Arabinoglucuronoxylan and Arabinoxylan Adsorption onto Regenerated Cellulose Films

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ABSTRACT

Cellulose and hemicelluloses have attracted increasing interest as renewable biopolymers because of their abundance. Furthermore, the recognition of biomass as a sustainable and renewable source of biofuels has driven research into the assembly and disassembly of polymers within plant cell walls. Cellulose thin films are useful in the study of interactions between cellulose and hemicelluloses, and quartz crystal microbalances with dissipation monitoring (QCM-D), surface plasmon resonance (SPR) and atomic force microscopy (AFM) are widely used to investigate polymer adsorption/desorption at liquid/solid interfaces.

In this study, smooth trimethylsilyl cellulose (TMSC) films were spincoated onto gold QCM-D sensors and hydrolyzed into ultrathin cellulose films upon exposure to aqueous HCl vapor. The adsorption of arabinoglucuronoxylan (AGX) and arabinoxylan (AX) onto these cellulose surfaces was studied. The effects of structure, molar mass and ionic strength of the solution were considered. Increasing ionic strength increased AGX and AX adsorption onto cellulose. While AGX showed greater adsorption onto cellulose than AX by QCM-D, the trend was reversed in SPR experiments. The combination of QCM-D and SPR data showed a greater amount of water was trapped within the AX films. Both adsorbed AGX and AX films were subsequently visualized by AFM. Images from AFM showed AGX and AX adsorbed as aggregates from water, while AGX and AX adsorbed from CaCl₂ yielded smaller xylan particles.
with more numerous globular structures on the cellulose surfaces. Images from AFM of xylan films on bare gold surfaces also showed layers of uniform aggregates that were consistent with AX and AGX aggregation in solution.
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Chapter 1

Overview

Increasing attention has focused on biopolymers as candidates for renewable energy and functional materials. Cellulose and xylan are two of these candidates that are the most and the second most abundant polysaccharides in nature.\(^1\) Research on the interaction between these two abundant polysaccharides is important because it helps to elucidate the architecture of the cell walls and should provide insight into better ways to isolate cell wall components.\(^2\) Consequently, the goal of this research was the study of xylan adsorption, arabinogluconoxylan (AGX) from spruce and arabinoylan (AX) from wheat, onto regenerated (RC) cellulose films, and the effect of electrolyte solutions on the adsorption process. A variety of surface science techniques, surface plasmon resonance (SPR), quartz crystal microbalance with dissipation monitoring (QCM-D), and in situ atomic force microscopy (AFM) have been utilized to study the interaction between xylan and RC surfaces.

This dissertation consists of five chapters. Chapter 2 provides an overall introduction and background related to this study, which begins with general information on plant cell walls including the components and structures. Subsequent discussion focuses on the thermodynamic treatment of the adsorption, adsorption isotherms and theoretical concepts of polymer adsorption onto solid surfaces. Finally, a brief introduction to several techniques including QCM-D, SPR and AFM are presented.

Chapter 3 summarizes materials used in the study, substrate preparation, and experimental procedures. The description of materials and experimental methods is not repeated in detail in subsequent chapters.
Chapter 4 describes AGX and AX adsorption onto RC films from different solutions. Results from QCM-D and SPR were combined to discuss the effect of salts on the adsorption process and the hydration of the adsorbed xylan layers.

Finally, Chapter 5 provides overall conclusions of this dissertation, and some suggestions for related future work.

References

Chapter 2

Introduction and Review

2.1 Introduction to Wood

Wood – a hard, fibrous, and complex plant tissue – is a natural material that is obtained from trees. There is about one trillion tons of wood on earth and it grows at a rate of about 10 billion tons per year. In a living tree, wood performs as a mechanical support tissue that allows the tree to grow large and remain erect. It also performs a conduction function which enables the transport of water and other nutrients from the roots to the leaves and other growing tissues. In addition, some parts of the wood store food for the living tree in case the energy is needed.¹

The systematic division of the trunk can be roughly split into three parts: wood in the center, bark on the outside and the cambial layer or cambium between the other two.² The cambial layer is only one cell thick and represents the growing part of the trunk since it produces wood on one side and bark on the other.² Annually, about eight to ten times more wood is produced than bark, and therefore wood represents the largest fraction of the volume of the tree trunk. Wood itself is usually further divided into dark-colored heartwood, which is located in the central region of the trunk, and light-colored sapwood, which is on the periphery to act as the pathway for water and soluble nutrients to pass through the trunk to the leaves.³ Sapwood is always formed before heartwood in all kinds of trees. As trees grow, sapwood is gradually altered by heartwood. Heartwood is dead once the formation is complete.⁴ Trees having a larger amount of leaves normally grow more quickly and have thicker sapwood.

Wood is widely distributed on earth and is often classified into two major groups as softwoods or hardwoods. Technically, gymnosperms whose seeds are borne naked are termed as
softwoods, and angiosperms whose seeds are borne in a fruit structure are termed as hardwoods.\(^5\) Sometimes an easier way to distinguish softwoods and hardwoods is according to the shapes of the leaves. Thus softwoods are referred to as evergreen trees (e.g. spruce) or conifers whose leaves are needlelike or scalelike, and hardwoods are referred to as deciduous trees (e.g. birch) whose leaves are normally broad or bladelike.\(^5\)

**2.2 Introduction to Plant Cell Walls in Wood**

The structure of a living woody plant is primarily comprised of plant cell walls, which account for 60% to 90% of the total volume. The plant cell walls are differentiated into primary and secondary cell walls. Figure 2.1 presents the complexity of cell walls. The middle lamella forms before either type of cell wall forms and represents the first formed layer. It is the intersection of two adjacent cells which holds the cells together and provides stability. The first layer inside the middle lamella is the primary cell wall. Primary cell walls form simultaneously with cell growth and have physical and chemical properties that accommodate cell division and enlargement. When the primary wall stops expanding the secondary cell wall is created,\(^6\) although not all plant cells form secondary cell walls. Figure 2.1B provides a representation of the plant cell wall hierarchy. The secondary cell wall is generally thicker and stronger than the primary wall. On the basis of different microfibril orientations, the secondary cell wall is divided into three layers. The layer adjacent to the primary wall is the first layer of the secondary cell wall, or the \(S_1\) layer. The \(S_2\) layer is the central layer and is the main contributor to the overall thickness of the secondary cell wall and hence the overall physical properties.\(^1,\(^7\) The outermost \(S_3\) layer is the thinnest layer.
Plant cell walls are complex networks of cellulose, hemicellulose, pectin, proteins and/or lignin. Though the thickness of the cell wall varies with the type of the plant, primary and secondary cell walls are approximately 100 nm and 1 µm thick, respectively. In primary cell walls, a cellulose and hemicellulose network is embedded in a pectin matrix, whereas in secondary cell walls, lignin replaces pectin to fill in the voids in the cellulose and hemicellulose network (Figure 2.2). Cellulose in cell walls is by far the most abundant component, which accounts for 43 ± 2% by mass in both hardwood and softwood. In addition, there are hundreds of thousands of proteins in the cell wall. Some of the proteins are ‘structural’ proteins, while others participate in morphogenesis.
Figure 2.2 Idealized drawing of cell wall layering showing chemical components. Adapted from Dahl.  

2.2.1 Cellulose

2.2.1.1 Cellulose in Nature

Cellulose is the most abundant material on earth, and is responsible for the strength and structure of plant cell walls.\textsuperscript{12,13} Figure 2.3 shows the structure of cellulose. It is a non-branching homopolysaccharide with linear chains linked by $\beta(1\rightarrow4)$ glucosidic bonds. While cellulose is made from glucose, cellobiose, a glucose dimer, is the repeating unit of cellulose because of the stereochemistry around the $\beta(1\rightarrow4)$ glucosidic bond.\textsuperscript{14} The chair conformation of the pyranose rings, equatorial hydroxyl groups\textsuperscript{13} and extremely large number of hydrogen bonds\textsuperscript{1} make cellulose the most stable polysaccharide in plant cell walls.\textsuperscript{15} Despite the simplicity of the structure, cellulose is found in a wide variety of places like green algal, fungal, cotton, wood
pulp, bast fiber and leaf fiber,\textsuperscript{16} and accounts for 20\% to 30\% of the dry mass in primary cell walls and 50\% of the dry mass in secondary cell walls.\textsuperscript{15} The degree of polymerization (DP) of cellulose varies widely from approximately 250-500 in primary cell walls to 10,000-15,000 in secondary cell walls.\textsuperscript{15}

\textbf{Figure 2.3} Cellulose chain repeating unit: cellobiose.

Cellulose has three hydroxyl groups per anhydroglucose unit (AGU) and a flat linear conformation which allows cellulose to form an extremely large number of intra- and interchain hydrogen bonds.\textsuperscript{14} Each AGU of cellulose has 2 interchain and 2 \~3 intrachain hydrogen bonds. Numerous infrared experiments have verified an absence of ‘free’ or “non-hydrogen bonded hydroxyl groups” within cellulose.\textsuperscript{17} As a consequence, cellulose chains strongly associate laterally. Approximately 30 to 50 physically interacting cellulose chains form cable-like cellulose microfibrils.\textsuperscript{15, 18} Both hydrogen bonding and van der Waals forces impart a parallel orientation of glucan chains within crystalline cellulose I structures\textsuperscript{19} and give them remarkable characteristics: high tensile strength (equivalent to steel),\textsuperscript{20} considerable stiffness, chemical stability, water insolubility and relative immunity to enzymatic attack.\textsuperscript{12} However, the cellulose chains are not perfectly packed. X-ray studies show that highly organized crystalline domains and amorphous (more precisely, non-crystalline) regions are alternating,\textsuperscript{12} and each crystalline region is about 600 \AA\ in length.\textsuperscript{1}
The layers that compose the plant cell wall (primary cell wall, S₁, S₂ and S₃ layers which were presented in Section 2.2.2) could be distinguished by the degree of angular dispersion of cellulose microfibrils.¹¹ Microfibrils in primary walls are mostly random, irregular and interwoven.⁵ In contrast, they are much more oriented and tend to have a specific microfibril angle in secondary cell walls.⁵ The S₁ and S₃ layers are arranged nearly perpendicular to the long axis of the cell, while the orientation in the S₂ layer is almost parallel to the long axis of the cell.⁷ Microfibril orientation, affects the stiffness, wood density and drying processes of the cell wall.⁷

Cellulose exists in six crystalline forms, however, cellulose I is the native form even though it is not the most thermodynamically stable one.²² Cellulose I is actually a mixture of two crystalline forms Iα and Iβ.²³ Cellulose Iα is a meta-stable phase with only one chain in a triclinic unit cell,²⁴ whereas less reactive cellulose Iβ has two conformationally distinct chains in a monoclinic unit cell.²⁵ Cellulose I occurs more commonly in nature as it is the predominant form found in higher plants (e.g. cotton, wood and ramie fibres),²⁶ whereas cellulose Iα is more prevalent in bacterial cellulose (contains 70% Iα), Valonia (contains 64% Iα), and algae.¹³ On the other hand, cellulose II is the most thermodynamically stable form and the second most commonly studied.¹³ Unlike cellulose I, where two cellulose chains lie parallel to each other in the unit cell, cellulose II has a monoclinic crystal structure with an arrangement of two antiparallel chains in the unit cell.¹³, ²² Figure 2.4 illustrates the parallel and antiparallel configuration of neighboring cellulose chains.
2.2.1.2 Cellulose Model Substrates

Cellulose model surfaces provide excellent means to examine cellulose/cellulose interactions and cellulose interactions with other materials in a simplified environment. Langmuir-Blodgett (LB) deposition and spin coating are the two most widely used methods on the basis of instrumentation, although several other methods have also been reported for the preparation of thin films of cellulose.

Schaub *et al.* introduced a way of preparing ultrathin model cellulose surfaces using the LB-technique in 1993. The LB-deposition of trimethylsilyl cellulose (TMSC, structure 1 in
Figure 2.5) onto various substrates with subsequent hydrolysis to cellulose has been widely used. As a hairy rod polymer, TMSC has a stiff cellulose backbone and short, hydrophobic TMS side chains that allows it to form monomolecular films on a Langmuir-trough and subsequent transfer onto hydrophobic substrates. Exposure of the TMSC layers to gaseous, wet 10% HCl solution for 30s results in the cleavage of TMS groups. In this way, cellulose was regenerated and the thickness of the regenerated cellulose (RC) films is directly proportional to the number of the monolayers.\textsuperscript{29-31} Other notable studies of cellulose films obtained by the LB-technique include work by Buchholz \textit{et al.} who modified this method to study hydroxyl accessibility\textsuperscript{32} and Holmberg \textit{et al.} who investigated surfaces forces.\textsuperscript{33-34}

![Figure 2.5](image)

\textbf{Figure 2.5} Regenerated cellulose (RC, 2) obtained from desilylation of TMSC (1).

Concurrently, Neuman \textit{et al.} reported the use of trifluoroacetic acid (TFA) as solvent for unmodified cellulose and spincoating to obtain cellulose films on mica.\textsuperscript{35} Compared with the LB-technique, which requires special equipment and demanding conditions,\textsuperscript{36} spincoating is a faster preparation method with greater reproducibility.\textsuperscript{26} Later on, this method was further developed by Geffroy \textit{et al.}\textsuperscript{37} and then Kontturi \textit{et al.}\textsuperscript{36, 38} and has become the most widely used method.\textsuperscript{31} The TMSC was dissolved in toluene and spincoated with a spinning speed 4000 rpm. Regeneration was completed by exposure to the vapor of 2.0 M aqueous HCl solution for about 1
min. The resulting cellulose film had a thickness of around 20 nm with a roughness of 3 nm. Films of RC formed under these conditions were the smoothest, but various factors like concentration and vapor pressure of the solvent and the spinning velocity of the substrate affected the thickness and uniformity of the RC films. After regeneration, RC films are hydrophilic with a water contact angle of 25°, amorphous as confirmed by infrared spectroscopy measurements and grazing incidence X-ray diffraction (GIXRD), and can swell considerably in humid air and water.

2.2.2 Hemicellulose

Hemicelluloses are defined by their method of extraction, that is, they can be extracted from higher plants by alkaline solutions. However, some hemicelluloess like β-(1→3,1→4)-glucans and arabinoxylans can be readily extracted by non-alkaline treatments. As a result, hemicellulose is not a very accurate term. More recently, some scientists proposed more proper names, like “heteropolysaccharides” or “non-cellulosic plant polysaccharides with β-(1→4)-linked, equatorial polypyranoose main chains.” However, the term hemicellulose is still widely used by most scientists.

Hemicelluloses comprise roughly 25% to 35% of most plant materials and are second to cellulose in abundance among natural polysaccharides. Hemicelluloses can be found in maize stems (28.0%), barley straw (34.9%), wheat straw (38.8%), rice straw (35.8%), rye straw (36.9%), etc. and form hydrogen bonds with the cellulose fibrils and covalent bonds with lignin. The entrapment of hemicelluloses is probably the reason for noncrystalline regions in cellulose microfibrils. Unlike cellulose, which is a linear polymer with degrees of polymerizations (DP) in excess of 250, hemicelluloses can be highly branched with lower DPs (generally below 200). In addition, hemicelluloses are heteropolysaccharides that can be
further classified into other groups of complex polysaccharides like xyloglucan, xylans and mannans. Some common monosaccharides that are incorporated into hemicelluloses are shown in Figure 2.6.¹⁵,⁴⁴

Figure 2.6 Common monosaccharides found in hemicelluloses.
2.2.2.1 Xyloglucan

The most abundant hemicellulose in primary cell walls by dry mass of spermatophytes except for grasses is xyloglucan – around 20% to 25% in dicotyledonous angiosperms such as Sycamore or *Arabidopsis thaliana*, around 10% in fir trees and around 10% of the bulb cell walls of onion.\(^{41}\) Xyloglucan still accounts for approximately 2% to 5% of the dry mass of grasses.\(^{41}\) In addition to the spermatophytes, xyloglucan can be found in all the land plant species except Charophytes. The backbone of xyloglucan is composed of 1→4 linked β-D-glucopyranose whereas its short side chains vary among plant species and tissues.\(^{12,41}\) The short side chains can be xylose, galactose, arabinose and fucose.\(^{12}\) A depiction of the xyloglucan structure from tamarind seeds is shown in Figure 2.7.\(^{45}\)

![Xyloglucan Structure](image)

**Figure 2.7** Xyloglucan from tamarind seeds (Megazyme Inc.) contains glucose (45%), xylose (35%), galactose (16%), and arabinose (4%).

Xyloglucans have been reported to coat the surface of cellulose microfibrils and adhere to them via hydrogen bonds.\(^{15}\) The length of xyloglucan can be up to 700 nm while the spacing
between cellulose microfibrils in primary cell wall is 20 to 40 nm, therefore xyloglucan may form tethers that can link several adjacent microfibrils together.\textsuperscript{46-47} The extensive cross-linking between cellulose microfibrils and xyloglucans results in less stiff and more extensible structure compared with cellulose alone.\textsuperscript{47} This structure is believed to be the major load bearing structure in primary cell walls.\textsuperscript{46} The cellulose – xyloglucan network appears to form a three dimensional lattice, around which other polysaccharides are interwoven.\textsuperscript{15,46}

\textbf{2.2.2.2 Xylans}

Xylans are the most common hemicellulose present in algae, cereals, grasses, wood, and herbs.\textsuperscript{48} Secondary walls of dicots are dominated by xylans with glycuronosyl and 4-\textit{O}-methyl glycuronosyl residues, while xylans with many arabinose branches are the most abundant in the primary cell walls of commelinid monocots. While there are many xylan structures, they share a backbone composed of $\beta$-(1$\rightarrow$4)-linked xylose residues as a common feature.\textsuperscript{41} The only exception is homoxylan, which contains $\beta$-(1$\rightarrow$3) or a mixture of $\beta$-(1$\rightarrow$3, 1$\rightarrow$4) glycosidic linkages. Homoxylan can be found in algae but is very rare in higher plants.\textsuperscript{48} Xylans in higher plants are usually substituted with other sugars and \textit{O}-acetyl residues.\textsuperscript{41,48}

Glucuronoxylans (GXs) are typical for dicot plants of the highest evolutionary level and are pervasive in secondary cell walls. Substituents on GX are 2-linked 4-\textit{O}-methyl-$\alpha$-D-glucuronic acid groups and/or $\alpha$-(1$\rightarrow$2)-linked glucuronic acid units, with wide variation in the degree of substitution among different hardwood species.\textsuperscript{41,48}

Arabinoglucuronoxylans (AGXs) exist in softwoods, and the lignified tissues of grasses and annual plants. Substituents on AGX are 2-\textit{O}-linked $\alpha$-D-glucuronic acid and/or 4-\textit{O}-methyl-
α-D-glucuronic acid and 3-linked α-L-arabinofuranosyl units. For example, Figure 2.8 depicts the structure of spruce AGX, which was isolated by the Gatenholm group.

Figure 2.8 Arabinoglucuronoxylan from spruce contains xylose (79%), 4-O-methyl-α-D-glucuronic acid (14%), and arabinose (7%).

Arabinoxylans (AXs) can be found in various cereal grains with xylopyranosyl residues monosubstituted at C3 and/or disubstituted at C2 and C3 by α-L-arabinofuranose residues. The distinction between AX and AGX is not always clear as some AXs also contain traces of glucuronic acid and/or 4-O-methyl-glucuronic acid residues. Figure 2.9 shows a depiction of the structure of wheat arabinoxylan from Megazyme Inc. AXs can be further classified according to their solubility. Compared with water insoluble AXs (wis-AXs), the number of arabinofuranose positioned on monosubstituted xylose residues of water soluble AXs (ws-AXs) is relatively greater.

Figure 2.9 Arabinoxylan from wheat contains xylose (62%) and arabinose (38%).
Heteroxylans (HXs) present in cereals, seeds and exudates are highly branched with a variety of different sugar branches. Aqueous HX solutions are therefore highly viscous and exhibit exudate gum properties.48

### 2.2.2.3 Mannans

Mannans are widely distributed in seeds, mosses, lycophytes, and early land plants, and are further differentiated into galactomannan, glucomannan and galactoglucomannan (GGM).42, 50 The backbone can be solely \(\beta-(1\rightarrow4)\)-linked mannose in mannans and galactomannans, or can be mixed \(\beta-(1\rightarrow4)\)-linked mannose and glucose in glucomannans and GGMs.42

The main hemicelluloses in softwoods are GGMs, which account for 10% to 25% (w/w) in dry wood. The GGMs contain a backbone of \(\beta-(1\rightarrow4)\)-linked glucosyl and mannosyl residues and \(\alpha\)-D-galactosyl residues attached to C6 of the mannosyl residues. The ratio of galactosyl residues to backbone mannosyl residues is between 0.1:4 and 1.3:4.15 Backbone mannosyl residues of GGMs are often acetylated at position 2 or 3 as \(O\)-acetylgalactoglucomannans.15,51 A depiction of the structure of spruce GGM is shown in Figure 2.10.51

![Figure 2.10](image.png)

**Figure 2.10** Norway spruce water-soluble51 \(O\)-acetylgalactoglucomannans contains **mannose** (73%), **glucose** (18%) and **galactose** (9%).
2.2.2.4 Mixed Linkage $\beta$-Glucans

Mixed linkage $\beta$-glucan (MLG) is another hemicellulose common to grasses, cereals, and related species.\textsuperscript{52} For example, primary cell walls of grasses contain 2\% to 15\% (w/w) MLG.\textsuperscript{50} The MLGs are linear cell wall polysaccharides and generally contain segments of two or three (1→4)-linked $\beta$-D-glucose residues separated by single $\beta$-(1→3)-linkages. The resultant structure is $\beta$-(1→3)-linked copolymer of cellotriosyl (58\% to 72\%) and cellotetraosyl (20\% to 34\%) units.\textsuperscript{53-54} Increasing interest in MLGs arises from their commercial and nutritional importance as well as their physical and physiological properties. Some studies show MLGs can reduce plasma cholesterol and control postprandial serum glucose levels in humans and animals.\textsuperscript{53} On the basis of their gelation property under certain conditions, MLGs can be thickening agents to modify the texture and appearance in gravy, dressing, and ice cream formulations.\textsuperscript{53}

2.2.2.5 Pectins

Pectin is one of the most structurally complex polysaccharides found in nature and is very common in higher plants.\textsuperscript{55-56} Pectins represent about 35\% of the dry mass of primary cell walls in dicots and non-graminaceous monocots, 2\% to 10\% of grass and other commelinoid primary walls, and up to 5\% of walls in woody tissue.\textsuperscript{56} In addition to their abundance in primary cell walls, another remarkable feature of pectins is their diverse function. Pectins are believed to contribute strength and support to higher plant cell walls. They are also thought to play a role in moving water and plant fluids through rapidly growing parts of the plant. Furthermore, they are related to the plant defense responses, turgor pressure and lignification.\textsuperscript{55,57} On the other hand, pectins also have a variety of applications in food and other industries. They are ingredients of
ice cream, jam, yoghurt, as well as degradable films, adhesives, paper substitutes, foams and plasticizers.\textsuperscript{56}

Pectins are hetero-polysaccharides that predominantly contain 1,4-linked \textit{\textalpha}-D-galactosyluronic acid (GalpA) residues.\textsuperscript{57-58} However, pectins exhibit tremendous compositional variation across different sources, locations and environmental conditions.\textsuperscript{55} For example, some GalpAs are methylated while others are not.\textsuperscript{57} Pectins in primary cell walls contain more oligosaccharide chains on their backbone and the side chains are longer than pectins in middle lamella.\textsuperscript{55} Generally speaking, there are three pectic polysaccharides that have been found and characterized, homogalacturonan (HG), rhamnogalacturonan-I (RG-I), and substituted galacturonans (SG).\textsuperscript{58} Substituted galacturonans can be further divided into xylogalacturonan (XGA), apiogalacturonan (AP) and rhamnogalacturonan-II (RG-II).\textsuperscript{59} Homogalacturonan (HG), a linear chain homopolymer whose backbone is composed of \textit{\textalpha}-(1→4)-D-galacturonic acid and comprises more than 60\% of the total pectin in plant cell walls.\textsuperscript{56,59} Homogalacturonan GalpA is partially methyl-esterified at the C-6 carboxyl, and/or O-acetylated at O-2 and/or O-3, and may contain other esters depending on the plant source.\textsuperscript{56} The primary structure of HG is shown in Figure 2.11A. Both esterification and acetylation are very important in determining the properties of pectin, the most important of which is the degree of cross-linking (less methyl-esterification leads to greater sensitivity to Ca\textsuperscript{2+} cross-linking).\textsuperscript{57} RG-I is another pectin component, comprising 20\% to 35\% of the mass of dry pectin.\textsuperscript{56} The main chain of RG-I is an alternating copolymer of D-galacturonic acid and L-rhamnose. For the RG-I backbone, 20\% to 80\% of the rhamnosyl residues in RG-I backbone are substituted mainly at C-4 with single [(\textit{\textbeta}-D-Galp-(1→4)] units and/or linear and branched \textit{\textalpha}-L-arabinofuranosyl (Araf).\textsuperscript{57-58} Other glycosyl residues like \textit{\textalpha}-L-fucosyl (Fucp), \textit{\textbeta}-D-glucuronosyl (GlcpA), and 4-O-methyl-\textit{\textbeta}-D-glucuronosyl
(4-O-Me GlcP A) may also be present among the side chains (Figure 2.11B). Rhamnogalacturonan II (RG-II) is a type of substituted HG. Like HG, the backbone of RG-II is made up entirely of 1,4-D-galacturonan. Considered to be the most structurally complex component of pectin, RG-II comprises ~10% of pectin. There are four types of branches (A through D) that have been identified, consisting of 12 different types of sugars (e.g., apiose, aceric acid, 3-deoxy-D-lyxo-2-heptulosaric acid (DHA), 3-deoxy-manno-2-octulosonic acid (KDO), etc.) with over 20 different linkages (Figure 2.11C). Molecules of RG-II molecules usually exist as dimers crosslinked via borate. Only apiose moieties of the side chain carbon atoms of RG-II are involved in borate cross-links in the plant. Another substituted HG, XGA, is substituted at C-3 with single β-linked xylose residues and is present in the walls of reproductive plant tissues. The AP, which is an HG substituted at C-2 or C-3 with D-apiofuraose, is present in aquatic monocots.
Figure 2.11 The primary structure of (A) homogalacturonan which is partially esterified and acetylated on backbone GalpA residues, (B) rhamnogalacturonan I with repeating GalpA and Rhap units as its backbone, and (C) rhamnogalacturonan II which has structurally different oligosaccharide side chains that are attached to backbone GalpA residues. Adapted from Mohnen.56
2.2.3 Lignin

Lignin is second to cellulose as the most abundant natural material\textsuperscript{60} and accounts for approximately 30\% of the organic carbon in the biosphere.\textsuperscript{61} Lignin is also the most abundant aromatic (phenolic) polymer.\textsuperscript{62} In principle, the functions of lignin are to provide stiffness and mechanical support of the cell wall, transport water and solutes through the vascular system, and protect plants against pests and pathogens.\textsuperscript{15,61} Additionally, more than 50 million tons per year of lignin are produced commercially, most which is used as a fuel with lesser amounts being used in adhesives, tanning agents and other products.\textsuperscript{63}

Lignification always takes place around the time of cell death as the last stage of cell differentiation, during which the aqueous phase of the cell walls is replaced by this hydrophobic polyphenolic polymer.\textsuperscript{15} Lignin may form chemical bonds with hemicelluloses and creates a hydrophobic environment. Secondary cell walls have the highest lignin content, though the lignin concentration is higher in the middle lamella and at cell corners.\textsuperscript{61} Nature lacks lignin-depolymerases and can only be degraded oxidatively through radical processes, which is probably the reason for unusually stable and structurally diverse lignin.\textsuperscript{64-65}

Lignins are complex amorphous aromatic heteropolymers composed primarily by three building blocks – p-coumaryl, coniferyl, and sinapyl alcohols (Figure 2.12). However, lignins incorporate more compounds than these three monomers. The amount and distribution of the monomers vary greatly in different plant sources and cell types. The structure of lignin is not stipulated. Instead, lignin polymer is created with a highly diverse series of radical coupling reactions, and this coupling is mostly found between preformed lignin oligomers.\textsuperscript{15,61}
**Figure 2.12** Three major monolignols: (A) p-coumaryl alcohol, (B) coniferyl alcohol and (C) sinapyl alcohol.

### 2.2.4 Plant Cell Wall Structure

Several models for primary cell wall structure were reviewed by Cosgrove in 2001. The first model proposed that xyloglucan, pectins and structural proteins are covalently linked to form a network, while cellulose is H-bonded to xyloglucan (Figure 2.13A). Another model, proposed by Hayashi and Fry, had xyloglucan chains tether the cellulose microfibrils together with pectin and structural proteins physically entangled in the cellulose-xyloglucan network (Figure 2.13B). A variant of this model proposed each microfibril is coated with a less-tightly bound polysaccharide layers, and the microfibrils are non-covalently linked to each other (Figure 2.13C). The final model reviewed by Cosgrove hypothesized that pectins are sandwiched in cellulose-hemicellulose lamellae (Figure 2.13D). While all of these models were based on some aspect of plant cell wall research, no consensus model exists. In fact, recent NMR studies call into question the fundamental underlying principle in Figure 2.13 that xyloglucan...
completely coats the cellulose fibers.\textsuperscript{71-72} Moreover, some recent studies have found evidence for the covalent linkages originally proposed by Keegstra \textit{et al.}\textsuperscript{67, 73}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{primary_cell_wall_models.png}
\caption{Primary cell wall models: (A), matrix polymers are covalently linked,\textsuperscript{67} (B) “tethered network”,\textsuperscript{68-69} (C) “multicoat” model,\textsuperscript{74} (D) “stratified” wall model.\textsuperscript{70} (Reprinted with permission from Ref. 66; Copyright 2001 American Society of Plant Physiologists)}
\end{figure}

Primary cell wall models have been tested more extensively than secondary walls, because of their importance to mechanics and cell growth.\textsuperscript{41} In secondary plant cell wall,
hemicelluloses are bonded to the surface of cellulose microfibrils, and play a role in linking lignin and cellulose together. The ligninification process occurs after the synthesis of cellulose fibers is complete, hence lignins are generally covalently bound to hemicellulose on the exterior of microfibrils. The lignins themselves are linked together via covalent ether and alkyl linkages. Disordered cellulose molecules and lignin, together with hemicellulose are located in the spaces between cellulose microfibrils.

2.2.5 Plant Cell Wall Materials and Renewable Biopolymers

With the characteristics of being biodegradable, biocompostable, biocompatible and having low toxicity, biopolymers have gained tremendous attention as sources of bioenergy and functional materials. Among all biopolymers, plant cell walls store enormous amounts of carbon by converting atmospheric carbon dioxide into more complex condensed phase materials through photosynthesis and subsequent biosynthetic pathways. Therefore, biopolymers are of interest as abundant sources of sustainable energy. Nowadays, bioconversion of cell wall materials into biofuels, biomedical materials, food, textiles, paper, etc. attains greater importance in daily life. In 2008, about 87 gigaliters of liquid biofuel was produced, which is roughly equal to the volume of liquid fuel consumed that year by Germany. The U.S. Department of Energy is set a goal to have 30% of the 2004 motor vehicle gasoline demand replaced by biofuels in the 2030. Likewise, the European Union has a target of 25% of the transportation fuel derived from biofuel. The first step of the conversion is always a pretreatment to break down the recalcitrant structures whereby enzymes like cellulases, xylanases and others convert the pretreated biomass into small molar mass sugars. In a subsequent step, the sugars undergo fermentation to ethanol or other biofuels.
2.3 Adsorption Phenomena

Adsorption is the adhesion of atoms, ions, or molecules from a gas, liquid, or dissolved solid onto a surface. Adsorbate is the name given to the material that adsorbed. Adsorpt or adsorptive is the name of the substance prior to adsorption. The adsorbent is the material on which adsorption takes place. Adsorption often occurs at the following interfaces: liquid–gas, liquid–liquid, solid–liquid and solid–gas. Adsorption can be further divided into physisorption and chemisorption. Physisorption is adsorption characterized by a physical interaction between the adsorbate and adsorbent and represents a case where the adsorbate is relatively free to diffuse and the adsorption equilibrium is quickly established. Chemisorption indicates adsorption dominated by a chemical reaction between the adsorbate and adsorbent where the adsorption energy is on the order of a chemical bond and the adsorbte is essentially immobile after adsorption in contrast to physisorption.

2.3.1 Thermodynamics of Adsorption

Adsorption of many vapors onto solid surfaces is typically spontaneous, with a negative change of the Gibbs free energy ($\Delta G_{ads}$):

$$\Delta G_{ads} = \Delta H_{ads} - T\Delta S_{ads} < 0$$  \hspace{1cm} (2.1)

where $T$ is temperature, $\Delta S_{ads}$ and $\Delta H_{ads}$ are the changes in enthalpy and entropy, respectively. $\Delta S_{ads}$ in the equation is generally negative because the adsorbed molecules lose a degree of freedom when they are confined at the interface. In order to obtain negative $\Delta G_{ads}$, $\Delta H_{ads}$ should be negative too. The case is different if adsorption occurs from solution. The prediction of the signs of $\Delta S_{ads}$ and $\Delta H_{ads}$ is complicated by effects of solvation, etc.
Since adsorption always involves an interface and the interface greatly influences all the parameters of a system, a deeper understanding of interfacial thermodynamics is essential. Gibbs developed a model, in which two bulk phases $\alpha$ and $\beta$ are separated by a third infinitesimal interface $\sigma$, to analyze the thermodynamics of the interface. Although this is an imaginary model, it works well for most applications. The volumes of the two separate phases are $V^\alpha$ and $V^\beta$, and because all the extensive quantities can be written as the sum of all the components, the internal energy of the interface is:

$$U'' = U - \mu^\alpha V^\alpha - \mu^\beta V^\beta$$  \hspace{1cm} (2.2)

where $\mu^\alpha$ and $\mu^\beta$ are the energies per unit volume in the two bulk phases, and $U$ is the total internal energy of the system. The number of the $i^{th}$ molecules that exist at the interface is defined as $N_i$. The surface concentration or the so called surface excess is expressed as:

$$\Gamma_i = \frac{N_i}{A}$$  \hspace{1cm} (2.3)

where $A$ is the interfacial area. The magnitude and sign of the value of $\Gamma_i$ is dependent upon the location of the dividing plane.$^{79,82}$

Figure 2.14 shows how a general property $P$ changes from bulk phase $\alpha$ through the dividing surface to another bulk phase $\beta$. The distance perpendicular to the interface is defined as $x$ and $\tau$ is the thickness of the zone of interface where the properties of phase $\alpha$ changes continuously to phase $\beta$. The dividing plane in the Gibbs model has no volume and is situated at some specific value of $x = x_0$. The hatched area to the left of $x_0$ shows the amount of $P$ that is overestimated as $P^\alpha$ in phase $\alpha$. Likewise, the hatched area to the right represents the underestimation of $P$ in phase $\beta$. By convention, $x_0$ is set so as that the two hatched areas can cancel each other for one component in the system, and therefore.$^{79,82}$
However, the dividing surface can only be picked once. As such, $\Gamma_1 = 0$ is usually defined for the solvent. As a consequence, $\Gamma$, for solutes are seldom also zero. For, $\Gamma_1 > 0$ there is normally an excess of species $i$ at the surface, whereas $\Gamma_1 < 0$ means there is a depletion of species $i$ from the interface.

\begin{equation}
\Gamma_1 = 0
\end{equation}

**Figure 2.14** Variation of a general property with the distance of $x$ near the interface between bulk phases $\alpha$ and $\beta$.

### 2.3.2 Adsorption Isotherms

The adsorption isotherm is a fundamental concept of surface science. It describes the equilibrium between the adsorption of a material at a surface and the pressure or concentration in the bulk fluid phase at constant temperature.\textsuperscript{80}
In terms of the Gibbs treatment that was mentioned in Section 2.3.1, the ideal model is only appropriate for fluid-fluid interfaces. Because surface tension changes at solid interfaces caused by composition changes cannot be determined experimentally, other isotherms like the Langmuir isotherm and Freundlich isotherm are used. Typical plots for Freundlich and Langmuir adsorption isotherms are shown in Figure 2.15.

![Figure 2.15 Schematic plots of (A) Freundlich, and (B) Langmuir adsorption isotherms. Partial pressure \( P \) or concentration \( c \) refers to adsorption from a gas or solution, respectively.](image)

The Langmuir adsorption isotherm was first proposed by Irving Langmuir, and is given as:

\[
\Gamma = \frac{\Gamma_{\text{mon}} K_L c}{1 + K_L c} \tag{2.5}
\]

where \( \Gamma_{\text{mon}} \) is the maximum surface coverage of a single monolayer, \( K_L \) is the Langmuir constant which is the ratio of adsorption rate constant to desorption rate constant, and \( c \) is the bulk adsorbate concentration. The Langmuir adsorption isotherm can be linearized as:

\[
\frac{c}{\Gamma} = \frac{c}{\Gamma_\infty} + \frac{1}{K_L \Gamma_\infty} \tag{2.6}
\]
The Langmuir adsorption isotherm is based upon very stringent assumptions: ideal and homogeneous surfaces that consist of definite and energetically equivalent adsorption sites on the surface at all surface concentrations that only accommodate a single molecule per site (Figure 2.16).\textsuperscript{79} Two limiting cases of Eqn. 2.5 are at infinite dilution where \( c \rightarrow 0 \) and therefore \( \Gamma / \Gamma_{\text{mon}} = K L c \), and at high solute concentrations or high surface affinities where \( K L c \geq 1 \) and therefore, \( \Gamma / \Gamma_{\text{mon}} \approx 1 \), which means the surface reached saturation. However, the majority of the practical situations are not ideal, instead, the binding sites are usually heterogeneous, and the system is not limited to the adsorption of a single monolayer.\textsuperscript{82, 84}

\begin{equation}
\Gamma = K_s \cdot c' \tag{2.7}
\end{equation}

\textbf{Figure 2.16} Schematic depiction of Langmuir adsorption. Adapted from Butt \textit{et al.}\textsuperscript{79}

Other models have been developed to address adsorption that is inconsistent with the Langmuir model. The Freundlich adsorption isotherm assumes that the surface is heterogeneous, i.e. some of the binding sites are of higher affinity than others. This case is equivalent to one where adsorbed molecules can have lateral interactions with neighboring adsorbates. A depiction of the Freundlich model is shown in Figure 2.17. The Freundlich adsorption isotherm equation is:
where $K_F$ is the adsorbent capacity, $c$ is the bulk adsorbate concentration, and $q$ is the adsorption affinity constant with a value less than one. The linearized form of Eqn. 2.7 is:

$$\ln \Gamma = \ln K_F + \frac{q}{\ln p}$$

(2.8)

The Freundlich adsorption isotherm was originally an empirical model and is closer to reality than the Langmuir model in most cases.\(^{79,81}\)

![Freundlich model](image)

**Figure 2.17** Schematic depiction of the Freundlich model. Adapted from Butt *et al.*\(^{79}\)

### 2.3.3 Polymer and Polyelectrolyte Adsorption onto Solid Surfaces

Polymer adsorption onto a surface is even more complicated. If the interaction between the polymer and the surface is less favorable than that of the solvent with the surface, depletion takes place.\(^{85-86}\) Polymers in the solution are usually considered as random walks on a periodic lattice\(^{87}\) and can assume a large number of conformations.\(^{81}\) Polymer chains have translational and rotational degrees of freedom, and a large number of conformational degrees of freedom.\(^{86}\)

The adsorption of a flexible macromolecule onto a solid surface will distort its conformational topology,\(^{88}\) and therefore a polymer loses both transitional and conformational entropy upon adsorption.\(^{89}\) Typical chain configurations of adsorbed polymer chains are illustrated in Figure
2.18, which shows adsorbed segment trains, free standing loops, and a substantial portion of non-adsorbed chain end tails.\textsuperscript{90-91} For strongly attractive potentials, that is to say, higher adsorption energy, the polymer chains are flat with most segments set on the surface, a small fraction in loops and a negligible trace in tails.\textsuperscript{92-93} With decreasing attractive interactions, the length of loops increases, while that of trains decreases, and that of tails become more important.\textsuperscript{86, 91}

![Schematic drawing of the structure of an adsorbed single chain. The ‘tail’, ‘train’ and ‘loop’ sections are indicated.](image)

\textbf{Figure 2.18} Schematic drawing of the structure of an adsorbed single chain. The ‘tail’, ‘train’ and ‘loop’ sections are indicated.

The adsorption of polymer is generally monomolecular and the amount of adsorption is largely dependent upon polymer concentration and molar mass. The loops are considered to form a thin layer adjacent to the bulk phase of the polymer solution,\textsuperscript{94} and the thin surface layer is typically 3 to 30 nm thick. The thickness is roughly proportional to the square root of the chain length.\textsuperscript{81} Due to the thickness of the first polymer layer, the chance for adsorbing the second polymer is usually negligible.\textsuperscript{81} On a per unit area basis, the amount of polymer adsorbed increases dramatically with increasing bulk concentration when the solution is dilute, and frequently reaches a plateau as the polymer concentration is further increased. On the other hand, the amount of adsorption increases proportionally to the molar mass of polymer when the
molecular weight is small, but exhibits a weaker dependence on the molar mass when the molar mass is high enough.\textsuperscript{94} Meanwhile, the time to reach adsorption equilibrium and the reversibility of adsorption are also dependent upon molar mass. Polymers with large molar mass need more time to achieve adsorption equilibrium and once adsorbed, are difficult to remove by neat solvent.\textsuperscript{81,94}

Polymers, are positively or negatively charged water-soluble polymers.\textsuperscript{95-96} If the charges are in a frozen configuration, that is, the charges do not depend upon pH but are only determined by the initial chemistry, they are usually discussed as strong polyelectrolytes. For weak polyelectrolytes, the amount and location of charged sites are changing as a function of pH.\textsuperscript{97-98} Electrostatic interactions play a crucial role in polyelectrolyte adsorption, where three factors are dominant: ionic strength, surface charge density, and linear charge density of the polyelectrolyte.\textsuperscript{93,99} At low salt concentrations, strong polyelectrolytes adsorb in a flat configuration on an oppositely charged surface and the amount adsorbed is small with no dependence on molar mass or ionic strength. If salt is added so that the media has a relatively high ionic strength, the adsorption increases as a function of the ionic strength and the molar mass. For weak polyelectrolytes, more tends to adsorb more when the ionic strength is high.

2.4 Experimental Techniques

2.4.1 Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D)

A quartz crystal microbalance with dissipation monitoring (QCM-D) is a simple and highly sensitive technique for investigating adsorption onto a surface. The instrument is an acoustic wave resonator\textsuperscript{100-101} which operates in the thickness shear mode. Changes in the mass of the crystal that arise from adsorption, such as the mass of adsorbed macromolecules from
solution, can be deduced from frequency changes, in real time. The development of quartz crystal microbalances (QCM) advanced greatly from the 1960’s after Sauerbrey discovered a linear relationship between the frequency change of a quartz crystal and rigidly adsorbed mass, and Warner and Stockbridge developed a quartz crystal balance. A QCM is mainly used in electrochemistry and biotechnology fields for controlled deposition of thin films, biomolecule adsorption kinetics, cell adhesion, DNA hybridization and many other applications. Later development made it possible to determine the viscoelastic properties in addition to the adsorbed amount.

A QCM-D consists of a thin disk-shaped AT-cut quartz crystal (Figure 2.19A) sandwiched between two metal electrodes (typically made of gold) oscillating in shear mode at a fundamental resonant frequency. The AT-cut quartz crystals are piezoelectric whereby the application of an alternating external voltage produces a shear deformation of the crystal lattice. Stable oscillations only occur at the resonant frequency of the crystal and at overtones that satisfy the condition where the thickness of the crystal is an odd multiple of half the acoustic wavelength of the excitation frequency. Figure 2.19B depicts the fundamental and third harmonic of the oscillation. The AT-cut angle is 35.25° relative to the optical axis of the crystal. This cut angle minimizes the thermal expansion coefficient, enhances high frequency stability, and most importantly, yields a shear strain that is perpendicular to the surface. Furthermore, the acoustic wave generates a standing shear wave because of the constructive interference between the incident and returning acoustic waves. Upon deformation, both surfaces move in parallel but opposite directions (as illustrated in Figure 2.19B) so that the quartz surfaces are antinodes of the acoustic wave, and the acoustic wave can also travel into the ambient medium.
Figure 2.19 Schematic of (A) an AT-cut quartz crystal and (B) a side view of the shear deformation of the crystal at overtone number n=1 and n=3.116-117 ((B) was reprinted with permission from Ref. 117; Copyright 2011 American Chemical Society)

The thickness shear mode of a quartz crystal is very sensitive to the accumulation and release of surface mass. Mass bound to or removed from the surface alters the resonance frequency of the crystal and can cause a frequency shift, Δf, which is related to the mass change, Δm (Figure 2.20). For rigidly adsorbed homogeneous films, the linear relationship is normally referred to as the Sauerbrey equation:

\[ \Gamma = \Delta m = -C_{QCM-D} \frac{\Delta f}{n} \]  \hspace{1cm} (2.9)

where \( \Gamma \) is the surface concentration and \( C_{QCM-D} \) is the mass sensitivity constant which is equal to 17.7 ng·cm\(^{-2}\)·Hz\(^{-1}\) at \( f = 5 \) MHz for the crystals used in this thesis, and \( n \) is the overtone number (1, 3, 5, …).105 The Sauerbrey equation works best for the adsorption of a film in vacuum or in gaseous environments where the adsorbed films are rigidly attached to the electrodes with no slip or deformation, evenly distributed, and sufficiently thinner than the penetration depth \( \delta = \frac{2\eta_f}{\omega \rho_f} \)\(^{0.5} \), where \( \eta_f \) is the viscosity of the surrounding medium, \( \rho_f \) is the density of the surrounding medium and \( \omega = 2\pi f \) is the angular frequency. Figure 2.21A shows the acoustic wave penetrates into the rigid bound film.102-103, 118 On the contrary, if the bound film is thick, soft and
viscoelastic, Eqn. 2.9 may not hold. Tremendous work has been done to explain these effects and a Voigt-based viscoelastic model is most commonly applied.\textsuperscript{119-122} The acoustic wave penetration into soft films is shown in Figure 2.21B.

In order to monitor viscoelastic properties of the material, a ring-down scheme was developed for the acquisition of the energy dissipation factor $D$.\textsuperscript{118} By periodically (~ 1 Hz) turning the power to the crystal on and off and monitoring the decay of the signal, $D$, can be calculated:

$$D = \frac{E_{\text{Dissipated}}}{2\pi E_{\text{Stored}}}$$ \hspace{1cm} (2.10)

where $E_{\text{Dissipation}}$ is the energy lost during one oscillation, and $E_{\text{Stored}}$ is the energy stored in the oscillating circuit. Numerous factors influence the value of $D$, but generally, a higher $D$ results from a soft material adsorbed onto the crystal surface. In contrast, rigidly adsorbed materials produce a lower $D$. Accordingly, the amplitude of oscillation, $A$, dies out exponentially when the driver circuit is disconnected. Figure 2.22 gives a depiction of amplitude decay of both rigid and soft films.
Figure 2.20 Resonance frequency of an unloaded crystal (in blue) and a loaded crystal (in red). To a first approximation, the change in frequency, $\Delta f (\Delta f = f_{\text{unloaded}} - f_{\text{loaded}})$ is due to the loaded mass and the broadening (identified by bandwidth $\omega$) is dependent upon the viscoelastic properties of the added layer. Adapted from Reviaikine et al.\textsuperscript{117}

![Figure 2.20](image1)

Figure 2.21 Acoustic wave penetration into the bound film. (A) A thin and homogeneous rigid film that satisfied the Sauerbrey equation. (B) A soft film where the decay is related to the viscoelastic properties of the adsorbed material.\textsuperscript{117} (Reprinted with permission from Ref. 117; Copyright 2011 American Chemical Society)
Figure 2.22 Typical decay curves of a rigid film (blue) and viscoelastic film (red). The decay of the amplitude of oscillation decays are proportional to $e^{-DfDt}$. Soft materials decay faster due to a relatively larger $D$ factor. Adapted from ReviaRen et al. and Rodahl et al.\textsuperscript{117-118}

2.4.2 Surface Plasmon Resonance (SPR)

Surface plasmon resonance (SPR) is a unique optical surface sensing technique which takes advantage of the sensitivity of a special type of electromagnetic field, a surface plasmon, to probe changes in the refractive index of media near a metal surface.\textsuperscript{123-125} Since the Kretschmann arrangement for SPR coupling was first proposed around 1970\textsuperscript{126-127} and the introduction of the BIACore\textsuperscript{®} SPR instrument during the 1990’s,\textsuperscript{128-129} SPR has been used for adsorption measurements,\textsuperscript{130} biokinetics,\textsuperscript{131} gas detection,\textsuperscript{132} immunosensing,\textsuperscript{133} SPR microscopy,\textsuperscript{134} refractive index measurements,\textsuperscript{135} and thin film characterization,\textsuperscript{136, 124} because of its high sensitivity, stability and speed of response.

A surface plasmon (SP) is a longitudinal charge density wave with a transverse-magnetic mode propagating along the interface between two different media where the real part of the
dielectric function changes sign across the interface, e.g. metal and air interface. There are
two main criteria for the existence of a SP at an interface. First, the electrons in the platform
should exhibit free electron behavior. Metals that meet this requirement are gold, silver,
copper, and aluminum. Another requirement is the real part of the relative permittivity (\( \varepsilon \)) of
the two media must be of opposite sign, i.e. the real part of the relative permittivity \( \varepsilon_M \) of the
metal should be negative and its absolute value must be smaller than the relative permittivity \( \varepsilon_D \)
of the dielectric medium in contact with the metal. The most commonly used metals are gold
due to its stability and silver because it provides a sharper SPR resonance peak. The SP
propagates along the interface and decays into surroundings dielectric medium. The wave vector
of the surface plasmon (\( k_{sp} \)) is defined as:

\[
k_{sp} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_M \varepsilon_D}{\varepsilon_M + \varepsilon_D}}
\]

where \( c \) is the speed of light in vacuum, \( \omega \) is the frequency of incident light, \( \omega/c = 2\pi/\lambda \) is the
free-space wavenumber, \( \lambda \) is the free-space wavelength, and \( \varepsilon_M \) and \( \varepsilon_D \) are the relative
permittivity of the metal and dielectric continuum, respectively.

The SP mode can couple with light under appropriate conditions, and in the application
of SPR, SPs are excited by an external light source. In order for coupling, the wave vector
of the incident light must have a component parallel to the interface. The light source is usually a
laser, and a helium-neon laser is the most common. Since the light is an electromagnetic wave
consisting of orthogonal oscillating electric and magnetic fields transverse to the direction of
propagation, \( p \)-polarized light, whose electric vector is parallel to the plane of incidence, is
suitable for coupling with SPs. The electromagnetic field of \( p \)-polarized light is illustrated in
Figure 2.23A. The reason why only \( p \)-polarized light can be used is that only the normal
component of the electric field \((E_Z)\) can create charge density waves at the interface (Figure 2.23B).
Figure 2.23 (A) A schematic diagram illustrating the electromagnetic field of p-polarized light. Only light waves with electric fields normal to the metal surface can impart energy on surface electrons. (B) A schematic depiction of the electron delocalization. The resulting SP is a surface charge density wave at the metal surface. Adapted from Willets et al. 143
The most popular SPR configuration is the Kretschmann set-up proposed around 1970. This configuration is based upon attenuated total reflectance (ATR) in a glass prism coated with a thin metal film on one side.\textsuperscript{144} Total internal reflection can occur when light travels from an optically dense medium (one with a higher refractive index, \( n_g \), e.g. glass prism with a relative permittivity, \( \varepsilon_g \)) to a medium with lower optically density (lower refractive index, \( n_D \), e.g. dielectric continuum with a relative permittivity, \( \varepsilon_D \)), provided the light is incident at the angle greater than or equal to the critical angle (\( \theta_c \)) as depicted in Figure 2.24. If the angle of incidence is at the critical angle or greater, the light will not cross the boundary (no refracted beam) and will totally reflect internally (Figure 2.24B). These conditions and Snell’s law gives a relation between these parameters:

\[
\sin \theta \geq \sin \theta_c = \frac{n_D}{n_g} = \sqrt{\frac{\varepsilon_D}{\varepsilon_g}}
\]  
(2.12)

Furthermore, Eqn. 2.13 gives the wave vector \( k_x \) (also shown in Figure 2.24) of the propagating light, for the component parallel to the surface:

\[
k_x = \left( \frac{\omega n_x}{c} \right) \sin \theta
\]  
(2.13)

However, a small portion of the incident light, the so called evanescent wave or evanescent field, penetrates from the interface into the less dense dielectric continuum to a distance of up to one wavelength (for light in the visible spectrum, the penetration depth is about 1 \( \mu m \)). These fields are not propagating, but instead are decaying in amplitude and oscillating at the same frequency as the incident radiation in a direction normal to the interface. The penetration depth of the evanescent field in the metal is so small that the thin metal films coated onto the glass should be no thicker than 60 nm.
Figure 2.24 Schematic depiction of (A) refraction and (B) total internal reflection of light when the incident angle $\theta$ is greater than the critical angle $\theta_c$. The wave vector $(k)$ of the incident light and it has two components ($k_x$ and $k_y$).

The evanescent wave arising from the incident light is able to resonate with the SP of the metal film at a specific incident angle $\theta$ where $k_x$ and $k_{sp}$ match (Figure 2.25):

$$n_g \sin \theta = \sqrt{\frac{\varepsilon_M \varepsilon_D}{\varepsilon_M + \varepsilon_D}}$$

(2.14)

All of the parameters on the right-hand side of Eqn. 2.14 are constants as is $n_g$, hence the only thing that can be controlled experimentally is the incident angle ($\theta$). At this particular $\theta$ value ($\theta = \theta_{sp}$), also named the SPR angle, reflected energy from the incident light is greatly attenuated as it is transferred to the surface plasmon (Figure 2.25). Experimentally surface plasmon resonance is observed as a sharp intensity minimum of the reflected light as a function of incident angle (Figure 2.26). This minimum in the reflected intensity can be detected by using a two-dimensional array of photodiodes or a charge coupled detector (CCD). The SPR angle is very sensitive to variations in the refractive index of the medium at or very near the surface. The dashed red line in Figure 2.26 shows the SPR angle shifts away from the original signal after a
thin film is adsorbed onto the surface. The change in the SPR angle in Figure 2.26 ($\Delta \theta_{sp} = \Delta \theta_{sp}(2) - \Delta \theta_{sp}(1)$) is the basis of most SPR adsorption sensors. Furthermore, $\Delta \theta_{sp}$ is proportional to the refractive index change ($\Delta n_D$), and $\Delta n_D$ can also be used to determine the surface concentration according to the equation of de Feijter et al.:\textsuperscript{145}

$$\Gamma = \frac{L \times \Delta n_p}{dn/dc} = \Delta \theta \left( \frac{1}{d\theta/dL} \right) \left( \frac{\Delta n_p}{dn/dc} \right)$$

(2.15)

where the refractive index increment ($dn/dc$) is material specific and can be measured independently with a differential refractometer. Values of $dn/dc$ vary from 0.1 to 0.3 ml·g$^{-1}$ ($\approx$ 0.2 ml·g$^{-1}$ for many proteins). The other terms in Eqn. 2.15 are the thickness of the thin layer bound to the surface ($L$), the resonance angle change under the condition of irreversible adsorption ($\Delta \theta_{sp}$), and the change in resonant angle with layer thickness ($d\theta/dL$).\textsuperscript{146} In Eqn. 2.15, the observed $\Delta \theta_{sp}$ must be corrected for bulk contributions:

$$\Delta \theta_s = \Delta \theta_{sp} - c \left( \frac{d\theta_{sp}}{dc} \right)$$

(2.16)

where

$$\frac{d\theta_{sp}}{dc} = \frac{d\theta_w}{dn} \frac{dn}{dc}$$

(2.17)

The quantity ($d\theta_{sp}/dn$) = 61.5$^\circ$ is an instrument specific parameter obtained through the calibration of the instrument and is instrument specific.\textsuperscript{125,147}

The great value of SPR is the ability to measure the surface concentration because of the small sampling depth. The electromagnetic field of SPR is concentrated at the metal-dielectric interface and decreases exponentially into both media. The field decays in a direction perpendicular to the interface with a penetration depth of 20 to 30 nm and 100 to 500 nm in the
metal and dielectric, respectively (Figure 2.25). Thus, SPR only sees a small volume in the immediate vicinity of the metal surface.

Figure 2.25 The resonant angle for SPR is obtained when \( k_{sp} = k_x \). (B) The electromagnetic field of the SP decays exponentially in both media with different penetration depths.
Figure 2.26 Two SPR curves obtained before and after adsorption. The intensity of the incident light exhibits a sharp minimum at the SPR angle and the SPR angle shifts with changing refractive index of the surrounding medium.

Figure 2.27A illustrates the configuration of an SPR instrument based upon the Kretschmann prism configuration and Figure 2.27B illustrates a typical example of an SPR adsorption profile. Initially, background signals ($\theta_{sp}(1)$ in Figure 2.26) are collected by injecting an appropriate solution. Once analyte solution comes into contact with the surface, adsorption can occur and changes in the SPR angle are observed. This change arises from the refractive index changes of the bulk solution and is enhanced by any analyte that adsorbs onto the sensor. A plateau is obtained after the adsorption saturates. Finally, some loosely bound materials (reversibly adsorbed) are removed by switching back to the neat solvent or buffer ($\theta_{sp}(2)$ in Figure 2.26). Surface concentration is calculated by using SPR angle changes $\Delta \theta_{sp}$ and gives the amount of material that is irreversibly bound to the surface for the final plateau.$^{148}$
Figure 2.27 A schematic depiction of (A) a typical SPR experimental set up employing the Kretschmann prism and (B) a typical example of SPR experimental profiles. The change in the SPR angle is used to monitor the surface concentration.
2.4.3 Atomic Force Microscopy

Atomic force microscopy (AFM) was invented in 1986 \cite{149} and became commercially available in 1989. It is one of the best tools for imaging, measuring, and manipulating nanoscale matter with high resolution under different environments. \cite{150} An atomic force microscope allows quantitative imaging of topology in three-dimensions on virtually any flat solid surface without the need for extensive surface preparation. \cite{150-151}

The heart of the atomic force microscope is a cantilever with a sharp microfabricated tip pointing in a [001] crystal direction that can deflect when interacting with the sample surface. The sample topography is monitored by measuring the cantilever deflection in several ways. The cantilevers are typically made out of silicon, silicon oxide or silicon nitride and the back faces are coated by a thin metallic layer (often gold) in order to enhance reflectivity. This cantilever spring is essential since it senses the force between the tip and the sample according to Hooke's law and the configuration of the cantilever is shown in Figure 2.28. The force constant of the tip tries to satisfy two competing criteria: a spring as soft as possible so that the maximum possible deflection is obtained for a given force, and a spring that is stiff enough for a high resonant frequency. \cite{149} Furthermore, the deflection is usually measured using a laser spot reflected from the mirror like cantilever surface into a position-sensitive photodetector which further constrains the range of detectable deflections.

Figure 2.29 illustrates an atomic force microscope, and a topology image of the sample is created by plotting the deflection of the cantilever versus its position on the surface. In most cases, feedback systems are used to adjust the tip-to-sample distance to maintain a constant force between the tip and the sample. The cantilever is actuated in the z direction via a feedback-controlled piezo while the sample is scanned in the x and y directions using a piezoelectric
The tip moves sideways (in the $x$ and $y$ directions) and the surface contour is depicted in Figure 2.29B. The interaction forces between the tip and the sample include both long- and short-range forces (up to 100 nm, e.g. van der Waals, electrostatic, and magnetic forces). The force experienced by the tip is zero until the tip-sample separation distance is small enough. Upon approaching and retracting from the surface, repulsive forces and adhesion “pull off” forces are dominant, respectively. Long range forces (up to 100 nm) are typical for van der Waals, electrostatic, and magnetic forces.

Figure 2.28 Schematic depictions of the top view of a microfabricated cantilever. Adapted from Giessibl et al.\textsuperscript{151}
Figure 2.29 (A) Depiction of an atomic force microscope.\textsuperscript{153-154} The cylindrical tube is the piezoelectric actuator that can perform minimal displacements on the order of 1Å.\textsuperscript{155} (B) A schematic depiction of the tip and the sample surface. The dashed line is the tip contour of the surface.\textsuperscript{149,151} ((A) was adapted from Hinterdorfer \textit{et al.},\textsuperscript{153} and (B) was adapted from Giessibl \textit{et al.}\textsuperscript{151})
Dependent upon the application, the AFM can be operated in a number of modes, like static or contact mode and a variety of dynamic modes. In the so called contact mode operation, the tip is almost always in contact with the sample where the net force is repulsive. The cantilever deflection is used as the feedback signal and the force between the tip and the surface is kept constant during scanning by displacing the sample in the z direction. In the dynamic modes, the tip hovers 50 – 150 Å above the surface and the overall force is attractive. The cantilever is mounted on an actuator so that it is deliberately oscillated while the sample is brought towards or away from the sample and adjustments are made to maintain a constant oscillation amplitude or frequency. The topology image is therefore obtained by measuring the tip-to-sample distance at each (x, y) data point.

Tapping mode is another dynamic mode and was a key advance in AFM since it overcame problems associated with friction, adhesion, electrostatic forces, and other difficulties that plague conventional AFM scanning methods. In this mode, the cantilever is externally excited to oscillate up and down by a piezoelectric driver at near resonant frequency. The oscillation amplitudes are greater than 20 nm, typically from 20 to 100 nm. The oscillating tip is moving toward the surface until it begins to lightly touch, or tap the surface, and the interaction of the forces lead to decreasing amplitudes. The feedback system is set to detect the perturbation of the amplitude when the tip touches the surface and the sample height is adjusted accordingly so that the oscillation amplitude is compensated. Therefore the tip contacts the surface intermittently and the measurement of the reduction in oscillation amplitude yields the surface topology. Additionally, in tapping mode AFM, phase imaging is a powerful tool that can map variations in surface properties such as elasticity, adhesion and friction. This phase mode
imaging monitors the phase shift between the driving force and the tapping movement of the cantilever, and it is taken simultaneously with monitoring the topographic image.\(^{159}\)

2.5 Reference


Chapter 3

Materials and Experimental Methods

3.1 Materials

Ultrapure water (Milli-Q Gradient A-10, Milli-Q, 18.2 MΩ·cm, < 5 ppb organic impurities) was used in all experiments. Arabinoxylan (AX) from wheat was purchased from Megazyme, Inc. (weight average molar mass $M_w \approx 56.7$ kDa, lot 120601a). The AX has a structure similar to one characterized extensively in the literature.\textsuperscript{1} Arabinoglucuronoxylan (AGX, weight average molar mass $M_w \approx 12.8$ kDa) from spruce was isolated and characterized as described elsewhere by the Gatenholm group at Chalmers University of Technology.\textsuperscript{2} Trimethylsilyl cellulose (TMSC, DS=2.71) was synthesized according to the literature.\textsuperscript{3} Calcium chloride ($\text{CaCl}_2$, 99.9%) was purchased from Aldrich Chemicals, and sodium chloride ($\text{NaCl}$, 99.9%) was purchased from Mallinckrodt Baker, Inc. For substrate cleaning, $\text{NH}_4\text{OH}$ (28% w/w), $\text{H}_2\text{O}_2$ (30% w/w), and $\text{H}_2\text{SO}_4$ (98% w/w), were purchased from Fisher Scientific, EM Science, and Fisher Scientific, respectively. All glassware was cleaned by immersion into a 5 L base bath (4:1 isopropanol to water, 300 g KOH) for at least 24 hours. The glassware was then rinsed with ultrapure water, followed by a rinse with 0.1 mM HCl and a final rinse with ultrapure water.

3.2 Thin Film Preparation

3.2.1 Substrate Preparation

Quartz crystal microbalance with dissipation monitoring (QCM-D) crystals with an ~100 nm thick gold layer on top of a thin layer of chromium (~2 nm) were purchased from Q-Sense AB. The gold QCM-D sensors have a fundamental resonant frequency of 5 MHz, a diameter of
14 mm, and a quartz thickness of 0.3 mm. Gold surface plasmon resonance (SPR) sensors were purchased from Reichert. The 14 mm x 14 mm square SPR sensor slides consist of a ~ 1 mm thick glass substrate, which is coated with a thin chromium layer (~ 2 nm), and then a thin layer of gold (~ 50 nm). Both gold sensors were cleaned by exposure of the surface to UV/ozone for 20 min, followed by boiling in a 1:1:5 v/v/v of NH₄OH:H₂O₂:H₂O solution at 80 °C for 1 h. Then, the substrates were rinsed with ultrapure water and dried with nitrogen gas. Sensors for SPR were further cleaned by immersion into a piranha solution (H₂O₂:H₂SO₄= 3:7 by volume) for 1 hour and rinsed exhaustively with ultrapure water and dried with nitrogen gas before spincoating.

3.2.2 Regenerated Cellulose (RC)

Uniform regenerated cellulose films were spincoated onto cleaned sensors from 10 g•L⁻¹ TMSC solutions in toluene with a spinning speed of 2000 revolutions per minute (rpm) for 60s. Exposure of the TMSC films to the vapor of an aqueous 10% by mass solution of hydrochloric acid for 2 min cleaved trimethylsilyl groups and yielded smooth RC films.⁴

3.3 Characterization Techniques

3.3.1 Atomic Force Microscopy (AFM) Measurements

An Asylum Research atomic force microscope (MFP-3D-BIO, Asylum Research) was used in tapping mode for imaging surfaces. Tapping mode images were collected under ambient conditions with a silicon tip (OMCL-AC160TS, Olympus Corp.) and reported without any image processing. The root mean square (RMS) roughness of the polymer films were obtained from 2 µm x 2 µm scan areas.
3.3.2 Refractive Index Increment Measurements

Refractive index increment (\(dn/dc\)) for arabinoglucuronoxylan (AGX) and arabinoxylan (AX) solutions in water were determined with a differential refractometer (Optilab rEX, Wyatt Technology Corp.). It was operated using a laser light source with a wavelength of 690 nm at 20°C. Sample solutions were pumped into the refractometer at a rate of 0.200 mL\(\cdot\)min\(^{-1}\) and the evaluated concentration range was 0 to 200 mg\(\cdot\)L\(^{-1}\).

3.3.3 Surface Plasmon Resonance (SPR) Measurements

Sample adsorption onto RC films was investigated by SPR (SR7000, Reichert Inc.). After the RC film was prepared on the sensor, the sensor was immediately placed into the SPR flow cell. Immersion oil (n = 1.5150) was used such that the sensor was refractive index-matched to the prism of SPR and a laser diode with an emission wavelength of 780 nm was used as the light source. All solutions were degassed for 1 hour and pumped via Teflon tubing into the flow cell at a rate of 0.200 mL\(\cdot\)min\(^{-1}\) and 20°C. The tubing was connected to a cartridge pump (Masterflex), which was linked to a switch valve that allowed solution switching without the introduction of the air bubbles.

Ultrapure water was initially introduced into the SPR system for several hours to obtain equilibrium swelling until a stable baseline was obtained. Another 10 min was spent to equilibrate the RC film in electrolyte solution. Sample solution was then pumped into the system until the adsorption plateaued, and was followed by a switch to neat electrolyte solution. Ultrapure water was subsequently pumped through the system. Each SPR experiment was performed at least three times, and data points are reported as averages with one standard deviation.
Relevant SPR parameters for data analysis were schematically shown in Figure 2.27B, and Eqn. 2.15 was used for the conversion of the resonance angle ($\theta_{sp}$) to surface concentration ($\Gamma$).\(^5\) The difference in refractive index ($\Delta n_D$) in Eqn. 2.15 was the difference between the refractive index of the adsorbed layer ($n_f$) and that of the solvent ($n$). The change in resonance angle with changing film thickness ($d\theta/dL$) was obtained from Fresnel calculations carried out with a computer simulation program written in Matlab. Values of key parameters used in Eqn. 2.15 are listed in Table 3.1.

### Table 3.1 Values used in the equation of de Feijter et al. (Eqn. 2.15) to calculate $\Gamma$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d\theta/dL$</td>
<td>0.043 deg•nm(^{-1})</td>
</tr>
<tr>
<td>$n$ (water)</td>
<td>1.328(^7)</td>
</tr>
<tr>
<td>$n_f$ (AGX)</td>
<td>1.45(^6)</td>
</tr>
<tr>
<td>$n_f$ (AX)</td>
<td>1.45(^6)</td>
</tr>
<tr>
<td>$dn/dc$ (AGX)(^a)</td>
<td>0.144 cm(^3)•g(^{-1})</td>
</tr>
<tr>
<td>$dn/dc$ (AX)(^a)</td>
<td>0.148 cm(^3)•g(^{-1})</td>
</tr>
</tbody>
</table>

\(^a\) Determined by differential refractometry.

### 3.3.4 Quartz Crystal Microbalance with Dissipation (QCM-D) Monitoring Measurements

The adsorption of various hemicelluloses was also investigated by QCM-D (Q-Sense E4, Q-Sense AB). The sensor crystal was immediately placed into the flow cell after the surface was prepared. Solutions were pumped into the flow cell by a cartridge pump (ISMATEC-ISM935) at the same rate (0.200 mL•min\(^{-1}\)) and same temperature (20 °C) as SPR experiments. The procedure was also similar to that of SPR. Background solvent flowed over the surface for several hours until a stable baseline was obtained. Sample solution was then injected into the
flow cell and afterwards background solvent was reintroduced into the system. Both frequency ($\Delta f$) and dissipation ($\Delta D$) changes for the fundamental frequency (4.95 MHz) and six odd overtones ($n = 3$ through 13) were monitored simultaneously. Each QCM-D experiment was performed at least three times and values are reported as averages with one standard deviation.

When rigid films coat the surface and $\Delta D < 2 \times 10^{-6}$, the adsorbed mass can be calculated from the Sauerbrey equation (Eqn. 2.9). In this work, the increase in $\Delta D$ was small, thus the Sauerbrey equation provided surface concentrations from QCM-D ($\Gamma_{\text{QCM-D}}$).

3.4 References


Chapter 4

Quartz Crystal Microbalance with Dissipation Monitoring and Surface Plasmon Resonance Studies of Arabinoglucuronoxylan and Arabinoxylan Adsorption onto Regenerated Cellulose Films

4.1 Abstract

Adsorption of spruce arabinoglucuronoxylan (AGX) and wheat arabinoxylan (AX) from water, calcium chloride (CaCl$_2$) and sodium chloride (NaCl) solutions onto regenerated cellulose (RC) surfaces was measured with quartz crystal microbalance with dissipation monitoring (QCM-D) and surface plasmon resonance (SPR), and the morphologies of the formed films were subsequently visualized by atomic force microscopy (AFM). The influences of CaCl$_2$ and NaCl on AGX and AX adsorption were examined and the results demonstrated that AGX and AX adsorption onto RC surfaces increased with increasing salt concentrations. Enhanced adsorption was chiefly attributed to lower xylan solubility in salt solutions. The adsorption isotherms were obtained from QCM-D and were empirically fit by Freundlich isotherms. The combination of SPR and QCM-D yielded water contents of ~ 75% and ~ 90% by mass for AGX and AX layers, respectively. The amount of water in the films was large and was dependent upon the solvent. In addition, AFM images of AGX and AX layers on RC films and bare gold surfaces were comparable. Similar root-mean-square (RMS) roughness values were obtained before and after xylan adsorption, an indication of uniform adsorption, although observed globular structures are smaller and more numerous on surfaces formed from CaCl$_2$ solutions. Films formed on bare gold surfaces had film morphologies similar to films adsorbed onto RC films. Furthermore, low RMS roughness of these films (< 2 nm) formed on gold surfaces can be
considered uniform enough to be “modified substrates” for future studies of AGX and AX interactions with other plant cell wall polymers.

4.2 Introduction

Hemicelluloses, which account for approximately a quarter to a third of the dry weight of the material in woody plants, together with other polysaccharides represent the majority of the dry mass of plant cell walls of vascular plants. The trend of utilizing biomass sustainably has led to increased research on the exploitation of natural polysaccharides like cellulose and hemicelluloses as biopolymer resources. Xylans are hemicelluloses and are the second most abundant polysaccharides after cellulose in nature. Indeed, arabinoxylans (AX), arabinose substituted xylans, are major components of dietary fiber. In many cereals, like wheat endosperm, AX is ~ 85% of the total nonstarch polysaccharides. Water soluble AXs are the main components of the cell wall material in wheat flour, 2 to 3% (w/w). The principal hemicellulose found in softwood is arabinoglucuronoxylan (AGX, 5-10% of the total dry weight) followed by galactoglucomannan (GGM). Xylans, including AGX and AX, have an inherent affinity for cellulose. As a consequence, xylans adsorb onto cellulose irreversibly and can affect the cellulose crystallinity. Studies have shown that xylans prevent the aggregation of the cellulose microfibrils and are therefore responsible for less ordered regions in cellulose bundles. Numerous studies have revealed xylan-cellulose interactions, and identified xylan molecular structure, botanical origin, purity, etc. as important factors that influenced xylan-cellulose interactions. In this study, AGX and AX from different sources offered two hemicelluloses with different structure and molar mass. Regenerated cellulose (RC) surfaces were prepared and the adsorption of these two xylans onto these surfaces was quantified. Highly purified spruce AGX (with only 2.3% other sugars) used in this study is the same sample isolated
and characterized by the Gatenholm group.\textsuperscript{14} The structure of AGX is shown in Figure 4.1A and the average ratio of $\alpha$-L-arabinofuranosyl (Ara) to 4-$O$-methyl-glucuronic acid (MeGlcA) to D-xylopyranosyl (Xyl) residues is 1:2:11. This polysaccharide consists of linear chains of $\beta$-D-(1,4)-linked Xyl residues, which can be substituted at the 3-$O$-positions with Ara or at 2-$O$-positions with MeGlcA residues.\textsuperscript{14} The AX (purity 95\%) used in this paper was directly isolated from wheat flour and is similar to the AX used elsewhere.\textsuperscript{15} It is made up of a backbone of $\beta$-(1\(\rightarrow\)4) linkages. The Ara residues are the predominant side chains. Roughly 1/3 of the Ara residues are found on Xyl monosubstituted at the C(O)-3 position, while 2/3 of the Ara are located on Xyl disubstituted at the C(O)-3 and the C(O)-2 positions. The Ara-to-Xyl ratio is 38:62 and it contains negligible amounts of other sugars (Figure 4.1B).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structure.png}
\caption{Principal structure of (A) spruce AGX and (B) wheat flour AX used in this study.}
\end{figure}

Because of the vast abundance of hemicellulose, it has become one of the most promising renewable sources for dietary materials and industrial processes, e.g., food packaging,\textsuperscript{16} edible
films, hydrogels, and thermoplastic xylan derivatives. Buchanan et al. produced optically clear films of esters of arabinoxylan and cellulose. Zhang et al. obtained an efficient moisture barrier for AX films plasticized with propylene glycol, glycerol, or sorbitol. Gröndahl et al. showed hydrophobic AX films could be obtained by surface fluorination. Numerous studies showed emulsification of lipids in AX films yielded more opaque films with lower water vapor permeability. Films of AGX also have potential as a food packaging material since it shows low oxygen permeability. Furthermore, cast AGX films were totally amorphous, transparent, and flexible. The addition of sorbitol to AGX films resulted in weaker but even more flexible films. In the present research, the water content and morphology of AX and AGX films assembled from different solution conditions were studied.

Andrewartha et al. and other pioneers showed the Ara/Xyl ratio was an important parameter that affected the properties of AX. Both the viscosity of AX solutions and solubility of AX were well studied and have been demonstrated to increase with increasing Ara-to-Xyl ratio. It was also shown that the swelling and viscosity of wheat flour dough arose from crosslinks between AX molecules themselves, and AX and proteins. Similar information has also been reported for AGX. Low degrees of substitution of both Ara and MeGlcA residues make AGXs more likely to self-associate. In this study, we investigated the relationship between water content, xylan structure, and how the structure affects film morphology.

In addition, research based upon the characteristics of xylan adsorption onto cellulose surfaces is meaningful. As one example, previous studies showed xylan is desirable in paper manufacturing since it enhances paper strength. Likewise, the affinity between xylan and cellulose complicates bio-ethanol production where cellulose is enzymatically converted into fermentable glucose. Therefore, another goal of this study was a greater understanding of AGX
and AX adsorption onto cellulose films, and how film formation is affected by parameters like ionic strength.

In contrast to numerous studies of xyloglucan adsorption onto cellulose microfibrils because of its abundance in the primary cell wall,\textsuperscript{34-37} xylan is less studied, although a few papers have studied the effects of structure and concentration on xylan adsorption onto cellulose.\textsuperscript{7,13} In this work, a quartz crystal microbalance with dissipation monitoring (QCM-D), surface plasmon resonance (SPR) and atomic force microscopy (AFM) were used to study xylan adsorption onto cellulose. The cellulose substrates were regenerated cellulose (RC) derived from spincoated films of trimethylsilyl cellulose (TMSC). Previous studies revealed that RC films can be easily created by spincoating and the films obtained were amorphous and swollen in water.\textsuperscript{38-40}

4.3 Experimental Section

Films of TMSC were spincoated onto gold QCM-D and SPR sensors as described in Chapter 3.2.2. Atomic force microscopy (AFM) studies of films and refractive index increment ($dn/dc$) measurements of solutions for AGX and AX were conducted as outlined in Chapter 3.3.1 and 3.3.2, respectively. Detailed procedures for SPR and QCM-D experiments were provided in Chapter 3.3.3 and 3.3.4, respectively. Water, electrolyte solutions (CaCl$_2$ and NaCl) and sample solutions flowed through the SPR and QCM-D flow cells at a rate of 0.200 mL·min$^{-1}$ at 20 °C. Water was initially flowed over films for several hours until a stable baseline was obtained. For CaCl$_2$ and NaCl solutions, a second equilibration with neat electrolyte solution followed. Then, different solutions were introduced into the system at the same rate and temperature as water. Finally, neat electrolyte solution and water (or just water for samples without electrolyte solutions) was flowed through the cell for the removal of bulk and reversibly adsorbed material. The remaining adsorbed material was regarded as irreversibly adsorbed.
4.4 Results and Discussion

4.4.1 AGX and AX Adsorption onto Regenerated Cellulose Surfaces from Water and Electrolyte Solutions by QCM-D

Representative raw QCM-D data for the adsorption of AGX and AX from water and 30 mM CaCl$_2$ with different concentrations onto regenerated cellulose surfaces are provided in Figures 4.2 and 4.3, respectively. As seen in Figures 4.2 and 4.3, the scaled frequency changes ($\Delta f/n$), where $n$ is the overtone number, increased with increasing xylan concentration, and the dissipation change ($\Delta D$) followed the same trend for AGX and AX, respectively. Since desorption ceased shortly after the rinse with water started, the remaining film was regarded as irreversibly adsorbed. A comparison of $\Delta f/n$ after the final rinse with water revealed a smaller absolute value of $\Delta f/n$ for xylan adsorbed from water than for xylan adsorbed from 30 mM CaCl$_2$ at the same concentration. The $\Delta D$ profiles revealed the same trends. Larger $\Delta D$ indicated softer films were formed from CaCl$_2$ solutions than from water at the same concentration. In comparison with AX at the same concentrations, AGX films were more rigid than films adsorbed from both water and CaCl$_2$ solutions. Therefore, the adsorption behavior was different. Differences were not surprising because the two xylans had different chemical structures, molar mass and solubility.
Figure 4.2 Representative QCM-D data, $\Delta f/n$ and $\Delta D$ for $n = 5$ as a function of time for AGX adsorption onto RC films from solutions in (A) water or (B) 30 mM CaCl$_2$ at 20 °C. Symbols correspond to AGX concentrations of ([-]) 6, ([-]) 13, ([-]) 25, ([-]) 55, ([-]) 80, ([-]) 100, and ([-]) 200 mg·L$^{-1}$. Arrows indicate where solutions were switched.
Figure 4.3 Representative QCM-D data, $(\Delta f/n)$ and $\Delta D$ for $n = 5$ as a function of time for AX adsorption onto RC films from solutions in (A) water or (B) 30 mM CaCl$_2$ at 20 °C. Symbols correspond to AX concentrations of (–) 6, (–) 13, (–) 25, (–) 55, (–) 100, (–) 200, and (–) 500 mg•L$^{-1}$. Arrows indicate where solutions were switched.
In order to representative clearly show profiles influence of ionic strength \((I)\) on AGX and AX adsorption onto RC films, adsorption from \(\text{CaCl}_2\) and \(\text{NaCl}\) solutions at the same ionic strength were provided in Figures 4.4 and 4.5, respectively. These figures show \(\sim\) 1.5 h was required for adsorption equilibrium (the maximum change in \(\Delta f/n\) or \(\Delta D\)) for 100 mg\(\text{L}^{-1}\) xylan concentrations. After the salt solutions and water rinses, \(\Delta f/n\) values indicated that electrolyte solutions promoted adsorption. Final values for \(\Delta f/n\) and \(\Delta D\) for irreversible xylan adsorption onto RC films from 100 mg\(\text{L}^{-1}\) solutions are summarized in Tables 4.1 and 4.2.

Overall, electrolyte solutions enhanced adsorption. It has long been stated that increases in ionic strength of a solvent can screen repulsive monomer–monomer interactions as well as the repulsive interactions between the weakly charged surfaces and polymer.\(^{41}\) These factors enhance adsorption and lead to less extended polymer chains.\(^{42}\) This explanation is consistent with the AGX adsorption results. For the case of AX, the monomer-solvent interactions make neutral AX molecules swell and form somewhat expanded conformations in water. In the presence of electrolyte solutions, the attractive interactions between monomers are favored, leading to collapse of the chains. The higher the salt concentration, the stronger these attractive forces are.\(^{42-43}\) These factors led to a result for AX that was similar to AGX: more and less extended polymer chains are adsorbed from salt solutions.\(^{42}\) Moreover, xylan has very limited solubility in electrolyte solutions, which means even weak attractions can enhance the adsorption.\(^{28,44}\) As seen in Table 4.1, both \(\Delta f/n\) and \(\Delta D\) for irreversibly adsorbed AGX films formed from \(I = 0.090\) mM solutions are around three times greater than films formed from water. Electrolyte had a smaller effect on irreversible AX adsorption than it did on AGX adsorption as \(\Delta f/n\) and \(\Delta D\) were only twice the values obtained for adsorption from water. Since QCM-D gives the total mass adsorbed on the surface, polymer plus bound water, QCM-D results
cannot fully explain which xylan is more affected by the electrolyte. Hence SPR studies were required to separate polymer adsorption from the amount of coupled water.

Comparison between the surface excess data listed in Tables 4.1 and 4.2 at the same ionic strength, shows CaCl$_2$ and NaCl may play different roles in the adsorption process. For the case of AGX, the adsorption from CaCl$_2$ trends toward higher values than the adsorption from NaCl at the same ionic strength, although these values are not statistically significant. In contrast, the adsorption of AX is essentially identical from CaCl$_2$ and NaCl at the same ionic strength. These observations probably indicate CaCl$_2$ plays a more important role in the AGX adsorption. The reason is that Ca$^{2+}$ can form crosslinks between AGX molecules through carboxylic acid groups, while no such crosslinks can be formed between neutral AX molecules.

Another key point is that layers adsorbed from all three solutions in Figure 4.4 seem to be stable as only a slight decrease of $\Delta D$ was observed upon final exposure to water. This observation probably resulted from a few loosely attached xylan molecules that were removed by the water rinse. Notice that the $\Delta D$ of both AGX and AX films irreversibly adsorbed from electrolyte solutions have equivalent values after the water rinse. Qualitatively these films should be comparably soft. With increasing salt concentration, more molecules adsorb onto the surface. Some of the adsorbed molecules may dangle into solution whereby they can interact with free xylan molecules. As such softer films are plausible. Upon water rinsing, only irreversibly bound xylan molecules remain at the surface, and all the layers probably have similar rigidity.

A comparison between irreversibly adsorbed AGX and AX films shows that AX films have larger values of both $\Delta f/n$ and $\Delta D$ for adsorption from the same solution at the same concentration. One possible reason for this observation is the higher molar mass of AX relative
to AGX. Molecules with higher molar mass adsorbed on a surface can have longer free loops and tails extending into the nearby solution. Dangling loops and tails can couple more water with the motion of the oscillating crystal. This possibility was explored through SPR experiments.
Figure 4.4 Representative $\Delta f/n$ and $\Delta D$ versus time plots for QCM-D studies of regenerated cellulose surfaces exposed to 100 mg•L$^{-1}$ AGX solutions with and without (A) CaCl$_2$ or (B) NaCl. Curves with markers correspond to $n = 5$. Arrows indicate where solutions were switched.
Table 4.1 $\Delta f/n$ and $\Delta D$ of AGX adsorbed from water, CaCl$_2$ and NaCl solutions at equilibrium adsorption and after the final water rinse$^a$

<table>
<thead>
<tr>
<th></th>
<th>$\Delta f/n$ /Hz</th>
<th>$\Delta D \times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equilibrium</td>
<td>After water rinse</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>-4.9 ± 1.3</td>
<td>-4.0 ± 0.6</td>
</tr>
<tr>
<td>10 mM CaCl$_2$ ($I = 0.030$)</td>
<td>-11.4 ± 1.0</td>
<td>-8.6 ± 1.0</td>
</tr>
<tr>
<td>30 mM CaCl$_2$ ($I = 0.090$)</td>
<td>-12.9 ± 2.0</td>
<td>-9.0 ± 2.0</td>
</tr>
<tr>
<td>30 mM NaCl ($I = 0.030$)</td>
<td>-10.1 ± 1.5</td>
<td>-6.4 ± 1.1</td>
</tr>
<tr>
<td>90 mM NaCl ($I = 0.090$)</td>
<td>-11.9 ± 1.8</td>
<td>-6.9 ± 1.6</td>
</tr>
</tbody>
</table>

$^a$Data for adsorption from 100 mg•L$^{-1}$ AGX solutions ($n = 5$), ± one standard deviation
Figure 4.5 Representative Δf/n and ΔD versus time plots for QCM-D studies of regenerated cellulose surfaces exposed to 100 mg•L⁻¹ AX solutions with and without (A) CaCl₂ or (B) NaCl. Curves with markers correspond to n = 5. Arrows indicate where solutions were switched.
Table 4.2 $\Delta f/n$ and $\Delta D$ of AX adsorbed from water, CaCl$_2$ and NaCl solutions at equilibrium adsorption and after the final water rinse$^a$

<table>
<thead>
<tr>
<th></th>
<th>$\Delta f/n$ /Hz</th>
<th>$\Delta D \times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equilibrium</td>
<td>After water rinse</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>-7.5 ± 0.5</td>
<td>- 5.1 ± 0.6</td>
</tr>
<tr>
<td>10 mM CaCl$_2$ ($I = 0.030$)</td>
<td>-11.1 ± 1.2</td>
<td>- 8.5 ± 0.6</td>
</tr>
<tr>
<td>30 mM CaCl$_2$ ($I = 0.090$)</td>
<td>-14.8 ± 2.0</td>
<td>- 10.6 ± 1.7</td>
</tr>
<tr>
<td>30 mM NaCl ($I = 0.030$)</td>
<td>-10.1 ± 1.4</td>
<td>-8.4 ± 0.8</td>
</tr>
<tr>
<td>90 mM NaCl ($I = 0.090$)</td>
<td>-14.3 ± 2.2</td>
<td>-10.2 ± 2.0</td>
</tr>
</tbody>
</table>

$^a$Data for adsorption from 100 mg•L$^{-1}$ AX solutions ($n = 5$), ± one standard deviation

4.4.2 AGX and AX Adsorption onto Regenerated Cellulose Surfaces from Water and Electrolyte Solutions by SPR

Adsorption of AGX and AX onto regenerated cellulose surfaces was studied by SPR. Representative raw SPR data for AGX and AX adsorption from water, CaCl$_2$ and NaCl solutions onto regenerated cellulose surfaces are provided in Figures 4.6 and 4.7. For AGX adsorption from water, maximum (equilibrium) SPR angles only increased by ~ 0.009° and decreased to ~ 0.007° after the surface was exposed to water (irreversible angle change). In contrast, the SPR angle increased to ~ 0.0020° at equilibrium adsorption for wheat AX and with an irreversible change ~ 0.0016° after the water rinse. However, $\Delta \theta_{sp}$ values were several times larger when xylan layers were adsorbed from electrolyte solutions. The largest SPR angle changes occurred for adsorption equilibrium from $I = 0.090$ mM solutions. As increases in SPR angle are due to increasing refractive index of the thin layer near the surface, both salt solutions and xylan made
contributions to the SPR angle change. For the case of switching from water to salt solution, a dramatic SPR angle change occurred. Hence, part of SPR angle change at equilibrium from electrolyte solutions arose from CaCl₂ or NaCl, and its removal contributed strongly to the \( \Delta \theta_{sp} \) decrease after the water rinse. Because water rinsed out extra salt solution and loosely bound polymers, SPR angle changes reverted to smaller values. The magnitudes of the SPR angle changes followed the same trends as QCM-D experiments: \( \Delta \theta_{sp} \) in water solution < \( I = 0.030 \) mM < \( I = 0.090 \) mM at the maximum angle change. Changes in SPR angle for xylan layers before and after the water rinse are summarized in Tables 4.3 and 4.4. As seen in Tables 4.3 and 4.4, the trend for \( \Delta \theta_{sp} \) after the water rinse (irreversible adsorption) was water < \( I = 0.030 \) mM ≈ \( I = 0.090 \) mM.

In order to compare the adsorption of AGX and AX by SPR, irreversible \( \Delta \theta_{sp} \) were converted into surface concentrations of irreversibly adsorbed xylan (\( \Gamma_{SPR, irr} \)) through the equation of de Feijter et al.\textsuperscript{45} Values of \( \Gamma_{SPR, irr} \) are presented in Tables 4.3 and 4.4. Detailed information about the procedure for the conversion can be found in Chapter 3.3.3. The adsorption trend illustrated in these two tables is similar to adsorption trends listed in Tables 4.1 and 4.2: at the same ionic strength, slightly more AGX adsorbed from CaCl₂ than from NaCl solutions, whereas essentially equivalent adsorption of AX was obtained from both types of salt solution. The amount of AGX adsorbed from electrolyte solutions was about 2.5 times greater than the amount adsorbed from water, while the amount of AX adsorbed from CaCl₂ and NaCl was around six times greater than the amount adsorbed from water. In contrast to QCM-D, the SPR signal only arose from the polymer as \( \Delta \theta_{sp} \) was measured relative to a water background.\textsuperscript{45} Consequently, electrolyte solutions have stronger influence on the adsorption of AX than AGX by SPR. Nonetheless, more AGX adsorbed onto cellulose than AX for the same solution
conditions. Combining the results from QCM-D, where the adsorption of AGX was less than that of AX, we propose that water plays a more important role in AX adsorption than AGX adsorption.

Figure 4.6 Representative $\Delta \theta_{sp}$ versus time for SPR studies of regenerated cellulose surfaces exposed to 100 mg•L$^{-1}$ AX in (A) (−−) water, (−−) 10 mM CaCl$_2$, and (−−−) 30 mM CaCl$_2$ solutions, and (B) (−−−) 30 mM NaCl, and (−−−−) 90 mM NaCl solutions. Arrows indicate where solutions were switched.
Table 4.3 $\Delta \theta_{sp}$ and $\Gamma_{SPR, irr}$ for AGX adsorbed from water, CaCl$_2$ and NaCl solutions before and after the water rinse$^a$

<table>
<thead>
<tr>
<th></th>
<th>$\Delta \theta_{sp} /^\circ$</th>
<th>$\Gamma_{SPR, irr} / \text{mg} \cdot \text{m}^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equilibrium</td>
<td>After water rinse</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>0.009 ± 0.002</td>
<td>0.007 ± 0.001</td>
</tr>
<tr>
<td>10 mM CaCl$_2$ ($I = 0.030$)</td>
<td>0.041 ± 0.004</td>
<td>0.020 ± 0.004</td>
</tr>
<tr>
<td>30 mM CaCl$_2$ ($I = 0.090$)</td>
<td>0.082 ± 0.009</td>
<td>0.021 ± 0.005</td>
</tr>
<tr>
<td>30 mM NaCl ($I = 0.030$)</td>
<td>0.036 ± 0.008</td>
<td>0.15 ± 0.004</td>
</tr>
<tr>
<td>90 mM NaCl ($I = 0.090$)</td>
<td>0.080 ± 0.010</td>
<td>0.017 ± 0.005</td>
</tr>
</tbody>
</table>

$^a$Average values for adsorption from 100 mg•L$^{-1}$ AGX solutions ± one standard deviation
Figure 4.7 Representative $\Delta \theta_{sp}$ versus time for SPR studies of regenerated cellulose surfaces exposed to 100 mg•L$^{-1}$ AX in (○-) water, (●-) 10 mM CaCl$_2$, and (■-) 30 mM CaCl$_2$ solutions, and (B) (○-) 30 mM NaCl, and (■-) 90 mM NaCl solutions. Arrows indicate where solutions were switched.
Table 4.4 $\Delta \theta_{sp}$ and $\Gamma_{SPR, irr}$ for AX adsorbed from water, CaCl$_2$ and NaCl solutions before and after the water rinse$^a$

<table>
<thead>
<tr>
<th></th>
<th>$\Delta \theta_{sp} /^{\circ}$</th>
<th>$\Gamma_{SPR, irr} /$mg•m$^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equilibrium</td>
<td>After water rinse</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>0.0020 ± 0.001</td>
<td>0.0016 ± 0.001</td>
</tr>
<tr>
<td>10 mM CaCl$_2$ ($I = 0.030$)</td>
<td>0.034 ± 0.01</td>
<td>0.010 ± 0.007</td>
</tr>
<tr>
<td>30 mM CaCl$_2$ ($I = 0.090$)</td>
<td>0.067 ± 0.01</td>
<td>0.012 ± 0.003</td>
</tr>
<tr>
<td>30 mM NaCl ($I = 0.030$)</td>
<td>0.03 ± 0.007</td>
<td>0.009 ± 0.003</td>
</tr>
<tr>
<td>90 mM NaCl ($I = 0.090$)</td>
<td>0.066 ± 0.011</td>
<td>0.013 ± 0.006</td>
</tr>
</tbody>
</table>

$^a$Average values for adsorption from 100 mg•L$^{-1}$ AX solutions ± one standard deviation

4.4.3 Adsorption Isotherms

Adsorption isotherms for AGX and AX adsorption onto regenerated cellulose from water and 30 mM CaCl$_2$ solutions were obtained from QCM-D experiments (Figure 4.8). Each solution was flowed over cellulose substrate for 2 hours and each measurement was repeated at least 3 times. Representative data for each isotherm data point was provided in Figures 4.2 and 4.3. Details for the conversion of the measured QCM-D data into surface concentrations ($\Gamma_{QCM-D}$) were provided in Chapter 3.3.4. In brief, the Sauerbrey equation was used for the calculation. However, the Sauerbrey equation is no longer valid if $\Delta D$ values become significantly greater than $2 \times 10^{-6}$. Thus, 0 to 200 mg•L$^{-1}$ was used as the concentration range for the isotherms as $\Delta D$ exceeded $2 \times 10^{-6}$ for higher concentrations (e.g. $\Delta D$ for 500 mg•L$^{-1}$ AX in water solutions is $\sim 2.3 \times 10^{-6}$, Figure 4.3A).
Several theoretical models that describe adsorption behavior were summarized in Chapter 2.3.1, and Freundlich adsorption isotherms were used for an empirical fit of the adsorption data of AGX and AX onto regenerated cellulose surfaces. Although the Freundlich model was derived for the case of small molecules adsorbing onto a heterogeneous solid substrates, the model frequently provides a good empirical fit to adsorption isotherms of polymers. The Freundlich model assumes heterogeneous surfaces with both high and low affinity binding sites and this semi-empirical Freundlich isotherm is expressed as:

\[ \Gamma = K_F C^{1/n_F} \]  

(5.1)

where \( K_F \) is the adsorbent capacity, \( C \) is the bulk adsorbate concentration, and \( 1/n_F \) is the adsorption affinity constant.\(^{47}\) Fitting parameters from Figure 4.8 are shown in Table 4.5. As is evident from Figure 4.8, the amount of adsorption increases from water to 30 mM CaCl\(_2\) solution at the same xylan concentrations.
**Figure 4.8** Adsorption isotherms from QCM-D experiments for adsorption of (A) AGX and (B) AX onto regenerated cellulose surfaces from (●) water and (■) 30 mM CaCl$_2$ solutions with one standard deviation error bars. The solid lines represent fits with Freundlich isotherms.

**Table 4.5** Adsorption isotherm parameters for AGX and AX adsorption onto regenerated cellulose from aqueous and 30 mM CaCl$_2$ solutions.

<table>
<thead>
<tr>
<th></th>
<th>AGX</th>
<th></th>
<th>AX</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_F$ (L·mg$^{-1}$)</td>
<td>$1/n_F$</td>
<td>$K_F$ (L·mg$^{-1}$)</td>
<td>$1/n_F$</td>
</tr>
<tr>
<td>Water</td>
<td>0.14 ± 0.04</td>
<td>0.37 ± 0.06</td>
<td>0.11 ± 0.01</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>30 mM CaCl$_2$</td>
<td>0.14 ± 0.05</td>
<td>1.91 ± 0.26</td>
<td>0.17 ± 0.03</td>
<td>0.52 ± 0.04</td>
</tr>
</tbody>
</table>

The relationship between solvent characteristics and xylan adsorption onto RC films is clearly seen through the adsorption isotherms. All four isotherms in Figure 4.8 are different. Each isotherm has a steep initial slope that becomes gentler with increasing concentration.
low xylan concentrations, the molecules most likely have flatter conformations (trains) on the RC surface, driven by the unsubstituted regions of the xylans.\textsuperscript{13, 15, 48} Flatter conformations at low concentration would be expected independent of whether or not CaCl\textsubscript{2} was added. At higher xylan concentrations, more molecules adsorb with a greater fraction of loops and tails. Since no plateau value was observed in Figure 4.8 at high surface concentrations, xylan-xylan interactions are expected.\textsuperscript{13, 49} Because CaCl\textsubscript{2} solutions screen repulsive interactions between negatively charged repeating anhydroxylose units (AXU) monomers, enhanced xylan-xylan interactions are expected. As a consequence, thicker films are observed for AGX. For the case of uncharged AX, less favorable AX-solvent interactions are expected with increasing CaCl\textsubscript{2} concentration (AX has lower solubility in CaCl\textsubscript{2} solutions than water). This feature also drives stronger xylan-xylan interactions.

\textbf{4.4.4 Water Contents within Adsorbed AGX and AX Films}

If ideal mixing can be assumed, the water content of the AGX and AX films adsorbed from water, CaCl\textsubscript{2} and NaCl solutions onto regenerated cellulose films can be calculated. Values of $\Gamma$ measured by QCM-D and SPR along with the assumption of ideal mixing yields:\textsuperscript{50}

\[
\% \text{water by mass} = \left(1 - \frac{\Gamma_{\text{SPR}}}{\Gamma_{\text{QCM-D}}} \right) \times 100\%
\] (4.2)

A further assumption of Eqn. 4.2 was that $\Gamma_{\text{SPR}}$ only represented adsorbed polymer,\textsuperscript{45} whereas $\Gamma_{\text{QCM-D}}$ contained adsorbed polymer plus coupled water.\textsuperscript{51} Water contents of AGX and AX adsorbed onto RC films from 100 mg•L\textsuperscript{−1} solutions in water, CaCl\textsubscript{2} and NaCl are summarized in Table 4.6.
Table 4.6 Water contents of adsorbed AGX and AX films.

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>10 mM CaCl&lt;sub&gt;2&lt;/sub&gt;</th>
<th>30 mM CaCl&lt;sub&gt;2&lt;/sub&gt;</th>
<th>30 mM NaCl</th>
<th>90 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGX</td>
<td>77 ± 8</td>
<td>74 ± 4</td>
<td>74 ± 8</td>
<td>74 ± 8</td>
<td>73 ± 10</td>
</tr>
<tr>
<td>AX</td>
<td>95 ± 1</td>
<td>87 ± 2</td>
<td>87 ± 4</td>
<td>88 ± 4</td>
<td>88 ± 7</td>
</tr>
</tbody>
</table>

Water contents of AGX films (~75%) were smaller than the corresponding values for AX films, which were about 90% by mass. From the water contents, it is clear that all the adsorbed xylan films contained large amounts of water. Nonetheless, the higher water content of AX films relative to AGX films may reflect the larger molar mass of AX, thereby allowing longer loops and tails into the surrounding medium. The difference in water content was statistically insignificant for AGX films adsorbed from electrolyte solutions versus water, but the trend was for lower values for films formed from the electrolyte solutions for AX. As Ca<sup>2+</sup> could serve as crosslinking sites between 4-O-methylglucuronic acid groups on xylan chains, swelling in water would be expected from a network structure. Similar behavior was seen by Liu, et al.<sup>52</sup> for the role of Ca<sup>2+</sup> in carboxymethylcellulose films. In contrast, AX films formed from water contained more water than films formed from electrolyte solutions. As discussed earlier, less favorable AX-solvent interactions are expected in salt solutions. These stronger interactions between AX units in the film state may be kinetically trapped. Hence, they are not easily broken upon the switch from CaCl<sub>2</sub> solutions to water.
4.4.4 AFM Images of Adsorbed AGX and AX Films

Images from AFM for AGX and AX films adsorbed onto RC films from 100 mg•L\(^{-1}\) solutions in water, 10 mM CaCl\(_2\) and 30 mM CaCl\(_2\) are provided in Figures 4.9 and 4.10. For the purpose of facilitating a comparison of the conformation of xylans adsorbed onto RC surfaces and testing if the xylans themselves can form uniform layers, Figure 4.10 also contains AFM images of AGX and AX layers adsorbed onto bare gold surfaces. The AGX and AX films on both regenerated cellulose and bare gold surfaces were prepared by exposure to xylan solutions in water, 10 mM CaCl\(_2\) and 30 mM CaCl\(_2\) solutions for 2 h, followed by rinsing and vacuum oven drying (45 °C). Bare RC and Au films are shown for comparison. No significant morphology changes are visible after the RC surface was treated with the xylan solutions. The root-mean-square (RMS) roughness changed from ~ 1.6 nm for the bare RC film to ~ 1.1 nm, ~ 1.0 nm and ~ 1.4 nm for AGX layers formed from water, 10 mM CaCl\(_2\) and 30 mM CaCl\(_2\), respectively (Figure 4.9). Similar observations were made for AX layers formed from water, 10 mM CaCl\(_2\) and 30 mM CaCl\(_2\), where the RMS roughness were ~ 1.9 nm, ~ 2.4 nm, and ~ 1.3 nm, respectively. For the gold surfaces, AGX and AX adsorption yielded similar trends with respect to surface roughness. Adsorption from water, 10 mM CaCl\(_2\) and 30 mM CaCl\(_2\) yielded RMS roughnesses of ~ 1.0 nm for AGX layers and ~ 1.5 nm for AX layers. More importantly, the images in Figures 4.9 and 4.10 show AGX and AX completely and uniformly cover gold.

In addition to the small RMS roughness changes, globular xylan particles are clearly evident on the AFM images. Smaller and more numerous xylan particles are present on the RC surfaces for AGX and AX adsorption from CaCl\(_2\) solutions. These results are in accordance with a previous adsorption study\(^{42}\) where more xylan adsorbed onto cellulose surfaces from CaCl\(_2\) solutions as well as the hypothesis that less extended polymer chains were present in the CaCl\(_2\)
solutions. In comparison with the AFM images of AGX layers, AX adsorption results in rougher surfaces and the AX aggregates have large sizes and less uniform coverage. Interestingly, xylan aggregates adsorbed directly onto gold surfaces were similar to those adsorbed onto cellulose. The absence of any significant differences between the cellulose and gold surfaces is strong evidence in support of the hypothesis by Linder et al. that xylan adsorption is dominated by aggregates in solution.⁴⁹
Figure 4.9 Representative AFM height images of bare RC and gold films exposed to 100 mg·L⁻¹ AGX in water, 10 mM CaCl₂ and 30 mM CaCl₂ solutions. The scans are over a 2 µm × 2 µm areas with 20 nm z-scales.
Figure 4.10 Representative AFM height images of bare RC and gold films exposed to 100 mg•L$^{-1}$ AX in water, 10 mM CaCl$_2$ and 30 mM CaCl$_2$ solutions. The scans are over a 2 µm × 2 µm areas with 20 nm z-scales.
4.5 Conclusions

This work demonstrated that the amount of xylan adsorbed onto a surface was strongly influenced by the solvent. Solutions of CaCl$_2$ and NaCl enhanced the adsorption of xylan onto cellulose. Highly hydrated xylan layers (water content ~ 75% by mass for AGX films and ~ 90% by mass for AX films) were observed for all solution conditions studied. Moreover, AFM images show smaller and more numerous xylan aggregates for layers formed from CaCl$_2$ solutions. The AGX and AX aggregates had different sizes on RC films with AGX more uniformly covering the RC surface. Analysis of xylan adsorbed onto bare gold surfaces showed the adsorbed xylan aggregates had similar structures to those observed on RC films. The absence of structural differences for aggregates on RC and gold suggested the adsorbed structure was dominated by the adsorption of aggregates from solution. Consequently, this study gives information about how electrolyte solutions influence the interaction between xylans and cellulose and provides a facile mechanism for the preparation of xylan-modified substrates.

4.6 References


Chapter 5

Conclusions and Suggestions for Future Work

5.1 Overall Conclusions

In this work, quartz crystal microbalance with dissipation monitoring (QCM-D) and surface plasmon resonance (SPR) were used to detect the adsorption of spruce arabinoglucuronoxylan (AGX) and arabinoxylan (AX) from wheat onto regenerated cellulose surfaces. Calcium chloride (CaCl$_2$) and sodium chloride (NaCl) solutions screen repulsive interactions between AGX sugar residues as well as between weakly charged AGX and the cellulose surface. As a consequence, enhanced AGX adsorption was observed with increasing electrolyte concentration. Similar adsorption enhancement is found for AX and electrolyte solutions, although this is attributed to poorer solvent quality that enhances attractive interactions between neutral AX monomers. The combination of QCM-D and SPR data gave water contents for the adsorbed layers and showed both were highly hydrated (~ 75% for AGX and ~ 90% for AX). Adsorption isotherms of both AGX and AX were fit by Freundlich adsorption isotherms. The failure of the adsorption to attain a constant maximum value for both xylans is believed to arise from interactions between adsorbed xylan and xylan molecules in the solution that can lead to multilayer formation.

Atomic force microscopy (AFM) visualized the surfaces of xylan layers and all of them were uniform. More numerous and smaller sized globular structures were obtained from CaCl$_2$ solution and this is in accordance with the result that CaCl$_2$ solutions enhanced adsorption by forming more loops and tails on the cellulose surfaces. On the other hand, morphologies of xylan films adsorbed onto bare gold surfaces were similar to those adsorbed onto cellulose, which
suggested that cellulose did not affect the structure of xylan on the surface. The adsorbed structure likely arose from an aggregated structure of xylans in solution as previously reported by Linder, *et al.* Equally important, the uniformity of the xylan layers formed on gold crystals means that they can be used as substrate to study interactions with other plant cell wall polymers.

### 5.2 Suggestions for Future Work

For completion of the AGX/AX story, dynamic light scattering experiments of AGX and AX aggregates in solution should also be conducted. These experiments will provide insight into the relative size of aggregates in solution versus the adsorbed state.

Future work will also be based on plant cell wall materials and QCM-D, SPR and AFM technologies. Similar to work presented in Chapter 4 for the adsorption of arabinoxylans onto thin RC films, other hemicelluloses in secondary plant cell walls are also of interest. Galactoglucomannan (GGM) from spruce will be the first one studied. As mentioned previously, GGM is the predominant hemicellulose in softwood and forms tight and strong association with cellulose. Adsorption experiments carried out by QCM-D and SPR are aiming at mimicking the natural interaction between GGM and cellulose. Furthermore, in Chapter 4 we demonstrated that smooth arabinoglucuronoxylan (AGX) films formed on the QCM-D sensors. Research on the adsorption characteristics of GGM onto AGX films is interesting because both of them exist in the secondary plant cell walls of spruce and are expected to interact with each other and lignin in the plant.

Future work will also include several enzymes and their activities on adsorbed hemicellulose films. Both QCM-D and SPR will monitor the hydrolysis of AGX, AX and GGM films by mannases, xylanases, and xyloglucanases. In addition, AFM will examine films before
and after hydrolysis, thereby providing insight into how effectively the enzymes can attack tightly bound hemicelluloses.

5.3 References
