallic films have been used for SPR including copper, aluminum, palladium, platinum, nickel, and cobalt. However, gold and silver films have optical properties superior to other materials, are relatively easy to prepare, and therefore most common. The real part of the dielectric permittivity must be negative for a particular material to be a candidate for use as the thin film covering the glass substrate. The material must also be able to support the oscillation of electrons on the surface.
As shown in Figure 1.11, light is passed through a p-polarizer, makes its way through the glass medium (which behaves as a waveguide), and travels toward the metal/sample interface of the prism. A waveguide is a physical medium capable of guiding light through total internal reflection with minimal leakage to the surroundings.
In order for propagation to occur, the guiding medium must have a refractive index higher than the refractive index of the surroundings or total internal reflection cannot occur. In this case, glass is the guiding medium with a refractive index of 1.33 compared with air whose refractive index is 1.0029. According to the law of refraction (also known as Snell’s law),

\[ n_1 \sin \theta_1 = n_2 \sin \theta_2 \quad \text{Equation 1.1} \]

where \( n_1 \) is the refractive index of medium 1 and \( n_2 \) is the refractive index of medium 2. Figure 1.12 shows a beam of incident radiation traveling through a glass medium and designates the angles addressed in Equation 1.1 and 1.2. In this case, the incident angle is below the critical angle and therefore a portion of the radiation is refracted into the air medium. Figure 1.13 demonstrates the occurrence of total internal reflectance when the incident angle, \( \theta_1 \), is greater than the critical angle, \( \theta_c \) producing a 90° angle of refraction. Assuming \( \theta_2=90^\circ \), Snell’s law can be rearranged to obtain an expression for the critical angle.\(^9\)

\[ \theta_c = \sin^{-1} \frac{n_2}{n_1} \quad \text{Equation 1.2} \]
Figure 1.12. Total internal reflectance will not occur if the incident angle is lower than the critical angle.\textsuperscript{91}

Figure 1.13. Total internal reflectance occurs when the incident angle is greater than the critical angle, producing a refracted beam at 90° to the normal. \textsuperscript{91}
In SPR, a monochromatic, p-polarized light source is used to generate the incident light beam. The light travels through the optically dense waveguide (glass) toward the less dense medium (air). If the incident angle is larger than the critical angle, total internal reflectance occurs and the light is reflected back into the glass. Some of the energy of a guided wave makes its way outside of the waveguide boundary and creates what is referred to as an evanescent field. This process is much like the tunneling effect that is observed in quantum mechanics for a modified particle in a box model possessing walls of a finite height. If the metal film is sufficiently thin, the evanescent field can penetrate it and couple with oscillating electrons on the metal’s surface to form a surface plasmon (SP). The surface plasmon is a wave that propagates along the metal/air or metal/solution interface and is described by the following wave vector,

$$k_{sp} = \frac{2\pi}{\lambda} \sqrt{\frac{\varepsilon_m \varepsilon_a}{\varepsilon_m + \varepsilon_a}} \quad \text{Equation 1.3}$$

where $\lambda$ is the wavelength of incident light, $\varepsilon_m$ the dielectric permittivity of the metal, and $\varepsilon_a$ the dielectric permittivity of the ambient medium.

The SPR experiment is designed to tune the propagation vector of the incident light that travels through the prism,

$$k = \frac{2\pi}{\lambda} \sqrt{\varepsilon_g} \quad \text{Equation 1.4}$$

where $\varepsilon_g$ is the dielectric permittivity of the glass prism. More precisely, the surface-parallel component of the incident light, $k_x$, is monitored for a given wavelength of light.
until $k_x$ coincides with $k_{sp}$. The surface parallel component, $k_x$, is described by the following equation,

$$k_x = k \sin \theta \quad \text{Equation 1.5}$$

where $\theta$ is the angle of the incident beam. Substituting Equation 1.4 for $k$ into Equation 1.5 yields,

$$k_x = \frac{2\pi}{\lambda} \sqrt{\varepsilon_g} \sin \theta \quad \text{Equation 1.6}$$

Recalling that $\sqrt{\varepsilon} = n$ for a non-polar insulator,$^9$ allows Equation 1.6 to be expressed in terms of refractive indices. By varying the angle of the incident light beam, the condition $k_x = k_{sp}$ can be obtained so that,

$$n_g \sin \theta_{sp} = \sqrt{\frac{n_m^2 n_a^2}{n_m^2 + n_a^2}} \quad \text{Equation 1.7}$$

At $\theta_{sp}$, most of the incident light is transferred to the surface plasmon and hence, there is a minimum in the reflected intensity. Since the refractive indices of the glass prism and the metal are not varied during the course of an experiment, the only way to alter the resonance angle, $\theta_{sp}$, is by altering the refractive index of the ambient medium, $n_a$. Therefore, if adsorption onto the surface of the metal occurs during an experiment, it causes a change in the refractive index of the ambient medium and shifts the resonance angle to a new value, $\theta_{sp}'$, as shown in Figure 1.14.
Figure 1.14. Change in resonance angle caused by adsorption.\(^90\)

The change in refractive index, \(\Delta n_a\), can be used to determine the surface concentration (\(\Gamma\)) of adsorbed material on the surface of the metal through the following formula developed by de Feijter\(^93\)

\[
\Gamma = L \cdot \frac{\Delta n_a}{\frac{dn}{dc}} \quad \text{Equation 1.8}
\]

where \(L\) is the thickness of the adsorbed layer, \(\frac{dn}{dc}\) is the refractive index increment of the adsorbed material, and \(\Delta n_a\) is the difference between the refractive index of the adsorbed layer and the buffer. Since the thickness of the adsorbed layer, \(L\), can not be obtained directly using SPR, a simulation of the change in SPR response due to
Increasing thickness of the adsorbed layer is used to determine $\frac{dL}{d\theta}$ which is then used along with the corrected SPR signal, $\Delta \theta_s$, to determine $L$.

$$L = \Delta \theta_a \frac{dL}{d\theta_{sp}}$$  \hspace{1cm} \text{Equation 1.9}

$$\Delta \theta_a = \Delta \theta_{sp} - c \frac{d\theta_{sp}}{dc}$$  \hspace{1cm} \text{Equation 1.10}

The change in angle due to surfactant adsorption, $\Delta \theta_a$, is determined from Equation 1.10 to compensate for bulk effects that occur at high concentrations where the change in angle occurs because of changes in the refractive index of the solution.\textsuperscript{94} Combining Equations 1.8 and 1.9 allows for a re-expression of the de Feijter equation as shown in Equation 1.11.

$$\Gamma = \frac{\Delta \theta_a (n_r - n_s)}{\left(\frac{d\theta_{sp}}{dL}\right) \left(\frac{dn}{dc}\right)}$$  \hspace{1cm} \text{Equation 1.11}
2. Experimental Methods

2.1. Cellulose Model Film Preparation

SPR sensor slides (Reichert, Inc. and EMF Corporation), consisting of 12 mm x 12 mm glass slides covered with 1 nm of chromium and 50 nm of gold, were removed from their packaging and rinsed with 18.2 MΩ Milli-Q water (Gradient A10, <10 ppb organic impurities) and dried under a stream of nitrogen gas. Each slide was cleaned by immersion in piranha solution, a 7:3 solution of concentrated sulfuric acid:hydrogen peroxide (30%) by volume, for 30 minutes. Each slide was removed from the piranha solution, rinsed thoroughly with Milli-Q water, and dried with nitrogen. The glass portion of the sensor slide was hydrophobized by exposure to 1, 1, 1, 3, 3, 3-hexamethyldisilazane (Sigma-Aldrich) in an 80 °C oven for 6 hours. Upon cooling, the slide was placed into a 1 mM solution of 1-dodecanethiol (Aldrich) in absolute ethanol for 2 hours according to a procedure described by Laibinis et al. 95

The Langmuir-Blodgett (LB) technique was used to transfer trimethylsilyl cellulose (TMSC, Jena Bioscience, DS=2.8) onto SPR sensor slides by the repeated transfer of monomolecular films from the surface of a water subphase. The LB-trough (KSV 2000) used for TMSC film preparation appears in Figure 2.1. The trough was housed in a Plexiglas™ box to minimize dust deposition onto the water subphase and to create a humid environment free of air currents thereby preventing premature drying of the Wilhelmy plate. The trough was set on a Vibraplane™ vibration-free table (Kinetic Systems, Inc.) located inside a softwall clean room equipped with a Hepa filtration system (Clean Rooms International, Inc.).
Cleaning of the LB-trough consisted of wiping the Teflon™ trough bed with dichloromethane and the Delrin™ barriers with isopropanol. After allowing adequate time for solvent evaporation, Milli-Q water was added to the trough and removed by suction using a vacuum pump (Gast). This procedure was repeated several times to ensure cleanliness of the trough. The final portion of water added to the trough was allowed to equilibrate for 15 minutes to reach a temperature of 22.5 °C. This temperature was maintained for all experiments by using a circulating thermostated bath (Polyscience) connected to the LB-trough. The sandblasted Wilhelmy plate was cleaned by immersion in either piranha solution or a 50:50 mixture of sulfuric acid:nitric acid and then rinsed generously with water and dried with nitrogen. The plate was dipped into the water subphase to ensure complete wetting and then suspended from the pressure sensor. The barriers were compressed to their maximum position at which point a portion of the...
subphase surface was suctioned to remove surface active contaminants. The barriers were expanded until they occupied their original positions. The balance was once again zeroed within the KSV-5000 software package immediately before beginning an experiment.

Using a micro syringe, TMSC was spread from a solution of \( \approx 5 \text{ mg of polymer in } 10 \text{ mL of chloroform} \) onto the equilibrated water subphase until a surface pressure of \( \Pi \approx 10 \text{ mN} \cdot \text{m}^{-1} \) was achieved. After allowing approximately 20 minutes for chloroform evaporation, the barriers of the trough were compressed at a speed of 50 mm\text{•min}^{-1} and held to produce a target surface pressure of 20 mN\text{•m}^{-1}. A prepared gold slide was removed from the thiol solution and rinsed with an ample amount of absolute ethanol to remove any traces of unbound thiol. The slide was dried with nitrogen, rinsed with water, and dried again before being mounted on the dipper head. It is important to note that the gold slides were not mounted directly to the dipper head of the LB-trough due to their small size. Instead, Teflon™ tweezers clamped to one of the corners of the slide were mounted to the dipper head (as shown in Figure 2.1.), to increase the slide surface area that could be covered with TMSC. The KSV software was used to control the movement of both the barriers and dipper head during monolayer transfer. Once the target pressure had been reached, the software reset the barrier speed to 10 mm\text{•min}^{-1} to maintain a constant target surface pressure following removal of material from the surface. The dipper head was set to a speed of 10 mm\text{•min}^{-1} and the desired number of layers to be transferred was programmed accordingly. Transfer involved the vertical movement of the slide through the subphase picking up a film one molecule in thickness. On the return upward through the subphase, another layer of molecules was deposited.
onto the substrate. Repetition of this continued until the desired number of layers had been deposited. At the commencement of the deposition process, the slide was rinsed with Milli-Q water, dried with nitrogen and placed in a glass vial for storage. Before regenerating cellulose, the glass portion of the slide was wiped with chloroform to remove TMSC. The film was then exposed to a solution of concentrated hydrochloric acid, which affords a wet HCl vapor, for 30 seconds to regenerate cellulose.

2.2. Pullulan Abietate Synthesis

Mr. Mohammed Aziz Hussain, University of Wuppertal, Germany synthesized the pullulan abietate (DS=0.027) used in this study. The preparation of this compound involved reacting pullulan with the acyl chloride of abietic acid in the presence of pyridine. This procedure was adapted from the work of Sunamoto et al. The pullulan abietate was used in these experiments without further purification.

2.3. Surface Plasmon Resonance

A Reichert™ SR 7000 Surface Plasmon Resonance (SPR) Refractometer was employed to monitor the docking behavior of pullulan (from Aureobasidium pullulans, Sigma) and pullulan abietate (DS=0.027) onto a model cellulose surface. Fluid delivery was controlled via a peristaltic pump composed of a Masterflex® L/S® 8-roller pump head system (Model # 07519-20) coupled to a computerized pump drive (Cole-Parmer Instrument Co., Model # 7550-50). Small cartridges (Model # 07519-85) operating with L/S® 13 PharMed® tubing were loaded onto the pump head to direct flow. Two cartridges were used simultaneously, one cartridge responsible for solvent flow, the other for sample flow. PEEK® tubing (Upchurch Scientific), 1/16" O.D. 0.02" I.D. (orange) was connected to the PharMed® tubing via peristaltic tubing adapters (Model # P-757) to
transport water from the water reservoir to the selection valve. Fluid exiting the solvent selection valve moved through PEEK® tubing, 1/16" O.D. 0.01" I.D. (blue) into the SPR flow cell. The sample line was composed of blue PEEK® tubing and followed the path mentioned above after reaching the solvent selection valve. A waste line composed of orange PEEK tubing transported solution exiting the flow cell to a waste collection container. The SPR setup appears in Figure 2.2.

Figure 2.2. Components of the SPR setup include a selection valve, peristaltic pump and SR 7000.

The SPR was switched on and allowed to warm up for approximately 90 minutes. Meanwhile, both sample and water lines were cleaned by flowing absolute ethanol through each line for 10 minutes followed by rinsing with Milli-Q water for an additional 10 minutes. The sapphire prism and flow cell body were cleaned with cotton soaked in absolute ethanol and blown dry with nitrogen. The gasket was rinsed in a stream of ethanol and blown dry. Following cleaning, an SPR sensor slide was refractive index-
matched to the sapphire prism of the SPR using immersion oil (n_D = 1.5150) (Reichert, Inc.). For calibration purposes, a bare gold slide cleaned in piranha solution was mounted to the SPR prism. For all other analyses, a sensor slide possessing a self-assembled monolayer (SAM) topped with a regenerated cellulose thin film on its surface was attached to the SPR prism. The flow cell body was equipped with a Viton gasket (Dupont Dow Elastomers, LLC) and mounted on top of the sensor slide. Solutions were pumped into the flow cell at a flow rate of 0.35 mL·min⁻¹ via PEEK tubing. The selection valve was used to switch between water and sample solution avoiding the introduction of air bubbles into the system.

The custom executable program “SPR v2.21 alpha” created by Reichert using National Instruments Labview™ software was used for data acquisition. The “Experiment Setup” menu located within the Labview™ instrument interface allowed the reading interval time (typically 9 seconds) to be set and file name to be entered. The “Set/Reset Buffers” icon was used to clear the Labview™ memory locations and send a RS232 data format command to the instrument. Once proper communication between computer and instrument had been established, a message “||SF KLZAIH” appeared in the “Instrument Output” window. Once this message appeared, the instrument was initiated by clicking the “Initiate” icon. The initiation procedure set an internal LED light level and saved a reference scan of the reflectivity array in the instrument memory. Following initiation, the instrument was calibrated by clicking the “Calibrate” icon which reset the resonance angle based on the index of the flowing solution, in this case water. Clicking “Read” followed by “Monitor Liquid On” started the data acquisition process. A steady baseline was established by running only water through the flow cell body.
During the calibration procedure, water was allowed to run for approximately 10 minutes before switching to ethylene glycol (EG) solutions of known mass concentration. These EG solutions were used to relate the pixel numbers recorded by the instrument to relevant refractive index values.

Once calibrated, experiments to monitor the docking behavior of pullulan and pullulan abietate (DS=0.027) were conducted. In these experiments, the regenerated cellulose thin film on the sensor slide was allowed to reach an equilibrium swelling thickness by flowing water until a steady baseline was established after which time sample was introduced into the flow cell. The solvent selection valve was used to switch back and forth between water and analyte solution. All solutions analyzed using the SPR technique were prepared using Milli-Q water and volumetric glassware. Typically, analyte solutions were allowed to run for 20-30 minutes before switching the valve back to water. As the signal did not return to the original baseline due to irreversible adsorption of analyte, water was allowed to flow until a new baseline had been established. For most experiments, solutions of higher concentration were pumped through the flow cell under the same conditions described above after a new baseline had been established.

2.4. Surface Tension Measurements

Surface tension measurements of aqueous pullulan and pullulan abietate solutions ranging in concentration from 0 to 10,000 mg•L\(^{-1}\) were conducted using the tensiometer from the LB-trough (KSV 2000). The surface tension was determined by the Wilhelmy plate technique using a sandblasted platinum plate. Each solution to be analyzed was placed in a specially designed glass jar that consisted of an inner cup containing the
solution and an outer jacket which allowed for insulation with 22.5 °C water flowing from a circulating thermostated bath. The tensiometer and glass jar were set inside a Plexiglas™ box resting on a vibration-free table located within the portable clean room. All glass jars were cleaned in a 50:50 concentrated nitric acid:concentrated sulfuric acid solution while the Wilhelmy plate was soaked in piranha solution for several minutes prior to analysis. The critical aggregation concentration (cac) for each material was determined by finding the intersection of the two trendlines fit to the linear portions of the surface tension versus concentration graph. Error estimates of ± one standard deviation for each cac value were obtained from the standard deviations of the trendlines obtained by Igor Pro.

2.5. Atomic Force Microscopy

Atomic Force Microscopy (AFM) images were obtained for the regenerated cellulose, pullulan (above and below the cac) on cellulose, and pullulan abietate (above and below cac) on cellulose. All of these materials were deposited on 1" x 1" slides consisting of a glass slide coated with 1 nm chromium, and 50 nm gold (EMF Corporation). The gold slides and LB-films were cleaned and prepared according to the procedure described in Section 2.1. Two surfaces each of pullulan and pullulan abietate were prepared to compare adsorption behavior above and below the cac. Pullulan was adsorbed onto substrates by submersion into solutions below the cac (23 mg•L⁻¹) and above the cac (633 mg•L⁻¹). Similarly, pullulan abietate was adsorbed onto two separate substrates by submersion into solutions below and above the cac, 22.8 and 699 mg•L⁻¹, respectively. Substrates were soaked in their respective solutions for approximately seven hours and rinsed generously with Milli-Q water before being dried with nitrogen.
and analyzed. Measurements were conducted in Tapping Mode™ on a Digital Instruments Dimension 3000 Scope with a Nanoscope IIIa controller using etched single crystal silicon probes.

2.6. Ultraviolet Spectroscopy

Ultraviolet spectroscopy was used to quantify the degree of substitution of pendant abietate groups on pullulan abietate. Solutions of known abietic acid, pullulan, and pullulan abietate concentration were prepared in a mixed solvent system composed of 44% water and 56% methanol by volume and analyzed in a 1 cm quartz cuvette using a Varian Cary 50 Bio UV-Visible Spectrophotometer. Wavelength scans between 220 and 400 nm were conducted in dual beam mode using two matched cuvettes at a scan rate of 720 nm•min⁻¹ using a data sampling interval of 0.15 nm and an averaging time of 0.0125 seconds. A baseline correction was used in which the 44% water and 56% methanol solvent system was used to set 100% transmission prior to analysis of each sample. The concentration of abietic acid was plotted against absorbance to prepare a calibration curve. Pullulan abietate solutions were analyzed and their absorbance values plugged into the calibration curve to determine the concentration of abietic acid substituted pullulan repeating units. These values were used to determine the mole fraction of substituted pullulan repeat units and in turn, the degree of substitution.

2.7. Refractive Index Increment Determination

The refractive indices at 20 °C (n_D²⁰) of varying concentrations of pullulan and pullulan abietate in Milli-Q water were determined using a Thermospectronic Abbe Refractometer. The data was corrected to 780 nm using an empirical model developed by Quan and Fry describing the dependence of the refractive index of water on
wavelength. The refractive index increment \( \frac{dn}{dc} \) of each material was obtained from the slope of a plot of concentration versus refractive index at 20 °C and 780 nm (\( n_{20} \)).

2.8. Reflectivity Simulations using Winspall™

Winspall™ version 2.20 is a simulation program developed at the Max Planck Institut für Polymerforschung (Mainz, Germany) that uses the Fresnel equations to model the theoretical SPR minimum for given values of refractive index and thickness of a particular system. Initially, the layer system shown in Figure 2.3 was used to determine the refractive index (RI) of gold by iteratively adjusting gold’s RI to fit experimental SPR data for the SPR minimum of this layer system. RI values for the sapphire prism, glass, and water were obtained from the literature. The self-assembled monolayer (SAM) was not added to the layer system used for the simulations as Sigal et al. determined that the introduction of the SAM had negligible effects on the magnitude of calculated \( \theta_{\text{sp}} \) values and therefore was omitted for simplicity. Thickness values were input as zero for the prism, glass and water as they were considered infinitely thick. The thickness of gold was known to be 500Å per manufacturer’s specifications. For all simulations, a hemispherical prism was chosen along with p-polarized light, a wavelength of 780 nm, a Pktanz value of 1000, and an X-axis ranging from 45 to 60. All RI values were given in the \( n; \kappa \) form where \( n \) is the real part of the refractive index and \( \kappa \) is the absorption coefficient. For this study, only the metallic gold layer possessed a \( \kappa \) value, all other layers had a \( \kappa \) value equal to zero. Once the RI of gold had been

---

**Figure 2.3.** Layer system used in determination of the index of refraction for gold.
calculated, a regenerated cellulose layer was added to the layer system between the water and gold layers (Figure 2.4). Once again, an iterative approach was used to determine the RI of both swollen and non-swollen cellulose based on experimental SPR data. Finally, a layer system containing 6 isotropic layers was constructed to represent adsorption of pullulan and pullulan abietate onto a regenerated cellulose surface on the sensor slide. Figure 2.5 represents the components of the model layer system along with refractive index and thickness values obtained from the simulation. A refractive index of 1.46 was assigned to represent the adsorbed pullulan and pullulan abietate films.

<table>
<thead>
<tr>
<th>Thickness (Å)</th>
<th>n</th>
<th>kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.32642</td>
<td>0</td>
</tr>
<tr>
<td>Variable</td>
<td>1.46</td>
<td>0</td>
</tr>
<tr>
<td>90</td>
<td>1.493</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>0.07</td>
<td>5.83</td>
</tr>
<tr>
<td>0</td>
<td>1.511</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>1.76074</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 2.5. Layer system configuration, thickness, and refractive index values used to simulate pullulan and pullulan abietate adsorption onto a cellulose surface.
2.9. Reflectivity Profiles

Tera Term Pro version 2.3 software is a terminal emulation program that was used to facilitate communication between the SPR Refractometer and the computer to which it was interfaced. This program was utilized in the programming of the SPR coefficients (determined in the ethylene glycol calibration procedure) and in the acquisition of reflectivity profiles. To perform both of these tasks, proper communication between the SPR and the PC had to be verified. Once the Tera Term program was opened, the COM 1 Serial connection was chosen from the menu that appeared. From the “Setup” pull down menu, the “Terminal” option was chosen in which the “Local Echo” box was checked. Local echo allowed typed commands to appear on the PC screen. Within the “Serial Port” setup window, the COM 1 serial port baud rate was set to 19200 to match the baud rate of the lower serial port of the SR 7000. The flow control was also set to “Xon/Xoff” within the “Serial Port” setup. All other settings were allowed to remain as default values. To test communication, the phrase “ping” was entered into Tera Term and transmitted by hitting the enter key. If communication was established, a response stating: i.) MAIN OK, ii.) LSA OK, and iii.) PELTIER OK appeared.

To enter the SPR coefficients once this message appeared, the command “sprcoef” was typed followed by the coefficients from the third order polynomial fit of the real instrument pixel vs. refractive index plot from the ethylene glycol calibration. The numbers were entered in scientific notation beginning with the coefficient of the $x^3$ polynomial and ending with the constant, separating each value with a space. The “Enter” key was used to transmit the data and a “Done” message was returned when the coefficients were accepted.
In order to obtain a reflectivity profile once communication was established, a file name had to be assigned to the data. The file destination was chosen by accessing “LOG” from the “File” pull-down menu and entering a file name and path. The command “dump” was input at which time the SPR sent the reflectivity profile data to the computer and saved it as the aforementioned text file. Reflectivity profiles of various thicknesses of regenerated cellulose (0, 10, 20, 40, 50 layers) were obtained in both air and water. Profiles were acquired on an initiated and calibrated SPR instrument prior to sample analysis due to the inability to operate Tera Term and Labview™ simultaneously. The data was then imported into either Microsoft Excel or Igor Pro for further analysis.

2.10. Reflectivity Profile Fitting

The custom executable program “Reflectivity Normalize” created by Reichert using National Instruments Labview™ software was used to fit the reflectivity profiles. An air scan (a reflectivity profile obtained with air as the background) was imported into the reflectivity normalize program by clicking on the “Set Air Scan” icon and choosing the file to import. Similarly, a water scan was imported by clicking on the “Set Sample Scan” icon and choosing a file. The program functioned by normalizing both the air and water arrays and finally generating a normalized water/normalized air array to determine the pixel number corresponding to the SPR minimum. The movable cursors were used to surround the parabolic portion of the SPR curve. Hitting the “Find Minimum” icon supplied the pixel number corresponding to the array minimum. This value was calculated using a third order polynomial fit to the portion of the graph selected by the positioning of the cursors. The pixel number was converted to an angle using the pixel to angle ratio determined during the ethylene glycol calibration procedure.
3. Solution Characterization Results and Discussion

3.1. Critical Aggregation Concentration Determinations

Prior to investigating the self-assembly of pullulan and pullulan abietate (DS=0.027) at a cellulose surface, their behavior in an aqueous environment was investigated using the Wilhelmy plate technique to determine surface tension. Beginning with pure water at 22.5 °C, the surface tensions of pullulan and pullulan abietate solutions ranging in concentration from 0 to 10,000 mg•L⁻¹ were determined. To determine the critical aggregation concentration (cac), the surface tension (γ) values were plotted as a function of concentration. The presence of surfactant acts to lower the surface tension of water as shown in Figure 3.1. The addition of surfactant continues to lower the surface tension relative to pure water until a point is reached where additional increases in concentration no longer produce significant decreases in surface tension. The concentration at which this occurs is known as the critical micelle concentration (cmc). The term critical aggregation concentration (cac) is used here rather than critical micelle concentration because it is unlikely that pullulan abietate forms traditional “micelles” due to the low degree of substitution of hydrophobic abietate substituents. Rather, it is believed that aggregated structures form in which the abietate groups attempt to minimize their contacts with the aqueous surroundings.

In Figure 3.1, the cac curves of pullulan abietate (DS=0.027) and pullulan are shown. The cac for each material was determined by calculating the point of intersection of the two linear portions of the surface tension vs. concentration curves. Error estimates for each cac value were calculated from the trendlines of each curve assuming ± one standard deviation. The substituted pullulan has a lower cac value, 50 ± 5 mg•L⁻¹,
compared to the cac of the homopolysaccharide, 240 ± 50 mg·L⁻¹. The presence of pullulan abietate in water has a more dramatic effect on the surface tension of water compared to pullulan. Also, the cac of pullulan abietate occurs at a much lower concentration than the cac of pullulan, indicating a strong tendency to self-aggregate, which was expected due to the presence of the pendant abietate groups.

Figure 3.1. Critical aggregation concentration curves for pullulan abietate and pullulan determined using the Wilhelmy plate technique at 22.5 °C.
3.2. Ultraviolet Spectroscopy Results

Ultraviolet spectroscopy was used to quantify the amount of pendant abietate groups on pullulan abietate and thereby determine the degree of substitution (DS). Degree of substitution is defined here as the average percentage of abietate groups per anhydroglucose unit (AGU) (Figure 3.2). The basic building block of cellulose, pullulan abietate and pullulan is the anhydroglucose unit; however, the repeating unit of each polymer is expressed with an adequate amount of AGUs to convey the stereochemistry between adjacent units. Pullulan abietate with a DS=0.02 would have 2 abietate groups for every 100 AGU units or 1 abietate for every 50 AGUs. The synthetic method used by Mr. Hussain, University of Wuppertal, produced a pullulan abietate with an estimated DS=0.02 based on ratios of reactants to products. NMR was used to quantify the DS, however, the NMR spectrum was non-descriptive in terms of the DS on account of the low abietate content relative to the polysaccharide.

Initially, a UV spectrum of pullulan abietate in water was obtained that provided clear evidence of the presence of the abietate substituents. In order to compare pullulan abietate to abietic acid, a common solvent had to be found. A solvent composed of 44% water and 56% methanol by volume was effective at dissolving both species. The UV spectrum of a 22.1 mg•L\(^{-1}\) solution of abietic acid in a 44% water / 56% methanol solvent system is shown in Figure 3.3 with the \(\lambda_{\text{max}}\) values labeled accordingly. A 2188 mg•L\(^{-1}\) solution of un-derivatized pullulan analyzed in the 200-400 nm wavelength range failed to demonstrate absorbance and as a result was not shown. Therefore, it was believed that
any absorbance by pullulan abietate came directly from the presence of the abietate groups. The UV spectrum of a 574 mg•L\(^{-1}\) solution of pullulan abietate in the water/methanol mixed solvent system appears in Figure 3.3.

![UV spectra of abietic acid and pullulan abietate in a mixed solvent system composed of 44% water and 56% methanol by volume. Un-derivatized pullulan does not absorb in this region and was therefore not shown.](image)

**Figure 3.3.** UV spectra of abietic acid and pullulan abietate in a mixed solvent system composed of 44% water and 56% methanol by volume. Un-derivatized pullulan does not absorb in this region and was therefore not shown.
The calibration curve of abietic acid (inset) was obtained by plotting abietic acid concentration against absorbance at 252 nm. This wavelength was chosen because both species have $\lambda_{\text{max}}$ values at 252 nm. From the slope of the calibration curve, the molar absorptivity ($\varepsilon$) of abietic acid was estimated as 6928 cm$^{-1}$M$^{-1}$ at 252nm. By substituting pullulan abietate absorbance values into the equation of the calibration curve, the effective concentration of abietic acid substituted repeating units contained in each solution was calculated. The molecular weights of the substituted and unsubstituted repeating units, 446.583 g•mol$^{-1}$ and 162.142 g•mol$^{-1}$ respectively, were used to convert concentration to mg•L$^{-1}$. The concentration of unsubstituted pullulan units was calculated by subtracting the concentration of substituted repeating units from the initial total concentration of pullulan abietate. Once both of these values were known, the following equation was used to calculate mole fraction and therefore determine the degree of substitution of pullulan abietate:

$$\text{Mole Fraction} = \frac{[\text{Substituted Pullulan}]}{[\text{Substituted Pullulan}]+[\text{Un-Substituted Pullulan}]} \quad \text{Equation 3.1}$$

The degree of substitution was determined to be 0.027 ± 0.001 using this method of ultraviolet analysis. This DS equates to having 1 abietic acid ester group for every 37 anhydroglucose units.
4. Surface Characterization Results and Discussion

4.1. Calibration of the Surface Plasmon Resonance Refractometer

The surface plasmon resonance (SPR) technique was used to monitor the docking behavior of a model lignin-carbohydrate complex, pullulan abietate, onto a model cellulose surface. The SR7000 surface plasmon resonance refractometer is equipped with a 3694 pixel CCD array which detects changes in refractive index at the surface and expresses these values in terms of a change in resonance angle. A calibration procedure to relate refractive index to pixel number and in turn, change in resonance angle was performed using ethylene glycol (EG) standards of known concentration. The resonance angle, $\theta_{sp}$, coincides to the angle of minimum intensity of the reflectivity profile. The instrument supplies output in terms of specific pixel sites and therefore the instrument must be equipped with a dip finding algorithm to determine the minimum of the SPR dip. Simply finding the pixel with the smallest intensity i.e., using an intensity algorithm, is not adequate as the sensitivity of this technique suffers with increasing refractive index values. Therefore, a third-order polynomial algorithm which also accounts for pixels symmetrically spaced around the minimum pixel is used to determine the position of the SPR dip. Instrument demo data supplied by Thomas Ryan, Reichert Instruments, was used to determine the third-order polynomial pixel for an ethylene glycol solution with $n=1.325953$ for the SR7000. The minimum pixel determined using an intensity algorithm for the TR SR7000 (Ryan Instrument) was subtracted from that of the AE SR7000 (Esker Instrument) yielding a value of 47.511 pixels. This value was added to the TR third-order polynomial pixel for the EG solution to obtain the AE third-order polynomial pixel. The difference between the AE and TR third-order polynomial pixel
values, 47.511, was termed the pixel correction factor and was used to correct the pixel numbers of the other ethylene glycol solutions used for the calibration. This was done by taking the TR third-order polynomial pixel values of the other EG solutions and adding the pixel correction factor to them.

The reflectivity simulation program Winspall™ was used to determine the theoretical minimum of the SPR dip in terms of angle for each EG solution using a 4 layer system composed of sapphire, glass, gold, and ethylene glycol. The theoretical SPR response obtained using Winspall™ for an EG solution with n=1.325953 appears in Figure 4.1. Plotting the AE third-order polynomial pixel value against the corresponding Winspall™ angle for each ethylene glycol solution produced a plot with a slope of -0.00546315 degrees\(\cdot\)pixel\(^{-1}\) and an intercept of 67.02 as shown in Figure 4.2. This pixel to angle conversion factor allows the instrument to detect a change in pixel number and express it as a change in resonance angle, a more meaningful quantity.
**Figure 4.1.** Theoretical SPR curve for an ethylene glycol standard obtained using Winspall™.

**Figure 4.2.** Pixel to angle relationship for ethylene glycol standards of known concentration.
To relate changes in pixel number to changes in refractive index, the SPR coefficients were determined. These coefficients were obtained by running solutions of varying concentration of ethylene glycol over a clean, bare SPR sensor slide and recording the response in pixels expressed as array cell numbers. The array cell number is defined as the total number of pixels in the CCD array (3694) less the real instrument pixel. The refractive index values of ethylene glycol solutions of mass concentrations varying from 0 – 4.11% were determined using a linear extrapolation of mass concentration vs. refractive index data gathered from the literature and corrected to 780 nm. Initially, water was allowed to flow over the bare slide to establish a baseline corresponding to an array cell number of 680.851. The ethylene glycol solutions were introduced into the flow cell causing a rise in signal corresponding to a change in refractive index. Each solution was allowed to flow until leveling off at which point water was reintroduced into the system to clean the lines. Each solution was allowed to flow twice for approximately 45-55 seconds each time before analyzing the next solution of higher concentration as shown in Figure 4.3. After allowing the first ethylene glycol solution to flow over the SPR sensor, water was reintroduced into the flow cell however the baseline did not return to its original value due to a baseline drift. Therefore, the difference between the peak maximum and the peak minimum were determined for each solution and then added to the original value of the baseline, 680.851, to correct for the drifting baseline. Each array cell number corresponding to a specific ethylene glycol solution was converted to a real instrument pixel number by subtracting it from the total number of pixels in the CCD array (3694). To determine the SPR coefficients, these real instrument pixel numbers were plotted against refractive index as shown in Figure 4.4. A
third-order polynomial fit to the data was used to extract the SPR coefficients: $Y = 6.8785196 \times 10^{-9} X^3 - 6.18001270 \times 10^{-5} X^2 + 1.84984112 \times 10^{-1} X - 1.83149905 \times 10^2$

where $Y$ is the refractive index and $X$ is the real instrument pixel.

**Figure 4.3.** Ethylene glycol calibration SPR output where A = water and B-F = ethylene glycol solutions of varying mass concentrations. The concentrations are: A=0, B=0.121, C=0.514, D=1.02, E=2.03, and F=4.11 % ethylene glycol by weight.
Figure 4.4. Determination of the SPR coefficients. Coefficients were extracted from a third-order polynomial fit to the data, Refractive Index = $6.87875196 \times 10^{-9} X^3 - 6.18001270 \times 10^{-5} X^2 + 1.84984112 \times 10^{-1} X - 1.83149905 \times 10^2$, where X is the Real Instrument Pixel Number.

The SPR coefficients were programmed into the instrument using the Tera Term software program. Once communication had been established between the PC and the instrument, the coefficients were entered in scientific notation with 8 decimal places. Once the SPR coefficients had been programmed, the SPR data could be expressed in terms of $\Delta \theta_{sp}$, the change in resonance angle relative to pure water.

4.2. Determination of the Optical Constants for Gold and Cellulose

SPR sensor slides possessing a dodecanethiol self-assembled monolayer (SAM) and a varying thickness of regenerated cellulose on their surfaces were used to determine the relationship between thickness (L) and $\theta_{sp}$ for cellulose. Slides with 0, 10, 20, 40, and
50 layers of regenerated cellulose were prepared using the Langmuir-Blodgett (LB) technique. Each slide was mounted onto the prism of the SPR and Tera Term was used to obtain a reflectivity profile in air (air array) and water (water array) for each slide immediately upon exposure to water. This water array obtained immediately after mounting was considered to describe the behavior of the regenerated cellulose in the “un-swollen” state before its thickness per layer and refractive index changed due to water absorption. After allowing water to run until a stable baseline had been established, another water scan was acquired and used to describe “swollen” cellulose. In order to determine the minimum pixel of the SPR dip, the Reflectivity Normalize.exe program was used. Figure 4.5 shows the air and water arrays for a slide possessing 20 layers of regenerated cellulose on its surface in terms of pixel while Figure 4.6 shows the same scans in terms of angle. Each scan was normalized to itself and then the normalized water array was divided by the normalized air array to create a smooth SPR reflectivity profile. Dividing the water scan by the air scan reduces noise and more clearly shows the critical angle component.

The scans were obtained in terms of pixel but converted to angular expressions by first importing the profiles into the Reflectivity Normalize.exe program and then entering the pixel to angle conversion factor. The critical angle is the incident angle which produces a 90º angle of refraction and above which total internal reflectance occurs. Figure 4.6 C shows the region where the critical angle occurs. The critical angle was used to scale the water scan / air scan by 0.774 so that the maximum reflectivity did not exceed an intensity of 1.0. The minimum of the SPR dip was also found using this program for each of the sensor slides by fitting a third-order polynomial function to the
parabolic portion of the curve similar to that shown in Figure 4.7. For the slide with 20 layers of “un-swollen” cellulose on its surface, a minimum of 2915 pixels (51.095 degrees) was calculated with an $R^2$ value of 0.989.
Figure 4.5. Reflectivity profiles for an SPR sensor slide with 20 layers of regenerated cellulose on its surface in terms of pixel number: A.) Normalized Air Scan, B.) Normalized Water Scan, C.) Normalized Water Scan/Normalized Air Scan, D.) Scaled Normalized Water Scan/Normalized Air Scan.
Figure 4.6. Reflectivity profiles for an SPR sensor slide with 20 layers of regenerated cellulose on its surface in terms of angle: A.) Normalized Air Scan, B.) Normalized Water Scan, C.) Normalized Water Scan/Normalized Air Scan, D.) Scaled Normalized Water Scan/Normalized Air Scan.
Figure 4.7. Approximation of the region fit with a third-order polynomial function to determine the SPR dip minimum for an SPR sensor slide coated with 20 layers of regenerated cellulose.

The reflectivity profile fit procedure was conducted for each slide in the un-swollen and swollen states to obtain the angles corresponding to the SPR minimum dip for each. The thickness of an un-swollen regenerated cellulose layer was assumed to be 4.2 Å\text{layer}^{-1} based on X-ray reflectivity experiments performed by Buchholz et al.\textsuperscript{64} Figure 4.8 displays experimental and simulation data for the relationship between $\theta_{sp}$ and thickness for un-swollen cellulose. The simulation data was obtained from Winspall\textsuperscript{TM} using the 5 component layer system shown in Figure 4.9.
Figure 4.8. Theoretical data obtained using $n=1.523$ for un-swollen cellulose compared to experimental data obtained by determining the SPR minimum of each reflectivity profile.

<table>
<thead>
<tr>
<th>Thickness (Å)</th>
<th>n-ref</th>
<th>kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0</td>
<td>1.32642</td>
</tr>
<tr>
<td>Regenerated Cellulose</td>
<td>4.2 Å / layer</td>
<td>Vary</td>
</tr>
<tr>
<td>Gold</td>
<td>500</td>
<td>0.17</td>
</tr>
<tr>
<td>Glass</td>
<td>0</td>
<td>1.511</td>
</tr>
<tr>
<td>Sapphire Prism</td>
<td>0</td>
<td>1.76074</td>
</tr>
</tbody>
</table>

Figure 4.9. Winspall™ layer system used to acquire the theoretical data in Figure 4.8 for un-swollen cellulose.
The traces in Figure 4.8 share neither a common slope nor intercept, therefore a self-consistent model allowing the theoretical data to better match the experimental data was developed. The first point to be matched was for a thickness of cellulose equal to zero, corresponding to a bare gold slide. Using a refractive index value of $n=0.17$; $\kappa=4.93$ for gold obtained from work conducted by Sigal et al.\textsuperscript{94}, Winspall\textsuperscript{TM} was used to obtain a theoretical $\theta_{sp}$ value ($\theta_{sp, theo}$) of 51.5616 degrees for the layer system lacking a cellulose layer on gold. The experimental value of $\theta_{sp}$ ($\theta_{sp, exp}$) for the same system was 50.7028 degrees. The self-assembled monolayer was omitted from the layer system used to simulate the SPR response. The justification for this treatment was the work of Sigal et al. who noted that the presence of the SAM had a negligible effect on the change in resonance angle and therefore could be ignored when conducting simulations.\textsuperscript{94} Sigal et al.’s finding was confirmed by examining a gold slide with only a SAM deposited on its surface and recording a $\theta_{sp, exp}$ of 50.7032 degrees for that system which is extremely close to the value recorded for the bare gold slide. The parameters for gold were manipulated to match the theoretical data to the experimental data. Increasing the imaginary portion of the refractive index, $\kappa$, relative to the value in Figure 4.9 while holding the real part, $n$, at 0.17 caused the theoretical theta minimum to shift to lower values and the reflected intensity of the dip to increase as shown in Figure 4.10. The optimal $\kappa$ value for the gold was determined to be 5.83. This value was determined by fitting the curve in Figure 4.10 describing the relationship between kappa and $\theta_{sp}$ with an exponential function and inserting the $\theta_{sp, exp}$ value of 50.7028 degrees into the fit equation to obtain the corresponding kappa value. The minimum of the “ideal” theoretical SPR dip should be as close to zero as possible; however, for $\kappa=5.83$, the
reflected intensity of the minimum shifted away from zero. In order to correct for this deviation, the real part of the refractive index, \( n \), was reduced to 0.07 while holding \( \kappa \) at 5.83 until the reflected intensity of the minimum once again approached zero. Increasing \( n \) did not shift the minimum angle, but did lower the reflected intensity at the minimum point as shown in Figure 4.11. To determine the value of \( n \) producing a reflected intensity at the SPR minimum closest to zero, the portion of the curve in Figure 4.11 close to the minimum was fit with a third-order polynomial. The first derivative of this fit was taken and set equal to zero, solving for \( n \) yielded a minimum \( n \) value of 0.07. The optimal parameters for a complex refractive index for gold were determined to be \( n=0.07 \) and \( \kappa=5.83 \) producing \( \theta_{sp}=50.7207 \) degrees with a reflected intensity of 0.0005.

**Figure 4.10.** The effect of \( \kappa \) on the minimum angle and its reflected intensity assuming \( n=0.17 \) for the gold layer.
Figure 4.11. Relationship between the real part of the refractive index, n, for gold and the reflected intensity of the SPR minimum for $\kappa=5.83$.

Following the determination of the “optimal” refractive index for gold, a regenerated cellulose layer with a thickness (L) of 4.2 Å•layer$^{-1}$ was added on top of the gold as shown in Figure 4.9. The literature stated that the refractive index of dry cellulose was 1.523$^{101}$ however; using this value produced a discrepancy between the experimental and theoretical data. Refractive index values of 1.49, 1.50, 1.501, 1.503, 1.505, 1.507, and 1.51 were used to generate thickness vs. $\theta_{sp}$ curves for 0, 10, 20, 40, and 50 layers of cellulose assuming a thickness of 4.2 Å•layer$^{-1}$. The trace corresponding to $n=1.505$ was chosen as its slope (0.0046578 degrees•Å$^{-1}$) most closely matched the slope of the experimental data (0.0046553 degrees•Å$^{-1}$) as shown in Table 4.1. This value was used to represent the refractive index of un-swollen cellulose corresponding to a thickness per layer of 4.2 Å.
Table 4.1. Slopes of theoretical thickness vs. $\theta_{sp, theo}$ plots along with corresponding refractive index values assuming a thickness of 4.2 Å per layer for cellulose.

<table>
<thead>
<tr>
<th>$n$</th>
<th>Slope</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.490</td>
<td>0.004239</td>
<td>8.95</td>
</tr>
<tr>
<td>1.500</td>
<td>0.004515</td>
<td>3.01</td>
</tr>
<tr>
<td>1.501</td>
<td>0.004515</td>
<td>3.01</td>
</tr>
<tr>
<td>1.503</td>
<td>0.004566</td>
<td>1.92</td>
</tr>
<tr>
<td>1.505</td>
<td>0.004658</td>
<td>0.0537</td>
</tr>
<tr>
<td>1.507</td>
<td>0.004648</td>
<td>0.165</td>
</tr>
<tr>
<td>1.510</td>
<td>0.004780</td>
<td>2.69</td>
</tr>
</tbody>
</table>

Experimental Slope = 0.004655

When regenerated cellulose swells in the presence of water, it also experiences a change in refractive index therefore, the values of L=4.2 Å•layer$^{-1}$ and $n=1.505$ are no longer valid. The experimental values of $\theta_{sp}$ were obtained for 0, 10, 20, 40, and 50 layers of swollen cellulose, however the actual thickness per layer and the refractive index were initially unknown. In an attempt to match theoretical to experimental data, both the refractive index and the thickness per layer were simultaneously altered. Assuming ideal mixing, refractive index values were calculated using the following formula:

$$n = \Phi_1 \cdot n_{cellulose} + (1 - \Phi_1) n_{water}$$

Equation 4.1

where $\Phi_1$ represents the volume fraction of cellulose and $n$ is the refractive index of each respective material. By assuming ideal mixing, the volume fraction of cellulose, $\Phi_1$, is equal to the ratio of the thicknesses of the dry cellulose film to the swollen cellulose film. This formula was used assuming a dry cellulose thickness of 4.2 Å with a refractive index of 1.505 while the refractive index of water was assumed to be 1.32642. The swollen thickness per layer of cellulose was varied from 4.3 to 6.0 Å in increments of 0.1 Å
producing refractive index values ranging from 1.501 to 1.451. Each thickness and corresponding refractive index value were used to produce a plot of thickness vs $\theta_{sp,\text{theo}}$. The experimental $\theta_{sp}$ values were plotted against the thickness calculated assuming the specific thickness per layer in question. Table 4.2 shows the experimental and theoretical slopes for each refractive index and thickness combination along with the % difference between the slopes. The refractive index and thickness combination providing the smallest difference from the experimental data were assigned as the values for swollen cellulose. It was determined that in the presence of water, regenerated cellulose swells to a thickness per layer of 4.5Å with a refractive index of 1.493.

**Table 4.2.** Slopes of experimental and theoretical thickness vs. $\theta_{sp}$ plots along with corresponding refractive index values for varying thicknesses per layer of cellulose.

<table>
<thead>
<tr>
<th>n</th>
<th>L (Å•layer$^{-1}$)</th>
<th>Experimental Slope</th>
<th>Theoretical Slope</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.501</td>
<td>4.3</td>
<td>0.004577</td>
<td>0.004540</td>
<td>0.771</td>
</tr>
<tr>
<td>1.497</td>
<td>4.4</td>
<td>0.004471</td>
<td>0.004364</td>
<td>0.784</td>
</tr>
<tr>
<td>1.493</td>
<td>4.5</td>
<td>0.004371</td>
<td>0.004338</td>
<td>0.770</td>
</tr>
<tr>
<td>1.489</td>
<td>4.6</td>
<td>0.004276</td>
<td>0.004343</td>
<td>1.18</td>
</tr>
<tr>
<td>1.486</td>
<td>4.7</td>
<td>0.004185</td>
<td>0.004226</td>
<td>0.968</td>
</tr>
<tr>
<td>1.483</td>
<td>4.8</td>
<td>0.004098</td>
<td>0.004138</td>
<td>0.969</td>
</tr>
<tr>
<td>1.479</td>
<td>4.9</td>
<td>0.004014</td>
<td>0.004054</td>
<td>0.967</td>
</tr>
<tr>
<td>1.476</td>
<td>5.0</td>
<td>0.003934</td>
<td>0.003973</td>
<td>0.969</td>
</tr>
<tr>
<td>1.473</td>
<td>5.1</td>
<td>0.003857</td>
<td>0.003895</td>
<td>0.968</td>
</tr>
<tr>
<td>1.471</td>
<td>5.2</td>
<td>0.003783</td>
<td>0.003828</td>
<td>1.18</td>
</tr>
<tr>
<td>1.468</td>
<td>5.3</td>
<td>0.003711</td>
<td>0.003756</td>
<td>1.18</td>
</tr>
<tr>
<td>1.465</td>
<td>5.4</td>
<td>0.003643</td>
<td>0.003678</td>
<td>0.968</td>
</tr>
<tr>
<td>1.463</td>
<td>5.5</td>
<td>0.003576</td>
<td>0.003619</td>
<td>1.18</td>
</tr>
<tr>
<td>1.460</td>
<td>5.6</td>
<td>0.003513</td>
<td>0.003555</td>
<td>1.18</td>
</tr>
<tr>
<td>1.458</td>
<td>5.7</td>
<td>0.003451</td>
<td>0.003492</td>
<td>1.18</td>
</tr>
<tr>
<td>1.456</td>
<td>5.8</td>
<td>0.003392</td>
<td>0.003484</td>
<td>2.65</td>
</tr>
<tr>
<td>1.454</td>
<td>5.9</td>
<td>0.003334</td>
<td>0.003403</td>
<td>2.02</td>
</tr>
<tr>
<td>1.451</td>
<td>6.0</td>
<td>0.003278</td>
<td>0.003318</td>
<td>1.18</td>
</tr>
</tbody>
</table>
4.3. The de Feijter Equation

As mentioned in Section 1, the de Feijter equation can be used along with SPR data to quantify adsorption onto a surface. This equation contains three main components: 1.) $\Delta \theta_a$, 2.) $\frac{dn}{dc}$, and 3.) $\frac{d\theta_{sp}}{dL}$. The first component, $\Delta \theta_a$, is defined as the change in SPR angle due to adsorption only and was obtained by analyzing a series of solutions with varying concentrations using the SPR technique and correcting for bulk effects. The second component, $\frac{dn}{dc}$, is termed the refractive index increment and was found by determining the refractive indices of several highly concentrated solutions. The third, $\frac{d\theta_{sp}}{dL}$, is the response of $\theta_{sp}$ to changes in thickness obtained theoretically using reflectivity simulation software and will be discussed first.

4.4. Pullulan Abietate Reflectivity Simulation

The layer system configuration shown in Figure 2.5 was used to determine the $\frac{d\theta_{sp}}{dL}$ parameter for pullulan abietate adsorbed onto 20 layers of regenerated cellulose. The value of the refractive index for the pullulan abietate thin film was estimated as 1.46. Although this is not an exact value, the refractive index was expected to be less than that of cellulose due to a greater flexibility of pullulan abietate chains compared to the rigid structure of cellulose. Figure 4.12 shows how changes in the thickness (L) of adsorbed pullulan abietate affect the theoretical resonance angle ($\theta_{sp}$). A linear fit was added to the data, the slope of which yielded the value of $\frac{d\theta_{sp}}{dL} = 0.00363$ degrees$\cdot$Å$^{-1}$ for pullulan abietate (DS=0.027). Simulations of pullulan abietate onto 30 and 40 layers of cellulose...
were also conducted (assuming a thickness of 4.5Å•layer⁻¹ for cellulose) yielding \( \frac{d\theta_{sp}}{dL} \) values of 0.00375 and 0.00388 degrees•Å⁻¹ respectively. The adsorption of unsubstituted pullulan onto 20 layers of cellulose was also modeled using a refractive index of 1.46. This resulted in a \( \frac{d\theta_{sp}}{dL} \) value of 0.00363 degrees•Å⁻¹ for the homopolysaccharide.

\[ y = 0.00363 \times + 51.091 \]

![Graph showing \( \theta_{sp, theo} \) vs Pullulan Abietate Thickness in Å](image)

**Figure 4.12.** Determination of \( \frac{d\theta_{sp}}{dL} \) for pullulan abietate on 20 layers of cellulose using reflectivity simulation data. The refractive index of the pullulan abietate was assumed to be \( n=1.46 \).

### 4.5. Refractive Index Increments of Pullulan and Pullulan Abietate (DS=0.027)

Initially, the solutions used for SPR analysis with concentrations ranging from 0 – 1000 mg•L⁻¹ were analyzed using an Abbe refractometer. Rather than witnessing the refractive index increase with increasing concentration, the values did not shift...
significantly from the value of pure water at 20 °C. Therefore, solutions with much higher concentrations were prepared spanning a range from 0 – 232,000 mg•L⁻¹ and analyzed. The Abbe refractometer is designed with an optical setup allowing one to obtain values at the sodium D line, λ=589 nm. The SPR operates at a wavelength of 780 nm which requires the refractive index data to be corrected before determining the refractive index increment. An empirical model (Equation 4.2) originally developed by Quan and Fry describing the dependence of refractive index on wavelength for water was used along with Equation 4.3 to convert $n_{D}^{20}$ to $n_{780}^{20}$ for each data point.

$$n(\lambda) = 1.31279 + \frac{15.762}{\lambda} - \frac{4382}{\lambda^2} + \frac{1.1455 \times 10^6}{\lambda^3}$$  \hspace{1cm} \text{Equation 4.2}$$

$$n_{780, \text{solution}} = n_{589, \text{solution}} + \left[ n(780) - n(589) \right]_{\text{water}}$$  \hspace{1cm} \text{Equation 4.3}$$

The concentration of each solution was converted to g•mL⁻¹ and plotted against its corresponding index of refraction to yield Figure 4.13. The slope of the trendline is equivalent to the refractive index increment of each material. Error estimates of ± one standard deviation for each refractive index increment were obtained from the standard deviation of the trendlines obtained by Igor Pro. Pullulan abietate (DS=0.027) has a slightly higher refractive index increment, 0.133±0.003 mL•g⁻¹ compared to that of the unsubstituted pullulan, 0.119±0.003 mL•g⁻¹.
Figure 4.13. Determination of the refractive index increments for pullulan and pullulan abietate (DS=0.027) at 20 °C and a wavelength of $\lambda=780$ nm.

4.6. Surface Plasmon Resonance Analysis

The surface plasmon resonance (SPR) technique was used to monitor adsorption and desorption events for pullulan and pullulan abietate (DS=0.027) on a regenerated cellulose surface. Figure 4.14 shows an SPR profile for the adsorption of pullulan
abietate (DS=0.027) onto 40 layers of regenerated cellulose where increases in $\Delta \theta_{sp}$ reflect changes during exposure to a pullulan abietate solution, while decreases in $\Delta \theta_{sp}$ reflect changes that occur after switching the solution to water. Each SPR peak corresponds to a specific concentration of solution used for the analysis. The adsorption behavior of pullulan abietate (DS=0.027) onto 20, 30, and 40 layers of regenerated cellulose was monitored using SPR. The adsorption of unsubstituted pullulan onto a cellulose surface 20 layers in thickness was also studied as a comparison.

Figure 4.14. Raw SPR data for the adsorption of pullulan abietate onto 40 layers of regenerated cellulose at 20 °C. The letters on the graph correspond to the concentrations of the pullulan abietate: A=27, B=56, C=106, D=212, E=524, F=1024, G=2000, H=3000, I=5000, and J=10000 mg•L$^{-1}$. 

64
4.7. Adsorption of Pullulan and Pullulan Abietate (DS=0.027) onto Cellulose

The maximum values of $\Delta \theta_{sp}$ for each solution were plotted against their respective concentrations to obtain Figures 4.15-4.17 representing pullulan abietate adsorption onto 20, 30, and 40 layers of cellulose, respectively. The adsorption of pullulan onto 20 layers of cellulose was also monitored and appears in Figure 4.18. Since SPR measurements detect changes in the refractive index of the medium within ~ 200 nm of the surface, this technique is sensitive to both adsorption of material at the surface as well as to the presence of molecules in the bulk. The bulk effect produces a displacement in $\theta_{sp}$ proportional to the concentration of the analyte due solely to changes in the refractive index of the bulk and not necessarily to refractive index changes at the surface.\textsuperscript{94} To obtain the correct amount of material docked on the surface, a correction had to be made for the bulk effect. In each case, $\theta_{sp}$ values were corrected by determining the slopes, $\frac{d\theta_{sp}}{dc}$, of the linear portions of Figures 4.15-4.18 and by using Equation 4.4 to calculate $\Delta \theta_a$ for each solution.

$$\Delta \theta_a = \Delta \theta_{sp} - c \frac{d\theta_{sp}}{dc}$$

Equation 4.4

These $\Delta \theta_a$ values were plotted against their respective concentrations to obtain the “corrected” data shown in Figures 4.15-4.18. With all pieces of the de Feijter equation now calculated, a quantitative description of the adsorption of each material onto cellulose can be developed.
Figure 4.15. “Maximum” and corrected $\Delta\theta_{sp}$ values for the adsorption of pullulan abietate (DS=0.027) onto 20 layers of regenerated cellulose at 20 °C. The “maximum” values contain contributions from irreversible and reversible adsorption as well as changes in the bulk refractive index. Corrected adsorption represents the change in $\Delta\theta_{sp}$ for irreversible adsorption.
Figure 4.16. “Maximum” and corrected $\Delta \theta_{sp}$ values for the adsorption of pullulan abietate (DS=0.027) onto 30 layers of regenerated cellulose at 20 °C. “Maximum” and corrected adsorption are defined in Figure 4.15.
Figure 4.17. "Maximum" and corrected $\Delta \theta_{sp}$ values for the adsorption of pullulan abietate (DS=0.027) onto 40 layers of regenerated cellulose at 20 °C. “Maximum” and corrected adsorption are defined in Figure 4.15.
**Figure 4.18.** “Maximum” and corrected $\Delta \theta_{sp}$ values for the adsorption of pullulan onto 20 layers of cellulose at 20 °C. “Maximum” and corrected adsorption are defined in Figure 4.15.

### 4.8. Adsorption Isotherms for Pullulan and Pullulan Abietate (DS=0.027).

Initially, surface excess, $\Gamma$, values were calculated for each system using Equation 4.5 knowing the corrected adsorption ($\Delta \theta_a$), the refractive index increment $\left(\frac{dn}{dc}\right)$, the change in SPR angle with thickness $\left(\frac{d\theta_{sp}}{dL}\right)$, the refractive index of the adsorbed film ($n_r$), and the refractive index of the buffer ($n_s$) for each. For all systems, a value of 1.32642 was used for the refractive index of water ($n_s$). Table 4.3 contains the data used.
in the calculation of \( \Gamma \) values for pullulan and pullulan abietate (DS=0.027) adsorbed onto cellulose surfaces of varying thickness.

\[
\Gamma = \frac{\Delta \theta_a (n_f - n_s)}{\frac{d\theta_{sp}}{dL} \left( \frac{dn}{dc} \right)} \quad \text{Equation 4.5}
\]

Table 4.3. Data used in the calculation of \( \Gamma \) values for each system studied. A value of 1.32642 was used for refractive index of water \((n_s)\) in all systems.

<table>
<thead>
<tr>
<th># of Cellulose Layers</th>
<th>Adsorbed Material</th>
<th>( \Delta \theta_a )</th>
<th>( n_f )</th>
<th>( \frac{d\theta_{sp}}{dL} ) (degrees•Å(^{-1}))</th>
<th>( \frac{dn}{dc} ) (mL•g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Pullulan Abietate</td>
<td>Figure 4.15</td>
<td>1.46</td>
<td>0.00363</td>
<td>0.133</td>
</tr>
<tr>
<td>30</td>
<td>Pullulan Abietate</td>
<td>Figure 4.16</td>
<td>1.46</td>
<td>0.00375</td>
<td>0.133</td>
</tr>
<tr>
<td>40</td>
<td>Pullulan Abietate</td>
<td>Figure 4.17</td>
<td>1.46</td>
<td>0.00388</td>
<td>0.133</td>
</tr>
<tr>
<td>20</td>
<td>Pullulan</td>
<td>Figure 4.18</td>
<td>1.46</td>
<td>0.00363</td>
<td>0.119</td>
</tr>
</tbody>
</table>

Simply using Equation 4.5 and substituting in the appropriate values for all variables produces \( \Gamma \) values with units of Å•g•mL\(^{-1}\). To convert \( \Gamma \) in terms of Å•g•mL\(^{-1}\) to the desired units of anhydroglucose units (AGU)•nm\(^{-2}\), each \( \Gamma \) was multiplied by a factor of 10\(^{-22}\) to convert length units to nm (assuming 1 mL = 1 cm\(^3\)), was divided by the average molecular weight for each anhydroglucose unit (MW) to convert to moles, and was finally multiplied by Avogadro’s number. A MW value of 169.823 g•mol\(^{-1}\) was used for pullulan abietate (DS=0.027), while MW = 162.143 g•mol\(^{-1}\) was used for the pullulan.

Values of \( \Gamma \) were plotted against their respective concentrations to obtain the adsorption isotherms shown in Figures 4.19-4.22. In each case, an exponential function (solid trace) was fit to each curve to obtain the number of anhydroglucose units per nm\(^2\) of cellulose surface area present at “surface saturation” \((\Gamma_{sat})\). Error estimates obtained from Igor.
Pro’s fitting routine represent ± one standard deviation. Equation 4.6 represents the Langmuir isotherm where $\Gamma$ is the number of AGUs adsorbed per nm$^2$ of cellulose surface area for a specific bulk concentration, $\Gamma_{\text{sat}}$ is the number of AGUs adsorbed per nm$^2$ of cellulose surface area at surface saturation, $K$ is the adsorption constant, and $c$ is concentration.

$$\Gamma = \frac{\Gamma_{\text{sat}} Kc}{1 + Kc} \quad \text{Equation 4.6}$$

The adsorption isotherms in Figures 4.19-4.22 were fit with this function to determine values of $K$ for each case. $K$ is defined as the ratio of the rate constant for association ($k_a$) to the rate constant for disassociation ($k_d$). The Langmuir adsorption isotherm function was programmed into Igor Pro as a user defined function requiring an initial guess for the $\Gamma_{\text{sat}}$ and $K$ coefficients. Values of $\Gamma_{\text{sat}}$ obtained from the exponential fits to each curve appear in Figures 19-22 and were held constant to fit the data to the Langmuir adsorption isotherm.
**Figure 4.19.** Adsorption isotherm of pullulan abietate (DS=0.027) on 20 layers of regenerated cellulose at 20 °C. The solid line is an exponential fit used to obtain $\Gamma_{sat}$, while the dotted line is the best fit of the Langmuir adsorption isotherm, Equation 4.6, to the data. The error bars on $\Gamma_{sat}$ and $K$ represent ± one standard deviation.
Figure 4.20. Adsorption isotherm of pullulan abietate (DS=0.027) on 30 layers of regenerated cellulose at 20 °C. The solid line is an exponential fit used to obtain $\Gamma_{\text{sat}}$, while the dotted line is the best fit of the Langmuir adsorption isotherm, Equation 4.6, to the data. The error bars on $\Gamma_{\text{sat}}$ and $K$ represent ± one standard deviation.
**Figure 4.21.** Adsorption isotherm of pullulan abietate (DS=0.027) on 40 layers of regenerated cellulose at 20 °C. The solid line is an exponential fit used to obtain $\Gamma_{\text{sat}}$, while the dotted line is the best fit of the Langmuir adsorption isotherm, Equation 4.6, to the data. The error bars on $\Gamma_{\text{sat}}$ and $K$ represent ± one standard deviation.
Figure 4.22. Adsorption isotherm of pullulan onto 20 layers of regenerated cellulose at 20 °C. The solid line is an exponential fit used to obtain $\Gamma_{\text{sat}}$, while the dotted line is the best fit of the Langmuir adsorption isotherm, Equation 4.6, to the data. The error bars on $\Gamma_{\text{sat}}$ and K represent ± one standard deviation.

For the adsorption of pullulan abietate (DS=0.027), it appears that between 9-10 anhydroglucose units adsorb per nm$^2$ of cellulose surface area. In comparison, approximately 6.6 anhydroglucose units of unsubstituted pullulan adsorb per nm$^2$ of cellulose surface area. It appears that the presence of the pendant abietate groups have a marked effect on adsorption, driving the self-assembly of pullulan abietate at the surface of cellulose. Although the adsorption of pullulan and pullulan abietate is irreversible, the low K values indicate that the rate of disassociation is larger than the rate of association for all cases. Given the hydrophilic nature of the regenerated cellulose surface, it is most
likely that the physical-adsorption of unmodified polysaccharides is driven by hydrogen bonding, whereas pullulan modified by hydrophobic substituents would also benefit from the sum of relatively weak van der Waal interactions that can drive polymer adsorption to surfaces. As the polymer diffuses from dilute solution toward the surface, a monolayer of flattened chains starts to develop. Over time, new chains form loops adjoining disconnected empty sites and a diffuse layer starts to build up as shown in Figure 4.23. Using the molecular area for a single anhydroglucose unit lying flat on a surface ($60\AA^2\cdot\text{AGU}^{-1}$ for cellulose) and assuming the monolayer thickness of pullulan and pullulan abietate to be the same as that of regenerated cellulose ($4.2\AA$) if they adsorb flat on the surface the values of $\Gamma_{\text{sat}}$ were used to calculate the thickness of the adsorbate. The theoretical thicknesses of pullulan and pullulan abietate on cellulose were also calculated by extracting $\theta_{\text{sat}}$ values from exponential fits to the corrected adsorption curves shown in Figures 15-18 and using $\frac{d\theta_{\text{sp}}}{dL}$ to determine adsorbed thickness values ($L_{\text{ads}}$). Table 4.4 shows the thicknesses of adsorbate on cellulose where method 1 involved the utilization of $\Gamma_{\text{sat}}$ values and molecular modeling, while method 2 involved the utilization of $\frac{d\theta_{\text{sp}}}{dL}$ values obtained through reflectivity simulations. The presence of the abietic acid substituents on pullulan abietate causes the build up of a slightly thicker adsorbed layer compared to the unsubstituted pullulan.
Figure 4.23. Schematic displaying the average thicknesses (obtained from Table 4.4) of the diffuse layers formed through adsorption of pullulan and pullulan abietate on cellulose.

Table 4.4. Thickness of adsorbed layers of pullulan and pullulan abietate on cellulose.

<table>
<thead>
<tr>
<th>Adsorbed Material</th>
<th>$\Gamma_{\text{sat}}$ (AGU•nm$^{-2}$)</th>
<th>$\theta_{\text{sat}}$ (degrees)</th>
<th>$\frac{d\theta_{\text{sp}}}{dL}$ (degrees•Å$^{-1}$)</th>
<th>$L_{\text{ads}}$ (Å) Method #1</th>
<th>$L_{\text{ads}}$ (Å) Method #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pullulan Abietate</td>
<td>10.1</td>
<td>0.1035</td>
<td>0.00363</td>
<td>25.5</td>
<td>28.5</td>
</tr>
<tr>
<td>Pullulan Abietate</td>
<td>9.7</td>
<td>0.1046</td>
<td>0.00375</td>
<td>24.4</td>
<td>27.9</td>
</tr>
<tr>
<td>Pullulan Abietate</td>
<td>8.9</td>
<td>0.09514</td>
<td>0.00388</td>
<td>22.4</td>
<td>24.5</td>
</tr>
<tr>
<td>Pullulan</td>
<td>6.6</td>
<td>0.06328</td>
<td>0.00363</td>
<td>16.6</td>
<td>17.4</td>
</tr>
</tbody>
</table>

The adsorption behavior of pullulan abietate (DS=0.027) onto 20 layers of cellulose was investigated as a function of the state of aggregation at the initial stage of adsorption. Figure 4.24 shows the adsorption of pullulan abietate (DS=0.027) onto 20 layers of regenerated cellulose beginning with initial exposure of the surface to a solution below the critical aggregation concentration (cac) when polymer chains behave as loose coils in solution. Figure 4.25 shows the adsorption of pullulan abietate (DS=0.027) onto 20 layers of regenerated cellulose beginning with initial exposure of the surface to a
solution above the cac when most of the molecules are in an aggregated state; however, some must also exist in the loose coil state as required by the thermodynamic equilibrium associated with the cac. These figures reveal that if adsorption begins with the deposition of molecular aggregates, a larger number of AGUs are able to dock at the surface. The apparent molecular area of the aggregated pullulan abietate is smaller allowing diffusion to the surface to occur more rapidly than the loose coils in solution. Starting below the cac, pullulan abietate chains lie down on the surface to form a monolayer at the stage of initial adsorption. Subsequent adsorption involves the deposition of polymer chains in loop/tail conformations. When the concentration is raised above the cac, the surface is already saturated with polymer chains and therefore accessibility for aggregate adsorption is hindered; however, they are still free to adsorb to material that has already docked as shown in Figure 4.26-4.27, although this docking is most likely reversible rather than irreversible.
Figure 4.24. Adsorption of pullulan abietate (DS=0.027) onto 20 layers of cellulose beginning with an initial exposure of the film to a solution below the cac at T=20 °C.

Figure 4.25. Adsorption of pullulan abietate (DS=0.027) onto 20 layers of cellulose beginning with an initial exposure of the film to a solution above the cac at T=20 °C.
Figure 4.26. Schematic illustrating the adsorption behavior of pullulan abietate on cellulose with a.) initial exposure to a solution below the cac and b.) progression of adsorption when the solution concentration is subsequently raised above the cac. Pink circles represent pendant abietate groups while black lines represent the pullulan main chain composed of repeating AGUs.

Figure 4.27. Schematic illustrating the adsorption behavior of pullulan abietate on cellulose with a.) initial exposure to a solution above the cac and b.) progression of adsorption when the solution concentration increases further.
4.9. Atomic Force Microscopy Results

Atomic Force Microscopy (AFM) was used to probe the surface of regenerated cellulose, pullulan adsorbed onto cellulose, and pullulan abietate (DS=0.027) adsorbed onto cellulose. For each polysaccharide, films were prepared by exposure to solutions above and below the cac to detect differences in surface roughness based on the state of molecules in solution (aggregated or non-aggregated). AFM was used to obtain the topographical images shown in Figure 4.28. These images represent the heights of structures found on the surface. The root-mean squared roughness (RMS) defined in Equation 4.7 is the standard deviation of all heights (Z-values) within the given area of 3.00 µm x 3.00 µm.

\[
\text{RMS} = \sqrt{\frac{\sum_{i=1}^{N} (Z_i - Z_{ave})^2}{N}} \quad \text{Equation 4.7}
\]
Figure 4.28. AFM height images for a) 20 layers of regenerated cellulose, b) pullulan abietate (c=22.8 mg•L$^{-1}$) exposed to 20 layers of cellulose, c) pullulan abietate (c=699 mg•L$^{-1}$) exposed to 20 layers of cellulose, d) pullulan (c=23 mg•L$^{-1}$) exposed to 20 layers of cellulose, e) pullulan (c=633 mg•L$^{-1}$) exposed to 20 layers of cellulose.
A small roughness value of 1.00 nm for example indicates a very smooth thin film. A large roughness value of 30 nm for example is characteristic of a film with three-dimensional structures raised from the surface. In the case of regenerated cellulose, a roughness value of 1.34 nm was obtained indicating a relatively smooth film, which was expected. In the case of pullulan abietate, smooth films were also detected. There does not appear to be a significant difference in the roughness of the surface with solution concentration. Pullulan abietate films prepared above the cac were just as smooth as those prepared below the cac. Both RMS roughness values of pullulan abietate are lower than those for pullulan. Again, the difference is not overly dramatic, however, this may indicate the propensity of pullulan abietate to seek out the cellulose surface and self-assemble allowing pendant abietate groups to minimize their interactions with water. The pullulan has an affinity for the surface but the larger RMS roughness values of 2.10 nm and 2.11 nm may indicate that even though they are attracted to the surface, the drive to completely cover the surface it is not as strong and therefore when the AFM comes in contact with the adsorbed film, it detects a patchy surface. Again, there does not seem to be a large effect for pullulan concentration on the roughness of the adsorbed films.
5. Conclusions

The surface plasmon resonance (SPR) technique has been utilized to quantify the adsorption of a model lignin-carbohydrate complex (LCC) on a model surface for cellulose fibers. The model lignin-carbohydrate complex, pullulan abietate, was found to have a degree of substitution (DS) of 0.027±0.001 indicating the presence of one abietic acid ester linkage per 37 anhydroglucose units (AGUs). Surface tension measurements indicated that pullulan abietate has a stronger tendency to aggregate compared to the parent polysaccharide pullulan. This feature is due to the presence of the pendant hydrophobic abietic acid groups. The substituted pullulan also has a lower cac value, 50±5 mg•L\(^{-1}\), compared to the cac of the homopolysaccharide, 240±50 mg•L\(^{-1}\).

Through the use of the de Feijter equation, theoretical and experimental data were compiled to produce adsorption isotherms for the adsorption of pullulan and pullulan abietate (DS=0.027) onto cellulose surfaces. These results revealed saturated surface concentrations, \(\Gamma_{\text{sat}}\), the adsorption of between 9-10 AGUs per nm\(^2\) of cellulose surface area for pullulan abietate compared to \(\Gamma_{\text{sat}} \approx 6.6\) AGUs per nm\(^2\) of cellulose surface area for unsubstituted pullulan. Using \(\Gamma_{\text{sat}}\) values and assuming the molecular areas of an anhydroglucose unit in pullulan and pullulan abietate were comparable to the molecular area of an AGU in unsubstituted cellulose and the thickness per layer was similar to that of cellulose, the thicknesses of each adsorbed layer were calculated. The limiting values of \(\theta_{\text{sp}}\) and the theoretical \(\frac{d\theta_{\text{sp}}}{dL}\) factors were also used to obtain thickness values (L). An average thickness of 25Å was calculated for the adsorbed pullulan abietate film compared to 17Å for the adsorbed pullulan film. These results indicate that the presence of the abietic acid groups drive adsorption to the surface and cause the formation of a slightly
thicker film. Hence, SPR has proven to be a highly effective, surface-sensitive technique capable of monitoring the docking behavior of self-assembling species at a surface.
6. **Suggestions for Future Work**

Surface plasmon resonance (SPR) has proven to be an effective tool to study the adsorption of polysaccharides at a surface. Pullulan and pullulan abietate are just two of a large range of sugars that may provide vital information about the interactions between cellulose, hemicellulose, and lignin and/or find commercial uses in the field of wood composite materials. As a basis of comparison, other pullulans with differing degree of substitution may be studied. Also, other systems containing a different parent polysaccharide may also be examined.

The system used in this project was designed to model the self-assembly behavior of the lignin-carbohydrate complex (LCC) onto a model surface for cellulose fibers. The “model” LCC, pullulan abietate, is not a native component of wood; however, LCCs have been extracted and isolated from wood using a variety of solvent and temperature conditions. Investigating the self-assembly behavior of a water-soluble LCC isolated from wood may provide further insight into the interactions between these wood components. The xylans are a large group of hemicelluloses found in wood that have the potential to better mimic the behavior of the carbohydrate portion of the LCC compared to pullulan. Derivatization of one of these xylans with a substituent more closely resembling lignin and studying its self-assembly behavior in the presence of cellulose is another possibility for future investigations.

In terms of the work reported here, size-exclusion chromatography can be utilized to determine aggregate sizes for pullulan and pullulan abietate. Ellipsometry measurements of pullulan and pullulan abietate adsorbed on cellulose would provide thickness values for the films as well as refractive indices in both the dry and wet states.
The refractive indices for pullulan and pullulan abietate were only estimated for use in the de Feijter equation so these quantities would be of great value.

The SPR technique finds many applications in the field of biochemistry where it is used to determine kinetic information about specific binding events such as antigen-antibody binding. A model cellulose surface coated with a material such as pullulan abietate may provide protection from certain enzymes including cellulase which rapidly attacks and degrades cellulose. SPR can be used to monitor the effect of introducing such a surface to cellulase and can also be used to obtain kinetic data about these processes.
References


93. de Feijter, J.A.; Benjamins, J.; Veer, F.A., "Ellipsometry as a tool to study the adsorption of synthetic biopolymers at the air-water interface", *Biopolymers*, 1978, 17, 1759-1772.

