2-Dimensional Assembly of Cellulose-Based Materials

Elina Niinivaara
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Elina Niinivaara

A doctoral dissertation completed for the degree of Doctor of Science (Technology) to be defended, with the permission of the Aalto University School of Chemistry, at a public examination held in lecture hall Ke2 at the Department of Biotechnology and Chemical Technology on 8 April 2016 at 12.

Aalto University
School of Chemical Technology
Department of Forest Products Technology
Materials Chemistry of Cellulose
Abstract

The objective of this thesis was to systematically investigate the two dimensional assembly of cellulose-based materials and the two dimensional response to various external stimuli. The motivation of studying such materials is the ever increasing trend in materials science to substitute synthetic polymers for greener materials. Studies such as the one presented here are essential to understand the fundamental behaviours and characteristics of bio-based polymers and to be able to utilize them in new functional materials.

Trimethylsilyl cellulose (TMSC), cellulose triacetate (CTA), cellulose acetate propionate (CAP) and cellulose acetate butyrate (CAB) were extensively studied by means of Langmuir- Schaefer deposition. The formation of nanostructures on the surface of solid substrates as a result of monolayer transfer from the air/water interface was investigated using substrates of varying total surface free energy. It was established that for TMSC, a decrease in substrate surface energy resulted in progressive dewetting of the transferred film, which eventually led to the formation of dendritic fractals. A similar pattern was not detected in the films of CTA, CAP and CAB however, it was found that the acetate and residual hydroxyl group content of the cellulose esters played a key role in the morphologies of the ultrathin films. The morphology of the cellulose derivative films could be tuned by regeneration, exposure to water or by altering film deposition surface pressure. The behaviour of the cellulose esters upon compression at the air/water interface was also thoroughly scrutinized by monitoring surface pressure-area Langmuir isotherms with a fast and slow compression. Results revealed the behavioural differences in monolayer assembly as a function of compression rate.

The intricate relationship between cellulose-based materials and water was also studied using cellulose in its native form. The water vapour sorption behaviours of ultrathin films of cellulose nanocrystals (CNCs) and regenerated amorphous cellulose (from TMSC) along with films with a combination of both were studied using a quartz crystal microbalance with dissipation monitoring (QCM-D) and spectroscopic ellipsometry (SE). Quantitative analysis of the results showed that hydration of CNC networks occurs through the envelopment of the individual crystals by three monolayers of water. The water vapour response of the cellulose films became unexpectedly complex when CNCs were mixed with amorphous cellulose. Relative humidity studies showed that the crystalline/amorphous ratio of films containing both types of cellulose played a critical role in water vapour adsorption. Adsorption in films with a similar ratio to that prevalent in the woody plant cell wall (~50/50) was promoted by the addition of CNCs, whereas in predominantly amorphous films it was inhibited.

Keywords cellulose derivatives, self-assembly, cellulose nanocrystals, LS-deposition, ellipsometry, QCM-D
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Elina Niinivaara

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Preface

This doctoral dissertation was carried out at the Department of Forest Products Technology of the School of Chemical Technology of Aalto University from 2012 to 2016 under the supervision of Prof. Eero Kontturi and was funded by the Aalto Starting Grant (917500). Funding was also received from the Emil Aaltonen Foundation for a research visit to Collège de France, Paris.

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And finally, my husband Joonas. Without you my world would crumble, you are my best friend, my rock and my inspiration. Thank you for always keeping my head above water and selflessly taking care of our little family. Your unwavering belief in me is my best source of strength. Jag älskar dig.

Helsinki, February 22nd, 2016

Eli
List of Publications

This doctoral dissertation consists of a summary and of the following publications which are referred to in the text by their Roman numerals


**Paper IV.** Niinivaara, Elina; Faustini, Marco; Tammelin, Tekla; Kontturi, Eero (2016) Mimicking the humidity response of the plant cell wall by using two-dimensional systems: the critical role of amorphous and crystalline polysaccharides. *Langmuir*, 10.1021/acs.langmuir.5b04264.
Author’s Contribution

**Paper I:** Elina Niinivaara was responsible for the experimental design, performed the main part of the experimental work, analysed the corresponding results and wrote the manuscripts. XPS and SEC measurements were performed by others.

**Paper II:** Elina Niinivaara was responsible for the experimental design, performed the experimental work, analysed the corresponding results and wrote the manuscripts. NMR measurements were performed by co-author.

**Paper III:** Elina Niinivaara was responsible for the experimental design, performed the experimental work, analysed the corresponding results and wrote the manuscripts.

**Paper IV:** Elina Niinivaara was responsible for the experimental design, performed the main part of the experimental work, analysed the corresponding results and wrote the manuscripts. SEC measurements were performed by others.
# List of Abbreviations and Symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ao</td>
<td>Limiting area of polymer</td>
</tr>
<tr>
<td>AGU</td>
<td>β-D-anhydroglucopyranose unit</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>CA</td>
<td>cellulose acetate</td>
</tr>
<tr>
<td>CAB</td>
<td>cellulose acetate butyrate</td>
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<tr>
<td>CAP</td>
<td>cellulose acetate propionate</td>
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<tr>
<td>CNC</td>
<td>cellulose nanocrystals</td>
</tr>
<tr>
<td>CTA</td>
<td>cellulose triacetate</td>
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<tr>
<td>DLA</td>
<td>diffusion-limited aggregation</td>
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<tr>
<td>DS</td>
<td>degree of substitution</td>
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<tr>
<td>DP</td>
<td>degree of polymerization</td>
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<tr>
<td>GIXRD</td>
<td>grazing incidence x-ray diffraction</td>
</tr>
<tr>
<td>HOPG</td>
<td>highly ordered pyrolytic graphite</td>
</tr>
<tr>
<td>LB</td>
<td>Langmuir-Blodgett deposition</td>
</tr>
<tr>
<td>LS</td>
<td>Langmuir-Schaefer deposition</td>
</tr>
<tr>
<td>NFC</td>
<td>nanofibrillated cellulose</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance spectroscopy</td>
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<tr>
<td>PA-IR</td>
<td>photoacoustic infrared spectroscopy</td>
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<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>SE</td>
<td>spectroscopic ellipsometry</td>
</tr>
<tr>
<td>SEC</td>
<td>size exclusion chromatography</td>
</tr>
<tr>
<td>SWE</td>
<td>single-wavelength spectroscopy</td>
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<tr>
<td>TMSC</td>
<td>trimethylsilyl cellulose</td>
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<tr>
<td>QCM-D</td>
<td>quartz crystal microbalance with dissipation monitoring</td>
</tr>
<tr>
<td>XPS</td>
<td>x-ray photoelectron spectroscopy</td>
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<tr>
<td>π</td>
<td>surface pressure</td>
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"Life is like topography, Hobbes. There are summits of happiness and success, flat stretches of boring routine and valleys of frustration and failure."

- Calvin and Hobbes
1. Introduction

Throughout recent history, cellulose has gained a great deal of attention in both science and industry as its worldwide abundance makes it an ideal biopolymer to study and utilise. Industrial applications of cellulose in both the macro- and nanoscale are vast and varying and as of late, its use in the production of bioethanol has also gained a fair amount of interest (for a review see Stöcker 2008 and references cited therein). In addition, as more materials scientists focus their attention on bio-based materials, the use of cellulose in many different areas of research is becoming increasingly relevant. One such area is that of ultrathin films; systems which can be used, for example as chemical and biological sensors (Stewart et al. 2008), transistors (Mitzi et al. 2004), super conductors (Zhang et al. 2010) and antireflective coatings (Walheim et al. 1999) with common synthetic polymers, to name a few.

The objective of the research carried out in this doctoral thesis is the two dimensional assembly of cellulose-based materials. Two dimensional assembly, in this context, involves the formation of ultrathin cellulose films on solid substrates using well established thin film preparation techniques. This research aims, not only to produce a systematic analysis of the naturally occurring self-assembly patterns of cellulose-based materials, but to also study the interactions of these materials occurring as the result of varying external stimuli (Figure 1). Identifying and examining these phenomena will provide a fundamental understanding on cellulose-based materials that can then be utilized in the design of new functional materials and provide a clearer understanding on the naturally occurring assemblies, structures and responses of cellulose in the woody plant cell wall. As a generalized overview of the main focus points of each of the four papers introduced in this thesis, Paper I and II mainly investigate the two dimensional assembly of various cellulose derivatives whereas Paper III and IV focus on the response of native cellulose to external stimulus, namely water vapour.
**Papers I and II** of this thesis focused on the surface induced two dimensional assembly of monomolecular layers of cellulose derivatives when transferred from the air/water interface onto a solid substrate using horizontal Langmuir-Blodgett (also known as Langmuir-Schaefer) deposition. In **Paper I**, monolayers of trimethylsilyl cellulose (TMSC) were deposited on to four substrate surfaces, each with a different total surface free energy. It was shown that the surface energy of the substrate greatly affected the morphology of the deposited monomolecular film. Most interestingly, when deposited on methylated silica, TMSC formed fractal-like structures leading to a previously unreported discovery. Tuning of the dimensions of these structures was carried out successfully through the regeneration of TMSC back to cellulose and the subsequent immersion of the cellulose film in water. The work in **Paper II** provides a direct follow up on Paper I as it introduces cellulose ester monolayers deposited on the same four substrates. The morphologies of these ultrathin films were also found to be substrate surface energy dependent. However, transfer from the air/water interface to the substrate was considerably different for the cellulose esters than for TMSC. The behaviour of the cellulose esters at the air/water interface was also comprehensively studied. Compression isotherms carried out with both a fast and a slow compression rate showed that each polysaccharide assembled in a unique manner when compressed.
Where the first two papers of this thesis concentrate on cellulose derivatives and introduce their complex relationships in regard to external parameters, including water, the remaining papers broaden the spectrum of the materials investigated to native crystalline cellulose, and focus on the water vapour uptake capability of both fully crystalline and semicrystalline model ultrathin films. In **Paper III** the water vapour adsorption of pure cellulose nanocrystal (CNC) networks was studied. CNCs provide an interesting platform for such studies as the crystals are impenetrable by water. Analysis of water vapour adsorption data revealed that hydration of the CNC network occurs through the envelopment of each individual crystal in a few monolayers of water, rather than through the wetting of the entire film. As a direct continuation to this work, in **Paper IV** the swelling behaviour of model films comprising of varying ratios of crystalline and amorphous cellulose components were studied; compositions varied from being either fully amorphous, similar in nature to that of the woody plant cell wall and then to predominantly amorphous. The systems studied focused on the naturally occurring hierarchical structure of cellulose, hemicellulose and lignin in the woody plant cell wall while maintaining chemical homogeneity between the two different phases. This setup allowed for the systematic analysis of the role of only the crystalline/amorphous ratio by eliminating any possible behavioural patterns caused by chemical differences, whilst reducing the hierarchical structure to the two dimensional realm of ultrathin films. It was shown that the swelling properties of the films were critically dependent on both the crystalline/amorphous ratio and the extent to which the two components entwined in the composite film. Swelling in the woody plant cell wall –like systems was promoted by the presence of the crystalline component whereas in the predominantly amorphous systems it was inhibited. Promotion of swelling in the woody plant cell wall –like systems was concluded to be caused by an excess of water vapour adsorption at the crystalline/amorphous interface.
2. Background

2.1 Plant cell wall

Due to its inexhaustibility and exceptional design, the plant cell wall has received ample amounts of research attention. Not only does it provide woody plants with excellent load bearing capabilities, it also allows them to withstand the harshest of environments. Despite extensive research, the structure of the plant cell wall still remains a popular topic, as scientists have yet to reach a consensus on its exact structure. It is, however, generally accepted that the woody plant cell wall can be divided into three main components: the middle lamella, the primary cell wall and the secondary cell wall, which is made up of three separate layers (S1, S2 and S3 layers) (Sjöström 1981). A distinction must be made between the cell wall structure of woody and herbaceous plants. Herbaceous plants are ones which often have no woody stem above the ground and do not require the same structural support as woody plants. As a result, the cell walls of these two plant types differ from one another – where the woody plant cell wall is composed of the aforementioned components, the cell wall of herbaceous plants consists of only the middle lamella and primary cell wall. However, for the sake of simplicity, from this point forth in this thesis, the woody plant cell wall will be referred to as the plant cell wall and no further mention of herbaceous plants will be made.

Cellulose, hemicellulose and lignin, the plant cell wall biopolymers, make up the majority of the composition of both the primary and secondary plant cell walls and coexist as a composite structure in which semicrystalline cellulose microfibrils are embedded into a network of lignin and hemicellulose (Figure 2) (Daniel 2007). Thickness wise, the secondary cell wall makes up the bulk of the plant cell wall whereas the primary cell wall is so thin that it is often considered in conjunction with the middle lamella as they are particularly difficult to distinguish from one another during structural analysis; together the primary
cell wall and the middle lamella are referred to as the compound middle lamella (Sjöström 1981).

The structural aspect of the plant cell wall which remains a topic of controversy is the orientation of the cellulosic elementary fibrils in the secondary cell wall; in the primary cell wall they are randomly arranged in a network-like structure (Sjöström 1981). Debate on this topic arises from the fact that this orientation differs between plant species and is also dependent on the technique used for analysis (Reza et al. 2014). In each of the three secondary cell wall layers, the elementary cellulose fibrils orientate at a different angle known as the microfibril angle as shown in Figure 2. The orientation of the microfibrils within the primary and secondary cell wall determine the majority of the physical properties of the plant itself, and as such is of utmost importance. The network structure of the primary cell wall microfibrils is believed to have evolved due to the fact that such a structure allows for more flexibility at the onset of the development of the secondary cell wall during cell growth (Sjöström 1981). The crossed structure of the secondary cell wall layers on the other hand is essential in providing the plant with the necessary axial stiffness to carry the weight of the growing plant whilst providing the plant cells with excellent collapse and burst resistance (Donaldson 2008).

Figure 2. Schematic representation of the layered structure of the woody plant cell wall and the composite structure of the primary and secondary cell wall biopolymers; cellulose microfibrils, hemicellulose and lignin.

Along with investigating the fundamental self-assembly patterns of cellulose and its derivatives in response to varying external stimuli, this thesis also focuses on the water vapour response of cellulosic systems. The natural ability of plant cells to retain their shape and integrity when exposed to water or water
vapour is a phenomenon which merits thorough exploration, an undertaking which has been performed in the latter part of this thesis.

Together, hemicellulose and lignin make up approximately 60% of the plant cell wall (Fengal and Wegener 1989, Hill et al. 2009) and for the sake of this thesis, represent the dissipative amorphous matrix within which cellulose is embedded. Hemicellulose is a heteropolysaccharide, the composition of which is wood species dependent. In softwood species hemicellulose consists of galactoglucomannans and arabinoglucuronoxylan whereas the corresponding units of hardwood species are glucomannan and glucuronoxylan. Lignin, on the other hand, is a polyphenolic polymer made up of coniferyl, sinapyl and coumaryl alcohols in varying proportions depending on the source of the lignin (Sjöström 1981). As an in-depth analysis of the structure of neither hemicellulose nor lignin is essential for this thesis, for more information on the subject the reader is advised to refer to Scheller and Ulvskov (2010) and Ralph et al. (2007).

2.2 Cellulose

Despite the almost ubiquitous presence of cellulose in everyday applications – paper and board, foodstuffs, buildings, pharmaceuticals and clothing – efforts are continuously being put forth to develop new ways by which to further exploit the its benefits. One of the allures of cellulose in both fundamental research and applications lies in the fact that not only is it predominant throughout the world, it is an easy to attain renewable resource that is biodegradable, biocompatible and non-toxic.

Cellulose is the main building block and structural component of wood, it is a linear homopolysaccharide made up of repeating units of β-D-anhydroglucopyranose units (AGUs), linked together by (1→4)-glycosidic bonds with hydroxyl groups at the C-2, C-3 and C-6 positions. The base unit of cellulose is considered to consist of 2 AGUs and is referred to as cellobiose. A key feature of cellulose is the abundance of hydrogen bonds occurring both inter- and intramolecularily (Figure 3) (Roman 2009).
Figure 3. Structure of the cellulose chain showing the intermolecular (red, dashed) and intramolecular (black, dashed) hydrogen bonding.

As previously mentioned, in the plant cell wall cellulose is found in the form of cellulose microfibrils which, essentially, are assemblies of cellulose chains bound together as planar sheets by intermolecular hydrogen bonds, which are connected via interplanar van der Waals forces (Nishiyama et al. 2002) (Figure 4). The cellulose chains making up these aggregates have a varying number of cellobiose units (known as degree of polymerization (DP)) depending on the source of the cellulose material.

Figure 4. Schematic representation of the cross-sectional, unit cell and top views of the crystalline structure and bonding of cellulose I\textsubscript{β}. (Adapted from Chundawat et al. 2011)

Independent of source, however, cellulose microfibrils exhibit regions of order (crystalline cellulose) and disorder (‘amorphous’ cellulose) (Klemm 1998). The structure of the cellulose microfibril is often alluded to using the fringed-fibrillar model, in which long stretches of ordered cellulose are disrupted by disordered regions (Mark 1940, Hearle 1963, Scallan 1971, Nishiyama 2003, Klemm 2005) (Figure 5) though it has been reported that these regions of disorder account for as little as 1% of the microfibril structure and are typically only 4 – 5 AGUs in length (Nishiyama 2003). Regardless, cellulose can adopt one of three
polymorphic forms, cellulose I, II or III. However, in the case of native cellulose, only cellulose I is relevant. Cellulose I exists in two different forms, $I_{\alpha}$ and $I_{\beta}$, which although coexist are present in varying ratios depending on the cellulose source, in bacterial and valonia, for example, cellulose $I_{\alpha}$ is the predominant forms whereas wood and plant celluloses are typically cellulose $I_{\beta}$ (Atalla and Vanderhart 1984, Horikawa and Sugiyama 2009).

![Figure 5. Exaggerated schematic representation of the fringed fibrillar model of cellulose chains with regions of highly ordered (crystalline) cellulose and regions of disorder (amorphous cellulose).](image)

### 2.2.1 Cellulose derivatives

The ordered supramolecular structure induced insolubility of cellulose in its natural form causes distinct difficulties in its utilization and as such its structure must be modified in order to render it more easily soluble. The derivatization of cellulose is a viable option with which to achieve this; by substituting the hydrogens of the AGU hydroxyl groups with functional groups such as esters (such as cellulose acetate) and ethers (such as carboxymethyl cellulose), it is possible to disrupt the hydrogen bonding network and attain derivatives that are soluble in certain common solvents such as water, acetone, toluene and chloroform (Klemm et al. 1998).

On an industrial scale, derivatization of cellulose is typically carried out through a heterogeneous modification in which functionalization reactions are performed on cellulose either in the solid state or when swollen (Klemm et al. 1998). Homogeneous derivatization is also possible to perform on cellulose in the dissolved state and can be carried out in one of two ways: i) cellulose is either dissolved into a derivatizing solvent where it is modified in situ or ii) it is first dissolved into a non-derivatizing solvent, which dissolves the cellulose only through physical interactions, and then transformed through substitution reactions in its dissolved state (Klemm et al. 1998, Heinze and Liebert 2004). Examples of a derivatizing and a non-derivatizing solvent are
dimethylformamide (DMF)/ nitrogen tetroxide (N₂O₄) and dimethylacetamide (DMA)/lithium chloride (LiCl), respectively. Whichever way derivatization is carried out, the complete dissolution of the cellulose raw material is essential in ensuring substitution occurs homogenously along the entire cellulose chain. Success of the substitution reaction is measured by the final degree of substitution (DS) of the cellulose derivative. DS can range from < 0 to 3 and is a measure of the average number of hydrogens of the 3 hydroxyl groups that have been substituted during derivatization. However, the complete functionalization of all of the hydroxyl groups is a rare occurrence and cellulose derivatives often contain a number of residual hydroxyl groups (Klemm et al. 1998).

The use of cellulose derivatives dates back to as far as the 1830s when the first variant of the explosive cellulose nitrate was discovered (Braconnot 1833). Since then, the development of new types of modifications has been a continuous focus in cellulose research and has led to the discovery of now commonly used derivatives like cellulose acetate. Cellulose acetates are not only used in fundamental research but have also been utilized in a variety of applications including textiles, cigarette filters, injection-moulded objects, display packaging and extruded plastic film, dialysis membranes, sheeting, lacquers, protective coatings and protective films in liquid crystal displays (Roman 2009, Zugenmaier 2004). In addition to their solubility and mouldability, possibly the most appealing feature of some cellulose derivatives, such as those examined in this thesis, is that they can relatively easily be regenerated back into cellulose. Regeneration not only provides a means by which to study pure cellulose systems but also allows for the manufacture of cellulose based materials, such a viscose fibres (Fink et al. 2001).

The cellulose derivatives used in this thesis were trimethylsilyl cellulose (TMSC), a cellulose silyl ether, along with cellulose triacetate (CTA), and cellulose acetate propionate (CAP) and cellulose acetate butyrate (CAB), which are cellulose esters and mixed esters, respectively (Figure 6). TMSC is typically prepared through a homogeneous modification in which purified cellulose is dissolved into a non-derivatizing solvent and then silylated (Schempp et al. 1984). In contrast, CTA can be synthesized using either a derivatizing or a non-derivatizing solvent with the acetylation step typically carried out with acetic anhydride or acetic acid (Heinze 2004). Mixed esters containing two or more different ester substituents are prepared by simultaneously deriving cellulose using two derivatization agents. In the case of
CAP, acetic anhydride/acid is mixed with propionic anhydride whereas for CAB, it is mixed with butyric anhydride (Hon 1997).

![Chemical structures of cellulose and its derivatives](image)

**Figure 6.** The chemical structures of cellulose and four of its derivatives – TMSC, CTA, CAP and CAB. (Paper II)

All of the cellulose derivatives used in this work are so-called ‘hairy-rod’ polymers, polymers which have a rigid backbone with a dense system of side chains. A characteristic property of these cellulose derivatives that supports their use in self-assembly studies like those presented here, is their amphiphilic nature (Wegner 1991). An amphiphile is a compound that contains both a hydrophilic (polar) and a hydrophobic (non-polar) moiety which, in the case of cellulose derivatives, are the glycosidic bonds, ring oxygen and ester linkages of the AGU backbone and the large hydrocarbon substituents, respectively. The extent of amphilicity of a molecule is enhanced by the increased hydrophilicity or -phobicity of the moieties; in cellulose derivatives hydrophobicity can be increased by extending the chain length of the hydrocarbon substituent and/or by increasing the degree of substitution of such moieties (Bartell and Ray 1952).

The amphiphilic nature of cellulose derivatives makes them an optimal polymer to use in thin film studies of bio-based materials. Due to the fact that cellulose derivatives are drawn to both air and water, they can easily form monomolecular layers at the air/water interface. In this thesis, the self-assembly patterns of the aforementioned cellulose derivatives are examined through the transfer of such monomolecular layers onto the surface of different solid substrates.
2.2.2 Nanoscaled cellulose

The nanoscaled cellulose that is the focus of this thesis, is cellulose nanocrystals (CNCs). CNCs were first introduced in 1951 by B.G. Rånby (Rånby 1951) when he proposed that the amorphous regions of the cellulose microfibril could be removed using a controlled acid hydrolysis, to leave intact only the crystalline regions. When prepared with sulphuric acid, an inherent property of CNCs is their anionic surface charge caused by sulphate ester groups, this surface charge provides the crystals with an electrostatic repulsion allowing them to form stable dispersions in water upon agitation through ultrasonication (Edgar and Gray 2003, Eichhorn 2011). CNCs are rod-shaped particles with a cross-sectional diameter ranging between 3 and 20 nm and lengths varying up to the micron scale. The size of CNCs is fully dependent not only on the hydrolysis conditions used but also on the origin of the raw material (Dong et al. 1998, Elazzouzi-Hafraoui et al. 2008, Eichhorn et al. 2010). Nevertheless, irrespective of raw material, all CNCs have an extremely high total surface area to volume ratio and a high aspect ratio.

CNCs exhibit a number of interesting characteristics: firstly, and most importantly for the work carried out in this thesis, CNCs – like all crystalline cellulose – are impenetrable by water (Saito et al. 2004, Kulasinski et al. 2015). Additionally, their high specific surface area and abundance of surface hydroxyl groups make them extremely susceptible to chemical modifications such as cationization, esterification, oxidation, silylation and polymer grafting, to name a few (Hasani et al. 2008, Habibi et al. 2010, Habibi 2014). Moreover, CNCs are intriguing in that they form chiral nematic liquid crystal dispersions above critical concentrations (Revol et al. 1992, Fleming et al. 2001). These liquid crystals also exhibit ordering and phase separation in the presence of an electric (Bordel et al. 2006) or magnetic field (Revol et al. 1994, Cranston and Gray 2006).

CNCs are not to be mistaken for cellulose nanofibrils (CNFs), which are a highly disintegrated fraction of cellulose first introduced by Turbak, Snyder and Sanberg in 1983 (Turbak et al. 1983). However, due to processing difficulties and low energy efficiency, CNFs did not gain widespread popularity at the time. It was not until the beginning of the 21st century, when interest in CNFs peaked as extensive research efforts introduced pre-treatments on the cellulosic material prior to disintegration (Pääkkö et al. 2007, Wågberg et al. 2008,
Saito et al. 2007) to significantly decrease the energy required to delaminate cellulose fibres and isolate nanofibrils with large specific surface areas and high aspect ratios (Ahola 2008). With the commercialization of CNFs, its research as various potential applications increased dramatically ranging from packaging (Rodionova et al. 2011) and barrier materials (Fukuzumi et al. 2009) to composites (Kim et al. 2009), electronics (Gao et al. 2013) and even to pharmaceuticals (Orelma 2012, Orelma et al. 2012a). In addition to plant-based CNFs, they also occur as the biosynthesis product of the gram-negative bacteria, *Acetobacter Xylinum* (Klemm et al. 2005).

### 2.3 Thin film model studies

With the development of soluble cellulose derivatives and cellulose dissolving solvents, it became possible to study cellulosic materials in the form of thin (< 1 μm) and ultrathin (<100 nm) films. Early works in the 1930s involving the investigation of cellulose model thin films mainly centred on contact angle measurements (Roman 2009) – measurements in which the wettability of surfaces is determined via the spreadability of a liquid at the solid/liquid/air interface. It was not until the 1980s that thin film model studies took on the popularity they have until this day. A pivotal point in these studies was the commercialization of surface analytical techniques like atomic force microscopy (AFM) (in the 1990s), ellipsometry, reflectometry and quartz crystal microgravimetry (in the 2000s), all of which are in constant use in today’s thin film model studies (Roman 2009). These techniques allowed for *in- and ex-situ* observations of phenomena occurring at the micro- and nanoscale to be carried out and have indeed provided the materials science community with invaluable information on the behavioural patterns of model thin films. A distinction must be made as to what constitutes a model thin film – a model thin film is one which reflects upon the fundamental behavioural patterns of a material in its bulk whereas studies involving more generalized investigations of material interactions are not considered model films.

With trends leaning toward greener chemistries, the use of bio-based polymers to substitute synthetic materials in thin film research has become more popular and as a result, research on cellulose thin films has also rapidly increased (for a review see Kontturi et al. 2006 and references cited therein). In general,
the attractiveness of model thin film research is the result of several factors. First and foremost, as mentioned, they have clearly defined chemistries and morphologies; these properties allow for thorough investigations into the properties of materials whilst eliminating the possible effects of the bulk materials. Additionally, they allow for the study of single component systems, as isolation of such materials from multicomponent systems often causes changes in the physical and even chemical properties of the material. Thin films in general, are also interesting systems in which the study of the interactions between different polymers is possible, for example, as polymer blend films (Kontturi et al. 2005, Nyfors et al. 2009, Taajamaa et al. 2011, Taajamaa et al. 2013). As well as being an excellent tool for fundamental research, thin films are also used in a variety of different applications which, as mentioned previously, include chemical and biological sensors (Stewart et al. 2008), transistors (Mitzi et al. 2004), super conductors (Zhang et al. 2010) and antireflective coatings (Walheim et al. 1999).

The substitution of synthetic materials with biopolymers in thin films naturally has both advantages and challenges. Bio-based materials are not only renewable, biodegradable and biocompatible, their intriguing structures provide an abundance of possibilities for materials science. Nevertheless, because of ever changing atmospheric conditions plants must adjust in order to survive, resulting in heterogeneous and somewhat unpredictable raw materials with which to work. Cellulose as a raw material in particular presents these kinds of challenges although they may, to some extent, be circumvented with the use of easily soluble cellulose derivatives and meticulous sample preparation.

As mentioned, in the work presented here, cellulose ultrathin films were used to examine the self-assembly patterns of cellulose derivative monolayers when transferred from the air/water interface onto the surface of solid substrates and to examine the water vapour response of native cellulose systems.
2.4 Self-assembly

Self-assembly is the process by which molecules or parts of molecules spontaneously form ordered supramolecular architectures that are governed by the structure of the molecules themselves. The driving force of self-assembly is thermodynamics, where molecules arrange themselves from an unstable form to form structures in the equilibrium state, or at least in the metastable state (Whitesides and Boncheva 2002). Self-assembly is mediated by van der Waals forces, electrostatic interactions (ionic bonds), hydrophobic interactions and hydrogen bonds, which are all weak and non-covalent interactions. Although as singular components, these interactions are extremely weak, their combined effects govern the formation of all biological macromolecules and influence the interactions occurring between molecules as well (Morris et al. 2004). In order for supramolecular architectures to occur, a balance must exist between the attractive and repulsive interactions that are at play. Additionally, the resulting association must be reversible in order to allow the components of the structure to re-order themselves once the structure has been formed (Whitesides and Boncheva 2002).

Self-assembled structures are all pervasive and as such play perhaps one of the most crucial roles in sustaining life. Additionally, these structures are also of utmost interest in the world of materials science as self-assembly phenomena have facilitated the development of technologies such as liquid crystal displays (for a review see Hoogboom et al. 2007 and references cited therein) and slow release drugs (Bulut et al. 2011). Nevertheless, in order to be able to utilize these phenomena, they must first be understood on a fundamental level, which makes work like the one presented here an essential stage in the raw material to end-use product process.

2.4.1 Interactions of cellulose-based polysaccharides

The interaction forces occurring between cellulose-based polysaccharides can be divided into DLVO and non-DLVO forces. DLVO, Derjaguin-Landau-Verwey-Overbeek, forces include attractive van der Waals forces and repulsive electrostatic forces, which interact in unison through the DLVO theory. The DLVO theory describes the combined effects of these two opposite interactions
when two bodies are brought into close proximity with each other (Figure 7) (Derjaguin and Landau 1941, Verwey and Overbeek 1948). The non-DLVO forces present in these systems include hydrophobic interactions and hydrogen bonds (introduced in Section 2.2).

![Diagram of DLVO theory](image)

**Figure 7.** Schematic graph representing the DLVO theory in which the total interaction force is the sum of the attractive van der Waals forces and repulsive electrostatic forces occurring between two similarly charged bodies.

*Van der Waals forces and electrostatic interactions*

Van der Waals (vdW) forces are omnipresent between all polarizable matter and predominantly act as an attractive interaction (Farinato et al. 1999). Their three main sources are: i) dipole-dipole interactions in which permanent dipoles orient themselves to form an attraction, ii) dipole-dipole induced interactions in which a permanent dipole induces a dipole polarization in another molecule and iii) the formation of attractive London dispersion forces as the result of simultaneous fluctuations in the distribution charges of neighbouring molecules (Barnes and Gentle 2010).

The magnitude of the vdw force between two bodies is dependent on both their Hamaker constant (A) and their geometries (Israelachvili 1992). In order to attain an accurate estimate of A, it must be calculated according to the Lifshitz theory (1956). This theory takes into consideration the possible effects of any
neighbouring surfaces, the medium through which the bodies interact and temperature. With an accurate \( A \), the total vDW forces acting between the surfaces can then be calculated using the Hamaker theory, in which the interaction energy between the atoms making up the bodies can be considered additive (Hamaker 1937). The geometries of the two interacting bodies must also be taken into account in the vDW force calculations as they play a key role in the size of the interaction. In this thesis, the two relevant geometries considered are those of two cylinders interacting in parallel and two cylinders interacting at a 90° angle to one another. The vDW force energies for these geometries can be calculated as follows (Israelachvili 1992):

Two parallel cylinders:

\[
V_{\text{vdW}}\text{ interaction energy} = \frac{-A}{12\sqrt{2}D^2} \left( \frac{R_1R_2}{R_1+R_2} \right)^{\frac{1}{2}}
\]  

(2.1)

Two cylinders at 90°:

\[
V_{\text{vdW}}\text{ interaction energy} = \frac{-A\sqrt{R_1R_2}}{6D}
\]  

(2.2)

where \( A \) is the Hamaker constant (1.1 \( \times \) 10\(^{-20} \) J for cellulose nanocrystals (Boluk et al. 2011)), \( R_1 \) and \( R_2 \) are the radii of the cylinders and \( D \) is the distance between them.

To counter balance the attractive vDW forces, similarly charged bodies also interact through repulsive electrostatic forces when in a liquid medium. These forces are the result of ions of the opposite charge to the body (counterions) present in the liquid medium; the counterions collect at the surface of the charged body and form an electric double layer consisting of a Stern layer in which counterions are bound to the surface and a diffuse layer in which they are free but in close proximity to the surface. When brought into sufficiently close contact, the electric double layers of two charges bodies will overlap causing osmotic pressure which amounts to repulsion. The magnitude of this force is dependent on the thickness of the electric double layer (Debye length), which is in turn governed by the charge of the body and the ionic strength of its surrounding medium (Derjaguin et al. 1987).
Hydrophobic interactions

Hydrophobic interactions occur when two non-polar molecules encounter one another in water or at a hydrophilic interface. As these types of molecules are not able to form hydrogen bonds, when exposed to a hydrophilic component they will be entropically driven to aggregate in order to decrease the number of molecule/hydrophilic component interactions occurring (Barnes and Gentle 2010).

In this thesis, hydrophobic interactions are utilized to study the self-assembly patterns in cellulose-based polysaccharide thin films induced by the surface properties of the solid substrate. The transfer of Langmuir monolayers from the air/water interface to the surface of a substrate causes a shift in the environment of the polymer and as a result it will re-assemble on the substrate in the most energetically favourable fashion possible. Using substrates of differing total surface energy (or, roughly speaking, hydrophilicity and -phobicity), it is possible to attain surface specific polysaccharide nanostructures.

2.5 Water vapour uptake of cellulose ultrathin films

Swelling of cellulose is an essential part of its isolation, dissolution and derivatization. In the presence of both liquid water and water vapour, cellulose swells significantly but, due to the microfibril orientation along the fibre axis, changes in fibril dimensions only occur in the transverse direction (Klemm et al. 1998). Reportedly, water and water vapour uptake of cellulose microfibrils occurs on three detectable levels, on the surface of the fibrils (tightly bound water) and in both the micro- and macropores of the plant cell wall structure (Aarne et al. 2012). The adsorption of water causes both physical and chemical changes in the structure of cellulose. From a physico-chemical point-of-view, the inclusion of water into the system causes an alteration in the hydrogen bonding network due to competition in hydrogen bond formation intermolecularly and between the cellulose polymer and water molecules. These chemical changes result in irreversible changes in the physical state of cellulose when the water is removed (also known as hornification) (Jayme 1944, Nazhad

Water uptake studies of cellulose-based materials have been extensively carried out using both liquid water and water vapour (Rehfeldt and Tanaka 2003, Fält et al. 2003, Kontturi et al. 2006 and references cited therein, Kittle et al. 2011, Tammelin et al. 2015, Kulanski et al. 2015). When exposed to liquid water, saturation of the material is achieved in a matter of minutes, if not seconds and as such studies are restricted to phenomena occurring at two extremes i.e. either in the wet state or in the dry state. By studying the same systems in the presence of water vapour, it is possible to attain information on the dynamic wetting of the material and gain a better understanding of the wetting mechanisms taking place as saturation of the system with water vapour takes considerably longer than with liquid water (Figure 8).

![Figure 8. Dynamic measurement of water vapour adsorption of spruce sulphite pulp at various relative humidities (Klemm et al. 1998 (originally published in a doctoral thesis by B. Philipp (TU Dresden) in 1952)).](image)

The water uptake capability of the plant cell whilst maintaining its structural integrity, is crucial in sustaining life on Earth. This property of the plant cell is enabled by the hierarchical structure of the plant cell wall described earlier (Section 2.1) and as a result, models of its structure are of great interest to materials scientists. One of the aspects of the plant cell wall that prevents plant
cells from rupturing upon swelling is its crystalline to amorphous ratio, controlled by the proportions of crystalline cellulose microfibrils and the amorphous lignin/hemicellulose matrix. Depending on the source, the plant cell wall has a crystallinity varying between 50 and 70% (Hermans and Weidinger 1949). The inherent difference in the water vapour uptake behaviours of the crystalline and amorphous components, is clear evidence that the degree of crystallinity of the plant cell wall plays a key role in its water and water vapour adsorption behaviour.

In this thesis, this vital aspect of the plant cell wall was investigated by exposing ultrathin films with varying ratios of crystalline and amorphous cellulose to water vapour. When studying the water vapour uptake behaviour of cellulose ultrathin films, hydration on all of the three detectable levels can be compressed to the two dimensional regime.
3. Experimental

The work carried out for this thesis centred on ultrathin films prepared using several cellulose-based polysaccharides; trimethylsilyl cellulose (TMSC) (Paper I and IV), various cellulose acetates; cellulose triacetate (CTA), cellulose acetate propionate (CAP) and cellulose acetate butyrate (CAB) (Paper II) and cellulose nanocrystals (Paper III and IV). Ultrathin films were deposited on four different substrates; hydrophilic silica (Paper I – IV), methylated silica, mica and highly ordered pyrolytic graphite (HOPG) (Paper I and II).

The ultrathin films were prepared using two well-established techniques: horizontal Langmuir-Blodgett (LB) (or Langmuir-Schaefer (LS)) deposition (Paper I and II) and spin-coating (Paper III and IV)

The main surface sensitive analytical techniques used were atomic force microscopy (AFM) for imaging (Paper I – IV) and spectroscopic ellipsometry (SE) (Paper III and IV) and a quartz crystal microbalance with dissipation monitoring (QCM-D) (Paper III and IV) were used for water vapour sorption studies. The surface energies of each of the substrates used was determined by contact angle measurements (CAM) (Paper I and II)

Supplementary techniques required for the characterization of the polysaccharides used in this work included photoacoustic infrared spectroscopy (PA-IR) (Paper I and IV), x-ray photoelectron spectroscopy (XPS) (Paper I and IV), size exclusion chromatography (SEC) (Paper I and IV), nuclear magnetic resonance spectroscopy (NMR) (Paper II). Grazing incidence x-ray diffraction (GIXRD) was used to determine thin film crystallinity (Paper I)
3.1 Materials

3.1.1 Cellulose derivatives

Trimethylsilyl cellulose (TMSC) was synthesized according to a method described previously (Tammelin et al. 2006). In short, microcrystalline cellulose powder from Spruce (Paper I) or cotton (Paper IV) was solvent exchanged using methanol and dimethylacetamide (DMA) after which it was dissolved into a DMA/LiCl solvent. Silylation of the cellulose hydroxyl groups was carried out using hexamethyldisilazane (HMDS). The synthesized TMSC was then recrystallized using tetrahydrofuran (THF), filtered and thoroughly washed with pure methanol. The dried synthesis product was then characterised using IR, XPS (for degree of substitution) and SEC (for average molecular weight (MW) and polydispersity (PD)).

Three different cellulose esters were used in this work (Paper II). Cellulose triacetate (CTA) was purchased from Acros Organics (New Jersey, USA) whereas cellulose acetate propionate (CAP) and cellulose acetate butyrate (CAB) were obtained from Sigma-Aldrich (Missouri, USA); all three acetates were used as received. Nuclear magnetic resonance spectroscopy (NMR) was used to determine the DS of each polysaccharide and they were found to be 2.85, 2.76 and 2.91, respectively (the compositions of which can be seen in Table 1). The chemical structure of all of the aforementioned cellulose derivatives are shown in Figure 6 (Section 2.2.1).

<table>
<thead>
<tr>
<th></th>
<th>CTA</th>
<th>CAP</th>
<th>CAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>0.15</td>
<td>0.24</td>
<td>0.09</td>
</tr>
<tr>
<td>Acetate</td>
<td>2.85</td>
<td>0.13</td>
<td>1.94</td>
</tr>
<tr>
<td>Propionate</td>
<td>0</td>
<td>2.63</td>
<td>0</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0</td>
<td>0</td>
<td>0.97</td>
</tr>
</tbody>
</table>
3.1.2 Cellulose nanocrystals

Cellulose nanocrystals (CNCs) were prepared using a well-established method (Rånby, 1951) and used in Paper III and IV. In brief, ground Whatman filter paper was hydrolysed using a strong acid, hydrolysis was quenched by the addition of water (purified by Millipore Direct-Q® 3UV (Millipore, Molsheim, France)) to the hydrolysis mixture. The resulting suspension was centrifuged and dialyzed after which, the CNC dispersion was counter-ion exchanged by the addition of sodium hydroxide (NaOH) and freeze dried. To remove any impurities from the surface of the CNCs, they were Soxhlet extracted with ethanol for 48 h, using a method originally reported by Labet and Thielemans (2011). Using conductometric titration, the surface charge of the CNCs was found to be 0.6 e nm⁻². In the final stage, the CNCs were then dispersed into milliQ water and subjected to a 15 min ultrasonication, using a probe sonicator (Digital Sonifier, Brason Ultrasonics Corporation, USA), to ensure homogeneity throughout the sample solutions.

3.1.3 Substrates

The cellulose-based thin films were deposited onto four different substrates; hydrophilic silica, methylated silica, mica and highly ordered pyrolytic graphite (HOPG). Of these, hydrophilic silica (silicon dioxide) (Figure 9) was used in each of the four papers (Paper I – IV). Approximately 1 × 1 cm² sized thin film supports were cut from a silicon wafer (Si 100 with a native oxide top layer) provided by Okmetic (Vantaa, Finland). Thin films were also prepared on AT-cut silicon dioxide coated QCM-D sensors (Q-Sense, AB, Gothenburg, Sweden) with a fundamental resonance frequency (f₀) of 5 MHz. Prior to use, the hydrophilic silica substrates were first dusted with a N₂ gas stream before cleansed in an UV ozone cleaner (Bioforce Nanosciences Inc., California, USA) for a minimum of 15 min.
Additionally, films were deposited onto methylated silica (Figure 10a), mica (Figure 10b) and HOPG (Figure 10c) (Paper I and II). The methylated silica substrates were prepared by methylating the native oxide layer of the pristine hydrophilic silica substrates; the hydrophilic silica was exposed to a mixture of dimethyldichlorosilane and xylene for a minimum of 48 h after which they were rinsed thoroughly with pure xylene. The chemical composition of the methylated substrates was analysed using XPS and results showed that on average 93.5% of the surface carbon was bound to silicon in the form of a C-Si bond indicating almost full coverage and demonstrating the successful methylation of the pristine hydrophilic silica substrate. Mica (Ruby red mica sheets, Electron Microscopy Sciences, USA) and HOPG (Grade – ZYB, Bruker, USA) were cleaved immediately prior to each deposition to ensure a clean substrate surface.
3.2 Methods

3.2.1 Ultrathin film preparation

**Spin coating** is a film deposition technique in which a droplet of solution is placed onto a solid substrate that is then set to rotate at high speeds. Due to the shear forces caused by the spinning, the droplet spreads out onto the substrate surface forming a liquid film, which then proceeds to thin by drying and form a solid thin film on the surface of the substrate. Thinning of the liquid film is primarily governed by rapid radial liquid flow at the onset of spin coating. However, when the liquid film is sufficiently thin, evaporation comes into play, which then drives the final thinning of the film (Figure 11) (Meyerhofer, 1978). Films prepared using the spin coating technique are not in thermodynamic equilibrium but adopt a forced, metastable configuration on the surface of the substrate due to the rapid evaporation of the solvent (Böltau et al. 1998).

![Diagram of liquid film thinning during spin coating](image)

**Figure 11.** Schematic representation of liquid film thinning during the spin coating process.

In this thesis, spin coating was carried out to prepare samples for spectroscopic ellipsometry and quartz crystal microbalance with dissipation monitoring analyses using a WS-650SX-6NPP/LITE spin coater (Laurell Technologies Corporation, North Wales, PA, USA). Prior to spin coating, all substrates were thoroughly cleansed in an UV Ozone cleaner (Bioforce Nanosciences Inc., California USA) for a minimum of 15 min and rinsed twice with milliQ water.
Spin coating was carried out at 3000 rpm with an acceleration of 2130 rpm s\(^{-1}\) for ca. 60 s. The duration of spinning was determined by the time required for the disappearance of the Newtonian rings (ca. 30 s) with an additional 30 s drying time. CNCs were spin coated from water dispersions with concentrations of 5, 10 and 20 g L\(^{-1}\) after which they were placed into an 80 °C oven in order to stabilize the films (Edgar and Gray 2003). For the two layered cellulose thin film systems, 10 or 20 g L\(^{-1}\) TMSC solutions (from toluene) were spin coated directly on top of the CNC layer. The CNC/TMSC films were then regenerated to cellulose through an acid vapour hydrolysis (Figure 12) (Schaub et al. 1993). For the SE measurements, samples were spin coated onto pre-cut 2 × 2 cm\(^2\) pieces of silica wafer whereas for the QCM-D measurements they were prepared onto purchased AT-cut silicon dioxide coated QCM-D sensors.

![Image](image.png)

Figure 12. Schematic representation of the acid vapour hydrolysis of TMSC to regenerated cellulose along with a schematic of film thickness decrease upon regeneration.

**Horizontal Langmuir-Blodgett (LB) deposition**, also known as Langmuir-Schaefer (LS) deposition, was first introduced by Langmuir and Schaefer in 1938 (Langmuir and Schaefer 1938) and has since become a standard method by which to deposit amphiphilic polymer/surfactant monolayers from the air/water interface onto the surface of a solid substrate (Figure 13).

The LS setup consists of a Langmuir trough filled with the water phase, two barriers with which to compress the polymer monolayer, a Wilhelmy plate to monitor surface pressure and a dipping mechanism to lower the substrate into contact with the monolayer at the air/water interface. Deposition was carried out by first spreading the corresponding 0.5 mg ml\(^{-1}\) cellulose derivative (TMSC, CTA, CAB or CAP) in chloroform on the surface of the water phase. Once
spread, the solvent was allowed to evaporate for 15 min after which the barriers were brought in toward one another at a rate of 5 mm min$^{-1}$ in order to pack the polymer molecules closer together and form a monolayer. The monolayer was compressed until the required surface pressure ($\pi$) – 5, 10 or 15 mN m$^{-1}$ – was achieved. The monolayer was then allowed to stabilize for another 15 min after which film transfer was carried out. The substrate was lowered, parallel to the water surface, into contact with the water surface at a rate of 5 mm min$^{-1}$ and left to maintain contact for 30 s. Upon contact, the monolayer was transferred onto the substrate surface and removed from the air/water interface simultaneously with the substrate; the substrate was lifted from the water surface at a rate of 5 mm min$^{-1}$. In order to maintain a uniform monolayer structure and a constant surface pressure throughout film deposition, the barriers continuously fluctuated back and forth at a rate of 1 mm min$^{-1}$. In order to ensure accurate monitoring of surface pressure, the Wilhelmy plate was burnt prior to each measurement to remove any impurities. The Langmuir set up used in this thesis was a KSV minitrough by KSV Instruments (Helsinki, Finland). As with the spin coated films, the LS deposited TMSC films were regenerated back to cellulose after film preparation was complete.

![Figure 13. Schematic representation of the different stages of horizontal LB (also known as LS) deposition.](image)
3.2.2 Total surface free energy

The total surface free energy of the four substrates were measured using the Fowkes' method (Fowkes 1964). Surface energy of a solid can be considered as analogous to the surface tension of a liquid. Energetically, the presence of surfaces must be less favourable than the material existing in the bulk in order to prevent sublimation. The excess energy at the surface in comparison to the bulk is the total surface free energy of the material.

The Fowkes’ method of determining total surface free energy divides it into two components; the dispersive component (\(\gamma^d\)) and the polar component (\(\gamma^p\)) which can be loosely regarded as the hydrophobic and hydrophilic components, respectively. Using a geometric mean approach, the contributions of both components can be combined using Young’s equation to provide the Fowkes’ equation for surface energy (Equation 3.1):

\[
\gamma_l(1 + \cos \theta) = 2 \left( \sqrt{\frac{\gamma_{lp}}{\gamma_{ls}}} + \sqrt{\frac{\gamma_{ld}}{\gamma_{ls}}} \right)
\]

(3.1)

Where \(\theta\) is the equilibrium contact angle and \(\gamma\) is the surface tension for the liquid, \(l\), and solid, \(s\). Simplified, the total surface free energy then becomes

\[
\gamma_s = \gamma_{sp} + \gamma_{sd}
\]

(3.2)

In order to attain data to calculate the total surface free energies, the contact angles of the substrates must be measured using a variety of different probe liquids. In this thesis, four probe liquids were used; water, diiodomethane, formamide and ethylene glycol (Table 2). The contact angle of a surface is the angle at which equilibrium is achieved between the liquid, air and solid phases at the three-phase contact point (Figure 14).
Advancing contact angle measurements were carried out using a CAM 200 contact angle goniometer (KSV Ltd., Helsinki, Finland). Contact angles were measured twice per probe liquid per substrate in order to ensure repeatability. The total surface free energies were calculated using the software provided by the manufacturer.

<table>
<thead>
<tr>
<th></th>
<th>$\gamma_{\text{tot}}$</th>
<th>$\gamma^d$</th>
<th>$\gamma^+$</th>
<th>$\gamma^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene Glycol</td>
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<td>47.00</td>
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<tr>
<td>Diododmethane</td>
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<tr>
<td>Formamide</td>
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<td>39.00</td>
<td>2.28</td>
<td>38.10</td>
</tr>
<tr>
<td>Water</td>
<td>72.80</td>
<td>21.80</td>
<td>25.50</td>
<td>25.50</td>
</tr>
</tbody>
</table>

Table 2. Surface tension values for probe liquids used to determine total surface free energy. Surface tension ($\gamma_{\text{tot}}$) values are further divided into their individual components; $\gamma^d$ the dispersive component and the Lewis acid ($\gamma^+$) and Lewis base ($\gamma^-$) components of the polar component, $\gamma^p$ (Volpe and Siboni 2000). (Paper I Supporting Information)
3.2.3 Atomic force microscopy

Atomic force microscopy (AFM), first introduced in 1986 by Binnig and co-workers, is a scanning probe microscopy technique which can be used to measure a variety of surface properties such as height, friction and magnetism by detecting the forces present between a nanoscaled probe and a surface (Binnig et al. 1986).

In the AFM, a laser beam is focused onto the end of a flexible cantilever with its tip either in close or direct contact with the surface of the sample. The laser beam is reflected off the top of the cantilever and detected by a quadrant photodiode. This detection causes a voltage, which is fed to a piezoelectric scanner (Figure 15); it is this feed-back loop between the photodiode and scanner which is the basis of AFM imaging. Whilst scanning the surface of the sample, the cantilever will deflect due to forces acting between the tip of the cantilever and the surface. The magnitude of this deflection (d) is governed by the strength of the force (F) and the spring constant (κ) of the cantilever through Hooke’s law,

\[ F = \kappa d \]  \hspace{1cm} (3.3)

Deflections of the cantilever, with respect to the sample surface, cause the reflected laser beam to be detected in varying regions within the quadrants of the photodiode. The collected light causes a voltage which is interpreted by both the imaging software, which produces the actual image, and the piezoelectric scanner, which controls the movements of the sample in the x, y and z planes. Due to the high sensitivity of the cantilever and the subnanometer precision of the piezoelectric scanner, the vertical resolution of the AFM is ca. 0.1 nm however, due to tip convolution the lateral resolution is only 30 nm.

AFM imaging can be carried out either in non-contact, contact or tapping mode. The AFM measurements performed for the work in this thesis, were all carried out in tapping mode where the tip oscillates up and down coming into contact with the surface when at its lowest point. The microscope used in this thesis was a MultiMode 8 Scanning Probe Microscope from Bruker AXS Inc. (Wisconsin, USA). Images were taken with an E scanner using NSC15/AlBS
silicon cantilevers from Ultrasharp μmasch (Tallinn, Estonia), which has a radius of curvature less than 10 nm and a resonance frequency of 325 kHz (information supplied by manufacturer). A minimum of 4 images were taken of each sample to assure repeatability. Images were analysed using Nanoscope Analysis 1.50 (Bruker Corporation) and Scanning Probe Image Processor (SPIP) 6.0.6 (Image Metrology, Lyngby, Denmark) softwares. Other than a simple flattening procedure, no image processing was carried out.

Figure 15. Schematic representation of the theory behind AFM in tapping mode.

3.2.4 Quartz crystal microgravimetry

In this work, the water vapour sorption behaviour of cellulose thin films was studied using a quartz crystal microbalance with dissipation monitoring (QCM-D). QCM-D measurements provide information on the changes of both the mass of the sample studied and its viscoelastic properties. The technique is based on the piezoelectric properties of the quartz crystal substrate; when a voltage is passed through the quartz crystal sensor it causes it to oscillate at a specific frequency (f), when mass is added to the sample, the frequency of oscillation decreases due to the Sauerbrey relationship (Sauerbrey 1959),
\( \Delta m = -C \cdot \frac{\Delta f}{n} \)  

where \( \Delta f = f - f_0 \) is the resonance frequency (where \( f \) is the measured frequency and \( f_0 \) the fundamental frequency of the sensor), \( n \) is the measurement overtone number (\( n = 1, 3, 5, 7... \)) and \( C \) is a constant which describes the sensitivity of the device to changes in mass (\( C \approx 0.177 \text{ mg m}^{-2} \text{ Hz}^{-1} \)). It should be noted though, that the Saurbrey relationship only holds true for rigid, uniformly covered thin films with a mass sufficiently less than the sensor itself.

On the other hand, when the voltage is cut off from the sensor, the oscillation of the crystal gradually decreases and eventually the sensor stops resonating. The energy lost from the system at this time is measured as the dissipation (D) of the substrate:

\[ D = \frac{E_{\text{Lost}}}{2\pi E_{\text{Stored}}} \]  

where \( E_{\text{Lost}} \) is the total energy dissipated during one oscillation cycle and \( E_{\text{Stored}} \) is the total energy. The measure of D provides information on the changes in the viscoelastic properties of the sample i.e. the more pronounced \( \Delta D \), the more viscoelastic the material.

The QCM-D device used in this thesis was specially equipped with a humidity chamber (QHM 401 humidity module). This involved the passing of a saturated salt solution with a given vapor pressure over a semi-permeable membrane placed above the sample and allowed the accurate control of the relative humidity (RH) that the thin film sample was exposed to (Figure 16).
Measurements were carried out by first determining the initial areal mass of each spin coated cellulose system using a method described by Peresin et al. (Peresin et al. 2012), in which the frequency responses in air (normal atmospheric conditions) before and after film deposition of the QCM-D sensor were measured. The collected frequency data was stitched together using the QTools software (Biolin Scientific, Stockholm, Sweden) and the areal mass was calculated according to the Sauerbrey equation (Equation 3.4). Prior to the mass change determinations, each sample was dried in an oven at 80 °C for 15 min.

The thin film systems were then stabilized overnight (ca. 18 h) inside the humidity module (Figure 16) at 11% RH by passing a saturated solution of LiCl through the module at a rate of 100 μl min⁻¹ (Tenhunen et al. 2014). After stabilization, water vapour adsorption experiments were carried out in six steps by gradually increasing the RH within the chamber using 5 different saturated salt solutions and milliQ water (Table 3). Each saturated salt solution was passed through the QHM 401 humidity module at a rate of 0.1 ml min⁻¹ for 30 min at 23 °C.

Δf and ΔD where measured by the QCM-D over a range of several overtones simultaneously. For the sake of comparability, all analyses were performed on the experimental values taken from the third overtone (15 MHz) of each measurement. To gain an overall view of the changes in Δf and ΔD, data from all of the overtones were compared.
3.2.5 Spectroscopic ellipsometry

In addition to QCM-D, spectroscopic ellipsometry (SE) was also used to monitor the water vapour uptake behaviour of cellulose thin films. SE is an optical technique that uses a beam of polarized light to characterize thin films through a range of different wavelengths. In this work SE was chosen as the spectroscopic technique as opposed to single-wavelength ellipsometry (SWE), for the reason that measuring over a broad spectrum of wavelengths of light provides more information on the changes occurring in the samples as a function of RH. Since information on two physical magnitudes at several wavelengths is gathered during an SE measurement, a higher number of unknowns can be solved through modelling than with only the one set of magnitudes attainable with SWE (Garcia-Caurel et al. 2013). Additionally, raw data from SE measurements can be modelled to take into account any changes in refractive index of the material caused by the adsorption of, for example, water vapour into the system (Boissiere et al. 2005).

When a beam of polarized light is reflected off a thin film surface, it undergoes a change in polarization which, when detected provides information on the properties of the film. As its name suggests, the technique utilizes elliptically polarized light; such light occurs when two electromagnetic waves with electric field vectors of different amplitudes or in arbitrary phases are combined. As a result, the polarized light has electric field vectors which oscillate both parallel (p-polarized) and perpendicular (s-polarized) to its plane of incidence. The

<table>
<thead>
<tr>
<th>Saturated Salt Solution</th>
<th>Relative Humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiCl(aq)</td>
<td>11</td>
</tr>
<tr>
<td>MgCl₂(aq)</td>
<td>33</td>
</tr>
<tr>
<td>Mg(NO₃)₂(aq)</td>
<td>53</td>
</tr>
<tr>
<td>NaCl(aq)</td>
<td>75</td>
</tr>
<tr>
<td>K₂SO₄(aq)</td>
<td>97</td>
</tr>
<tr>
<td>MilliQ(0)</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. RH of each saturated salt solution and water used in the QCM-D RH cycles (Greenspan, 1977). (Paper III and IV Supporting Information)
The enabling principle of ellipsometry is based on the fact that p- and s- polarized light will reflect differently from the surface of the sample; ellipsometry measures the complex reflectance ratio of p- and s- polarized light (Garcia-Caurel et al. 2013):

\[
\frac{R_p}{R_s} = \tan(\Psi)e^{i\Delta}
\]  

(3.6)

Where \(R_p\) and \(R_s\) are the Fresnel reflection coefficients for the p- and s- polarized light, \(\tan(\Psi)\) represents the magnitude of the ratio and \(\Delta\) the phase difference between the p- and s- reflected light.

The ellipsometry setup used in this thesis was specially equipped with a moisture control chamber which allowed for the in-situ monitoring of changes in thin film thickness as a result of changes in RH (Figure 17). Measurements were initiated by first aligning the reflected beam of polarized light to the detector and then performing a spectroscopic data measurement in order to attain the initial theoretical thickness and refractive index of the film using a Cauchy model. The measured data was fitted to the model data to ensure a good fit. The RH cycle was then started and the ellipsometric measurements were performed dynamically during the cycle. RH was controlled by supplying the moisture control chamber with a continual flux of air (3 l min\(^{-1}\)) at a controlled partial water pressure. Each RH cycle had a duration of ca. 45 min; 20 min for water vapour adsorption, 1-2 min for stabilization at the highest RH and 20 min for desorption - RH was incremented by 2% RH every 20 s. The measurements were performed using a UV-visible (401.39-998.85 nm) variable angle spectroscopic ellipsometer (VASE-2000U) (Woollam, Nebraska, USA) and data analyses were performed with the Wvase32 software using a Cauchy model.
3.2.6 Supplementary techniques

Grazing incidence x-ray diffraction (XRD) were carried out to determine the crystallinities of the TMSC and regenerated cellulose LS-films in paper I. The measurements were conducted at the Beamline W1.1 at the Hamburger synchrotronstrahlungslabor of the Deutsches Electronen Synchrotron (DESY). The beam was monochromatized with a double crystal Si(111) monochromator, and X-ray energy 10.5 keV was used. The beam was narrowed to 0.2 × 1 mm with slits, and the diffraction pattern was recorded with a flat image plate at a distance of 297 mm. The angle of incidence was \( \omega = 0.12^\circ \).

X-ray photoelectron spectroscopy (XPS) was used to characterize the synthesized TMSC and to evaluate the composition changes between the hydrophilic and methylated silica substrates. XPS data was collected using a Kratos AXIS Ultra electron spectrometer with an aluminium anode (mono Al K\(\alpha\)) operated at 100W. Positive charges caused by photoelectric emission were neutralized and samples were pre-evacuated overnight. For the methylated silica, two spots on two samples were measured and for the hydrophilic silica, three spots on two samples. Data was collected from an area of ca. 1 mm in diameter at an electron take-off angle of 90°. Low-resolution measurements (160 eV and 1 eV step) were used to analyse elemental compositions whereas high-resolution measurements (20 eV and 0.1 eV step) were used to analyse surface chemistries. The spectra from the methylated and hydrophilic substrates were compared by correcting the methylated substrate
spectra with the Si 2p position from the hydrophilic substrate spectra. This was carried out to determine the binding energy of Si-C on the methylated silica.

**Size exclusion chromatography (SEC)** was carried out to determine the average $M_w$, polydispersity and degree of substitution of the synthesized TMSC. Measurements were carried out using chloroform with 2% triethyleneamine as eluent with an elution speed of 1 ml min$^{-1}$ using the following system: PLgel precolumn and PLgel, 104, 105, 103, and 102 Å columns (Polymer Laboratories, Varian, Inc., Massachusetts, USA). Relative changes were determined with a Waters RI-detector (refractive index) against polystyrene standards at 35 °C.

**Photoacoustic infrared spectroscopy (IR)** was used to determine the chemical structure of TMSC. Measurements were performed using a Bio-Rad FTS 6000 Spectrometer (Bio-Rad, Massachusetts, USA) with a Gasera PA301 photoacoustic cell (Gasera Ltd., Turku, Finland).

**Nuclear magnetic resonance spectroscopy (NMR)** was used to determine the DS of the cellulose acetate derivatives. All spectra were acquired at 27 °C on a Varian UNITY INOVA 600 MHz spectrometer, using a 5 mm probe-head which is direct-detect for broad-band nuclei ($^{13}$C & $^{31}$P). Spectra were processed and integrated using MestReNova Version 10.0.1-14719 by Mestrelab Research S.L. After phasing, 3rd degree polynomial baseline correction was applied. For $^1$H spectra (600.0 MHz, 100mg in 1 ml of CDCl$_3$), 4 transients were collected, using a relaxation delay of 5 s, acquisition time of 1.7 s at a 45° pulse flip angle. For $^{13}$C spectra (150.9 MHz, 100mg in 1 ml of CDCl$_3$), 2000 transients were collected, using a relaxation delay of 1 s, acquisition time of 0.87 s at a 45° pulse flip angle. Decoupling was applied over the whole pulse sequence. For quantitative $^{13}$C spectra (150.9 MHz, 100mg in 1 ml of CDCl$_3$), 2000 transients were collected, using a relaxation delay of 4 s, acquisition time of 0.87 s at a 45° pulse flip angle. Inverse-gated decoupling was applied. A d1 array was performed to determine the appropriate d1 value for complete relaxation of the peaks being integrated (at 18.2 & 20.5 ppm). For quantitative $^{31}$P spectra (242.9 MHz, CDCl$_3$), 64 transients were collected, using a relaxation delay of 10 s, acquisition time of 1.2 s at a 45° pulse flip angle. Inverse-gated decoupling was applied. A literature procedure was followed for $^{31}$P NMR analysis and degree of substitution determination for the different cellulose samples (King et al. 2010).
4. Results and Discussion

4.1 Self-assembly of cellulose derivatives

The following section describes the parameters that effect the two dimensional assembly of cellulose-based polysaccharides. It introduces surface induced nanostructures formed during the transfer of cellulose derivate monolayers onto a solid substrate from the air/water interface (Paper I and II) and methods by which to tune a selection of the films presented (Paper I). Additionally, the behaviour of cellulose esters at the air/water interface is examined in detail (Paper II).

4.1.1 Surface induced nanostructures of cellulose derivatives

The rationale behind investigating the effect of substrate surface chemistries on the morphologies of transferred monolayers originated from a preliminary investigation into the transfer behaviour of LS monolayers with respect to direction of deposition. Due to the manner in which amphiphilic polymers arrange at the air/water interface – with their hydrophilic moeity in contact with the water phase and their hydrophobic one in contact with the air – it was hypothesized and indeed confirmed that the morphology of the monolayer would depend on whether deposition was carried out from above or from within (below) the water subphase (Figure 18). From the considerable differences in the morphologies of the two films, it was postulated that the substrate surface must, to some extent, play a role in LS deposition.
As mentioned, LS deposition has become a standard method by which to transfer monomolecular layers of amphiphilic molecules or polymers to the surface of solid substrates. Historically, LS deposition dates as far back as 1917 when Irving Langmuir proposed the first film balance (Langmuir 1917) after which in 1919 he introduced the transfer of fatty acid monolayers from the water surface of the balance onto glass – this research, carried out with Katherine Blodgett (Langmuir 1920), was the first milestone in both LB and LS (Roberts and Pitt 1982). Films deposited using either the LB or the LS methods are typically thought of as exact replicas of the monolayer formed during compression at the air/water interface (Lee et al. 1992) however, as shown in the results below, this is not necessarily always the case.

In order to determine the effects of the solid surface on the supramolecular structures of cellulose derivative monolayers, substrates of varying total surface free energy were used as solid supports. In total, four different substrates were used – HOPG, methylated silica, hydrophilic silica and mica (Figure 19). Figure 20 shows AFM images of TMSC monolayers deposited on each of these four substrates at a surface pressure of 15 mN m⁻¹ with a deposition time of 30 s and as can be seen, the morphologies of the films differ from each other significantly. Upon deposition onto a substrate of high total surface free energy, such as hydrophilic silica or mica, the LS deposited TMSC forms smooth uniform films, representative of those reported in previous studies (Kontturi et al. 2003, Orelma et al. 2012b, Taajamaa et al. 2012). With a decrease in the total surface energy of the substrate, a significant shift occurs in film morphology, for example, on HOPG the homogeneity of the monomolecular TMSC layer is disrupted and it forms a network-type morphology. Film morphologies like this are often the result of a dewetting phenomenon.
Dewetting is the process by which sufficiently thin metastable or unstable films break. In thicker polymer films, stability is provided by gravitational forces but, in significantly thinner films, the molecular forces between the polymer segments begin to dominate and cause the film to dewet (Seemann et al. 2005). Instability or metastability in thin films is caused by a lack of affinity of the polymer toward the substrate surface, in which case the polymer will preferentially aggregate or dewet in order to minimize contact with the surface. Dewetting of TMSC occurs when it is deposited onto the surface of a low total surface free energy substrate and progresses as the surface energy decreases. As a result, on the methylated silica the enhanced dewetting induces the formation of a dendritic fractal morphology in the film.

Until now, fractal patterns emerging from cellulose-based polysaccharides have remained unreported, although their formation in the crystallization of synthetic polymers has been extensively studied – poly(ethylene oxide), for example, is well known to crystallize into fractal structures (Wang et al. 2003, Zhang et al. 2008, Jin et al. 2009, Amir et al. 2011). Since crystallization is a known cause of fractal pattern formation, the crystalline structure of the TMSC fractals were analysed using GIXRD – these measurements were carried out on films in which the TMSC had been regenerated back into cellulose (Figure 21). Results revealed however, that the driving force of fractal formation the case of TMSC was not crystallization as no long-range order was visible in the diffraction spectra, which resembled those previously reported for amorphous spin coated films of regenerated cellulose (from TMSC) (Kontturi et al. 2011). A lack of crystallinity in the films is interesting not only in that the observed fractal patterns of TMSC were not caused by crystallization but also because
regenerated cellulose thin films prepared using the LS deposition method are typically crystalline cellulose II (Aulin et al. 2009). A speculative answer to this anomaly could be the shortage of material in the monolayer films – it is possible that crystallization requires a critical amount of polymer material in order to occur. The films studied by Aulin et al. (2009) were ones that consisted of 98 consecutively deposited layers of TMSC, whereas in contrast the films presented here were formed by the deposition of a single monomolecular layer. Examination of the critical amount of material required for crystallization would be worthy of further study. Additionally, it must also be taken into consideration that the lack of material in the sample will definitely affect the intensities and hence, interpretation, of the peaks in the diffraction spectra.

Figure 20. 5 × 5 μm² AFM height images of monolayers of TMSC on HOPG, methylated silica, hydrophilic silica and mica deposited at 15 mN m⁻¹. (Paper I)
TMSC is an interesting cellulose-based polysaccharide to utilize in LS deposition due to its highly amphiphilic nature – the TMSC used in these studies had a DS of 2.2 indicating that an average of 0.8 of the AGU hydroxyl groups remained unchanged. The relative abundance of these unsubstituted groups provides TMSC with true amphiphilicity making it an ideal polymer to study at the air/water interface though, fully substituted cellulose esters have also been studied in LS experiments regardless of their lack of hydroxyl groups (Adam 1933, Borgin and Johnson 1953, Kawaguchi et al. 1985, Itoh 1992, Cohen-Atiya 2007). As a comparison to the behaviour of TMSC when transferred onto the surface of HOPG, methylated silica, hydrophilic silica and mica, films of CTA, CAP and CAB (Figure 6 in Section 2.2.1) were also prepared onto the same substrates using identical LS deposition conditions (15 mN m⁻¹ surface pressure and 30 s deposition time).

Figure 22 shows AFM height images of monolayers of CAP transferred onto the surface of the four different substrates. Unlike with the TMSC, CAP did not form homogeneous films on the substrates with high total surface free energies.
In fact, adsorption of CAP onto hydrophilic silica was completely inhibited by its high surface energy. When transferred onto mica and HOPG, CAP formed seemingly unimolecular aggregates and with a further reduction in surface energy (methylated silica), a pearl necklace-like morphology was observed.

Aggregation, as opposed to spreading, in LS films occurs either during compression of the monolayer or after deposition in cases where there is a lack of affinity between the surface of the substrate and the polymer material. From the lack of plateau regions in the compression isotherms of CAP (presented in detail in Section 4.1.3), it can be concluded that aggregation did not occur at the air/water interface. As such, the aggregates visible in the AFM images must have formed through one of two possibilities: i) when the substrate was brought into contact with the monomer on the surface of the water or ii) during the drying of the sample after the monolayer had been transferred onto the surface of the substrate. Aggregated films of CAP have been previously reported as a result of annealing of the films above the glass transition temperature of the polymer (Kosaka et al. 2007).

Figure 22. 5 × 5 μm² AFM height images of monolayers of CAP on HOPG, methylated silica, hydrophilic silica and mica deposited at 15 mN m⁻². (Paper II)
The morphologies of the cellulose ester films change significantly when the acetate content of the derivative was increased from 0.13 in CAP to 1.94 and 2.85 in CAB and CTA, respectively and coupled to a concurrent decrease in the number of residual hydroxyl groups – 0.24, 0.15 and 0.09 for CAP, CTA and CAB, respectively. This dependence of film morphology on acetate and hydroxyl content is shown in the AFM images of Figure 23 and Figure 24. Surprisingly, CTA was unable to adsorb onto either of the hydrophilic substrates whereas CAB, although not adsorbed onto mica, formed an unstable film when deposited onto hydrophilic silica – the substrate of the highest total free surface energy. The instability of the CAB film is evident from its ruptured morphology, which like the CAP films, presumably occurred during the drying stage of sample preparation.

![Figure 23. 5 × 5 μm² AFM height images of monolayers of CTA on HOPG, methylated silica, hydrophilic silica and mica deposited at 15 mN m⁻¹.](Paper II)

Deposition of CTA and CAB onto the hydrophobic substrates resulted in some very intriguing morphologies. In addition to CTA on methylated silica – which formed a fibrillar-like network structure – both the CTA and CAB films associated on the hydrophobic substrates to form striated morphologies of elongated aggregates displayed in Figure 23 and Figure 24. Similar structures have previously been reported in solvent cast CTA films where they were ascribed to the helicoidal cholesteric properties of cellulose esters (Ritcey et al. 1989, Davé et al. 1992).
In the case of TMSC, an obvious relationship exists between the degree of dewetting of the monolayer and total surface free energy of the substrate – as surface energy decreases, the effects of dewetting increase. To understand this progressive dewetting, one must consider the thermodynamic phenomena occurring in the films. TMSC film formation must be examined from the point-of-view of the thermodynamic equilibrium existing between two systems: i) the initial system in which deposition has yet to be carried out and ii) the system in which the monolayer has been transferred onto the surface of the solid substrate.

According to the second law of thermodynamics, the total entropy of any naturally occurring process will always increase through the following relationship:

\[
T \Delta S = \Delta U - \gamma_n \Delta A - \Sigma \mu_i \Delta n_i \tag{4.1}
\]

where \( T \) is temperature, \( S \) is entropy, \( U \) is internal energy, \( \gamma_n \) is the interfacial energy between the substrate and TMSC, \( A \) the surface area (or work coefficient in LS deposition), \( \mu \) is chemical potential and \( n \) is the molar amount of transferred material.

Figure 24. 5 × 5 µm² AFM height images of monolayers of CAB on HOPG, methylated silica, hydrophilic silica and mica deposited at 15 mN m⁻¹. (Paper II)
For the sake of comparability, both the temperature and the amount of material transferred (confirmed by the deposition transfer ratios) are kept constant and thus, the internal energies and molar amount of transferred material are identical for all four TMSC/substrate systems. As a result, equation 4.1 can be simplified to:

\[ \Delta S \propto \gamma_n \Delta A \quad (4.2) \]

where the work coefficient also remains constant throughout the depositions.

Hence, as the interfacial energy between the substrate and TMSC increases with decreasing total surface free energy of the substrate, the total entropy of the system increases. This increase in entropy is evident in the AFM images – the increasing morphological uncertainty of the thin films with decreasing surface energy finally leads to the formation of dendritic fractals, structures which possess the highest amount of uncertainty because of their non-differentiability (Kohmoto 1988, Gonzalez et al. 2003).

### 4.1.2 Tuning of ultrathin film morphology

Although not the cause of fractal pattern formation, the process by which it occurs can be explained through the diffusion-limited aggregation (DLA) theory (Witten and Sander 1981, Witten and Sander 1983, Sander 1986). This theory utilizes a Monte Carlo approach and stipulates that the probability that a particle, in continuous and random motion (diffusion) will collide and attach with another particle, is very high. Fractal patterns emerge in situations where mobile particles collide with either an artefact or a stationary particle (a 'seed particle') that is present in the same plane. As more particles collide with the seed particle a cluster will evolve, eventually forming a fractal structure (Witten and Sander, 1981 and 1983). In order to confirm whether the TMSC fractals were indeed formed through the DLA model, monolayers were deposited using three different deposition times – 10, 30 and 120 s. The results showed that a fractal pattern was formed during each deposition but since the time scales measureable on the LS setup did not have a high enough resolution to monitor those required in the formation of nanoscaled patterns, it was not possible to...
determine whether the formation of the fractals was in fact diffusion-limited and so the DLA model could not be ruled out as a possibility. Instead, image analysis of the TMSC fractals revealed that they had a fractal dimension – a measure of the fractal complexity – of 1.86 which is very similar to that reported for fractals known to have formed through the DLA process (1.7). The fractal patterns of TMSC could successfully be tuned by exposing the film to 2 M hydrochloric acid vapour to regenerate it back to cellulose (Figure 12 in Section 3.2.1) before then immersing the regenerated cellulose film into water for 15 min and subsequently drying it for 48 h in a desiccator (Figure 25).

![Figure 25. 5 × 5 μm² AFM height images (with height profiles) of fractal patterns of regenerated cellulose and regenerated cellulose immersed in water and consecutively dried on methylated silica (deposited at 15 mN m⁻¹). (Paper I)](image)

When regenerated back to cellulose, TMSC films typically decrease in volume by ca. 40% due to the significantly smaller size of hydroxyl groups in comparison to silyl ether groups (Kontturi and Lankinen 2010). This decrease can clearly be seen when the morphologies of the TMSC and regenerated cellulose films are compared as the cellulose fractals are more sparsely distributed than the TMSC fractals. Upon immersion into water and subsequent drying, the cellulose fractals visibly contract in width but increase in height – indicating a conservation of matter, which was later quantitatively confirmed (Table 4 and Figure 26). A response to the presence of water by the regenerated cellulose film is not surprising as a substantial amount of research has been carried out on the structural changes occurring in cellulose fibers upon wetting and subsequent drying. As mentioned, the removal of water from swollen fibers results in their
irreversible hornification, which can be relatively easily quantified using water retention value measurements. (Jayme 1994, Hubbe et al. 2007, Pönni et al. 2012). Although the packing of the fractals could be tuned with ease, the complexity of the fractals remained unchanged with fractal dimensions of 1.73 and 1.81 for the regenerated cellulose and water immersed cellulose films, respectively. These slight variations in the fractal dimensions are the result of image analysis being carried out on three different samples, rather than on one sample over a given area.

Table 4. Quantified data on the coverage and material volume of TMSC, regenerated cellulose and regenerated cellulose immersed in water on methylated cellulose (deposited at 15 mN m\(^{-1}\)). (Paper I Supporting Information)

<table>
<thead>
<tr>
<th></th>
<th>Coverage %</th>
<th>Material Volume cm(^3) (x 10(^{-14}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMSC</td>
<td>43</td>
<td>1.83</td>
</tr>
<tr>
<td>Regenerated Cellulose</td>
<td>41</td>
<td>2.90</td>
</tr>
<tr>
<td>Regenerated cellulose immersed in water</td>
<td>35</td>
<td>2.82</td>
</tr>
</tbody>
</table>

The morphologies of LS deposited monolayers can also be tuned by varying the surface pressure at which deposition is carried out. Figure 27 shows the morphologies of deposited monolayers of CTA, CAB and CAP on HOPG at three different surface pressures – 5, 10 and 15 mN m\(^{-1}\). As can be seen, the morphologies of the CTA and CAP films are altered with increasing surface pressure. In the case of the CTA films, the changes in morphology are somewhat
as expected as the film becomes denser with increasing surface pressure. Additionally, with increased surface pressure, a clear orientation can be observed in the elongated aggregates. The opposite effect can be seen for CAB as the morphology of the films remains unchanged even at high surface pressures. Based on the increasingly dense morphology of the films, with a higher surface pressure it is possible that a homogenous monolayer of CTA could be transferred onto the HOPG substrate. The morphologies of the CAP films are particularly interesting, as can be seen in the AFM images, at a sufficiently low surface pressure (under 10 mN m⁻¹) CAP also forms films with a striated structure. When deposited at a low surface pressure, CAP appears to form a stable film on the surface of the HOPG at higher surface pressures, however, the transferred films appear to exist in an unstable/metastable state and as a result rupture to form aggregates in the final stages of film formation in the solid state.

Figure 27. 1 × 1 μm² AFM height images of CTA, CAB and CAP monolayers LS deposited on HOPG at surface pressures of 5, 10 and 15 mN m⁻¹. (Paper II)
4.1.3 Cellulose esters at the air/water interface

An interesting feature of cellulose esters is their ability to spread at the air/water interface despite the subtlety of their amphiphilic properties. To further examine their behaviour on the surface of the water subphase of the Langmuir trough, CTA, CAP and CAB monomers were compressed into monolayers using two different rates of compression. The formation of Langmuir monolayers is carried out by compressing randomly spread monomers at the air/water interface into an ordered structure. Prior to compression, when randomly spread on the water subphase, the monomers are in what is known as the gaseous state. Upon compression, the monomers are forced toward one another and begin to form a homogeneous monolayer that initially exists in a liquid expanded (LE) state and then, with a slightly higher compression, shifts into a liquid condensed (LC) state. At a high enough surface pressure, the monolayer will collapse and the monomers will stack on top of one another (Figure 28). Moreover, compression of the monolayer may in some cases cause an accelerated crystallization of the polymer material (Brinkhuis and Schouten 1991a, Brinkhuis and Schouten 1991b).

Figure 28. Schematic representation of a n-A Langmuir isotherm representing the different states of a polymer monolayer at the air/water interface upon compression.
Figure 29 shows the surface pressure-area ($\pi$-A) isotherms of each cellulose ester carried out using a slow compression ($0.1 \text{ mm s}^{-1}$) and a fast compression, ($5 \text{ mm s}^{-1}$). As can be seen, the self-assembly behaviour of the monomers at the air/water interface is dependent on the speed at which compression is carried out. In each of the three cases, the transitions in state are more pronounced during the slower compressions, due to the extensive amount of time allowed for monomer ordering. During the faster compressions these transitions are not as obvious and in fact vary in clarity depending on the cellulose ester in question. The transition from LE to LC state occurs at 17, 31 and 21 mN m$^{-1}$ during the slow compression for CTA, CPA and CAB, respectively, and correspondingly at 21, 28 and 25 mN m$^{-1}$ during the fast compression. As was the case with the morphologies of the transferred monolayers onto solid substrates, CAP behaved in a different manner than the other two cellulose esters during compression. Where CTA and CAB experienced a phase transition at higher surface pressures during the fast compression than the slow one, the opposite was true for CAP. Within the surface pressures used for the $\pi$-A isotherms (0 – 40 mN m$^{-1}$) none of the monolayers experienced a structural collapse, however, a slight disruption of the CTA films occurred at surface pressures above ca. 26 mN m$^{-1}$ as can be seen by the fluctuations in the isotherms of both compression rates.
An interesting parameter of Langmuir monolayers is the limiting area of the polymer repeat units, $A_0$ — a variable that provides information on the spreading capability and orientation of the monomers at the water surface. The spreading capability of monolayers is dependent on a variety of factors, which include the amphiphilicity of the monomers, the size of the repeating units' substituents, its degree of substitution (Adam 1933, Borgin and Johnson 1953), the temperature at which measurements are carried out (Kawaguchi et al. 1985) and the solvent into which the polymer was originally dissolved (Itoh et al. 1992).
Values for $A_0$ are derived by extrapolating the steepest section of the $\pi$-$A$ isotherm to zero surface pressure. For cellulose esters in particular, the magnitude of $A_0$ is important since their paucity of hydrophilic moieties causes difficulties for their spreadability. The $A_0$ determined for CTA, during both compressions was smaller than those reported previously, with a value of 33 Å$^2$ compared to ones ranging from 40 to 54 Å$^2$ (Adam 1933, Kawaguchi et al. 1985, Cohen-Atiya et al. 2007) underlying the challenges of homogeneous spreading of cellulose esters over water.

Table 5. $A_0$ determined through $\pi$-$A$ isotherm extrapolation to zero surface pressure for CTA, CAP and CAB when compressed at a rate of 0.1 and 5 mm s$^{-1}$. (Paper II)

<table>
<thead>
<tr>
<th></th>
<th>Limiting area, $A_0$</th>
<th>0.1 mm s$^{-1}$ compression</th>
<th>5 mm s$^{-1}$ compression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTA (DS 2.85)</td>
<td></td>
<td>33.0</td>
<td>33.1</td>
</tr>
<tr>
<td>CAP (DS 2.76)</td>
<td></td>
<td>42.9</td>
<td>44.1</td>
</tr>
<tr>
<td>CAB (DS 2.91)</td>
<td></td>
<td>41.6</td>
<td>39.6</td>
</tr>
</tbody>
</table>

Evidence of the poor spreadability of cellulose esters also lies in the fact that the $A_0$ of CAB was systematically smaller than that of CAP despite that, on a molecular scale, the butyrate substituent groups are larger than propionate. Although spreading is typically enhanced by larger substituent groups, due to a decrease in molecular cohesion (Borgin and Johnson 1953), in the case of the two cellulose esters here, spreading is seemingly governed more by the degree of substitution of the polymers than the size of the substituted functional group. Evidently, the spread of CAP is aided by its significant portion of residual hydroxyl groups, whereas the shortage of which hinders the spreading of CTA and CAB (Table 1 in Section 3.1.1).
4.2 Water vapour sorption of ultrathin cellulose films

As has been shown by the quantitative analysis involving changes occurring as an exposure of regenerated cellulose films to water (Figure 25), the relationship between cellulose-based polysaccharides and water is relatively complex and becomes even more so in systems where cellulose is not the sole component, such as in the plant cell wall. Although the cellulose derivatives examined thus far provide vital fundamental information on responses occurring as a result of varying parameters, their interactions do not completely reflect the behaviour of cellulose in its native form.

The following section describes the water vapour sorption behaviour of purely crystalline and semi-crystalline ultrathin cellulose films from both a fundamental and a model film standpoint. It introduces a qualitative analysis of the hydration mechanism of CNC ultrathin film networks (Paper III) and provides insight into the intricacies of the role of the crystalline/amorphous ratio in the water vapour adsorption of ultrathin cellulose films (Paper IV).

4.2.1 Hydration of cellulose nanocrystals

Regardless of the fact that cellulose is used in a multitude of different areas its hydrophilic and hygroscopic nature prevents it from being utilized to its full potential in numerous other applications. For the time being, synthetic materials are superior to cellulose-based ones in terms of water resistance and as such are primarily used in water-sensitive applications such as packaging and barrier materials. In order to be able to better exploit all of the environmental benefits that motivate the use of green materials, fundamental knowledge on their water uptake behaviour must first be attained. While the uptake of water in its liquid state is of extreme importance in functional materials, the sorption of water vapour is also highly significant as it accounts for up to 3% of the Earth’s atmosphere.

Highly crystalline cellulose is an intriguing material; despite its acute hydrophilicity it is impenetrable by water, allowing it to be used in studies concerned with water vapour adsorption at cellulose surfaces. CNCs in particular provide an excellent platform for such studies as they possess a high surface to volume ratio, which results in a vast number of possible adsorption
sites. Figure 27 shows the normalized changes in thickness of three different ultrathin CNC films of 5, 10 and 25 nm as a function of RH. Normalization of thickness changes was carried out in order to be able to compare the changes occurring in the films during the measurements and is defined as the change in thickness relative to the original thickness of the film, \((h-h_i)/h_i\).

![Normalized thickness as a function of RH](image)

Figure 30. Normalized \(\Delta h\) thickness as a function of RH of a) 10, b) 15 and c) 25 nm thick films of CNC measured by SE. \(\Delta h\) is the actual change in thickness of the film at 97% RH. (Paper III)

As can be seen, each of the three films exhibited an increase in thickness with increasing RH. This change in thickness of the films was an unexpected result. Spin coated CNC films have the morphology of a network structure in which the CNCs are randomly orientated with voids between them. Since the crystallites are impenetrable by water, the hydration of the network should intuitively occur in such a way that any water vapour adsorbed into the structure should
condense into the voids. As more water vapour is adsorbed, the voids should gradually fill with condensed water and at such a stage where the voids are filled to their maximum capacity, any additional water vapour introduced to the system should simply run off the top of the film. Under this hypothesis, no thickness change should be detectable with increasing RH. Evidently, this assumed mechanism is not the means by which the CNC network becomes hydrated. The increase in thickness of the samples is an indication that water vapour adsorption takes place not in the voids of the network but between the individual crystals (Figure 31). In fact, a quantitative analysis of the SE results in combination with results attained from QCM-D measurements revealed that hydration of the CNC network occurs by the envelopment of each individual CNC by three monolayers of water.

As a complementary technique to SE, QCM-D was used to monitor the changes of mass in the ultrathin CNC films with increasing RH by monitoring the changes in the resonance frequencies ($\Delta f$) of the samples (Figure 32) as $\Delta f$ is inversely proportional to changes in mass through the Sauerbrey equation (Equation 3.4 in Section 3.2.4). Information on the hydration mechanism of the CNC network was attained from data recorded at the point of saturation of the films which, for the QCM-D setup used, was presumed to occur at 97% RH as this was the highest achievable RH without the effects of water condensation within the humidity module (Figure 16 in Section 3.2.4). As can be seen by the

Figure 31. Schematic representation of the previously assumed mechanism by which CNC networks of ultrathin films uptake water vapour and the proposed mechanism.
significant changes in resonance frequencies, each sample adsorbed a substantial amount of water vapour. As the values of $\Delta f$ are relative to the initial mass of the film, based on the similarity of the maximum $\Delta f$ for each sample, it would seem that all three CNC films adsorbed approximately the same amount of water vapour. In other words, the results show that the mass of adsorbed water vapour was relative to the initial mass of the CNC film, further underlining the presumption that hydration by water vapour occurred at the surface of the CNCs rather than in the macroscopic features (voids) of the film. This is also evident from the fact that the mass uptake due to exposure of the films to water vapour was significantly less than that which has previously been reported for similar films in the presence of liquid water where the water does fill the network voids (Kittle et al. 2011, Dremeier and Bragatto 2013).

![Figure 32. $\Delta f$ of a) 10 nm, b) 15 nm and c) 25 nm CNC thin film as a function of time (and RH) monitored by QCM-D. (Paper III)](image-url)
Calculations using the intrinsic physical properties of both water molecules and CNCs were carried out in order to model the conformation of the adsorbed water molecules at the air/CNC interfaces in the films. These calculations were carried out by first determining the initial mass of the CNC networks and with that, the mass of the water vapour adsorbed by the films when hydrated. These masses were then converted into total specific surface areas of each component by calculating the number of individual crystals and water molecules present in their respective masses and then multiplying by their surface areas. Surprisingly, the total surface area of water was three times that of the CNCs for the 15 and 25 nm thick films. It should be noted that the thicker films of CNC provided full coverage of the QCM-D sensor, whereas the 10 nm film did not and as such a larger mass of water is adsorbed by this sample due to the hydrophilic nature of the exposed silica substrate (Figure 33). Nevertheless, from the thicker samples it is evident that upon hydration, each CNC of the thin film network became enveloped in three monolayers of water molecules.

Evidence of this finding is provided by the SE results. Given that the thickness of a single CNC is approximately 7 nm (Majoinen et al. 2011) it can roughly be estimated that the 10, 15 and 25 nm CNC films are made up of 1-2, 2-3 and 4-5 layers of CNCs, respectively. Upon hydration, each of these layers will be surrounded by three monolayers of water resulting in three monolayers of water underneath the CNC layer and three on top of it. Since the diameter of a single water molecules is ca. 0.275 nm (Pierotti 1965), each hydrated CNC layer will increase the thickness of the film by ca. 1.7 nm (i.e. six monolayers of water). A comparison of the hypothetical changes in film thickness upon hydration and the changes measured by SE show the results to be in good agreement, confirming the proposed hydration model of the CNC networks (Table 6).
Of course, this proposed model requires a number of assumptions and as such is purely hypothetical. As the techniques used are not able to monitor the conformation of water adsorption, it must be presumed that the water molecules are distributed evenly throughout the CNC network and are not tightly packed – sometimes the first hydration layer of cellulose materials the density of water can be up to three times that of its bulk density (Matthews et al. 2006). Regardless, the results attained are consistent with previously reported studies involving the water vapour adsorption of bulk cellulose materials (Urquhart and Williams 1924, Jeffries 1960, Li et al. 1992, Driemeier and Bragatto 2013).

Table 6. SE data on film porosity (row 1), initial film thickness (row 2) and QCM–D data (rows 3 and 4) used to quantify the mass of water adsorbed onto the CNC films at 97% RH (rows 5 – 7) and a comparison of the calculated change in thickness caused by the adsorbed water vapour compared to the measured change in thickness from SE data (rows 8 – 10). (Paper III)

<table>
<thead>
<tr>
<th></th>
<th>10 nm CNC</th>
<th>15 nm CNC</th>
<th>25 nm CNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Film porosity, % vol</td>
<td>27</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Initial thickness of film (SE) ± 0.1%, nm</td>
<td>10.4</td>
<td>15.3</td>
<td>25.6</td>
</tr>
<tr>
<td>Initial areal mass (QCM–D) ± 16%, ng cm⁻²</td>
<td>857</td>
<td>1200</td>
<td>1875</td>
</tr>
<tr>
<td>Areal mass hydration vapor ± 16%, ng cm⁻²</td>
<td>680</td>
<td>434</td>
<td>532</td>
</tr>
<tr>
<td>CNC total surface area ± 16%, cm²</td>
<td>2.5</td>
<td>3.5</td>
<td>5.4</td>
</tr>
<tr>
<td>Water total surface area ± 16%, cm²</td>
<td>17.8</td>
<td>10.5</td>
<td>13.3</td>
</tr>
<tr>
<td>Ratio of surface areas (CNC : water)</td>
<td>1 : 7.2</td>
<td>1 : 3</td>
<td>1 : 2.5</td>
</tr>
<tr>
<td>No. CNC layers in thin film</td>
<td>1 – 2</td>
<td>2 – 3</td>
<td>4 – 5</td>
</tr>
<tr>
<td>Calculated change in thickness, nm</td>
<td>1.7 – 3.3</td>
<td>3.3 – 5.0</td>
<td>6.6 – 8.3</td>
</tr>
<tr>
<td>Measured change in thickness (SE) ± 0.1%, nm</td>
<td>1.9</td>
<td>3.7</td>
<td>12</td>
</tr>
</tbody>
</table>

According to the DLVO theory, the CNC film is held in equilibrium by van der Waals forces and electrostatic forces. In addition to these forces, hydrogen bonds are also present in the system. Nonetheless, due to the long range of electrostatic forces – originating from the sulphate groups on the CNC surfaces – and the short range of hydrogen bonds (Nishiyama et al. 2003), their contribution to the equilibrium state of the hydrated CNC film can be considered negligible as the CNCs are separated by approximately 2 nm of water. At this range, the predominant forces at play are the attractive van der
Waals forces (Figure 34) which, it would seem, prevent the inclusion of any additional water vapour into the CNC network after the point of hydration.

Figure 34. Calculated interaction energy of van der Waals forces between two cylindrical CNCs crossed at 90° and in parallel per unit length (Israelachvili 2011). The Hamaker constant used in the calculations was $1.1 \times 10^{-20} \text{J}$ (Boluk et al. 2010). [Paper III]

### 4.2.2 Water vapour response of cellulose systems

To further examine the water vapour uptake behaviour of ultrathin cellulose films, two layered systems with varying crystalline/amorphous ratios were studied using SE and QCM-D. These thin film systems can be separated into two categories: i) films in which the crystalline/amorphous ratio was similar to that which is prevalent in the plant cell wall, with similar portions of both components (Fengel and Wegener 1989, Hill et al. 2009) and ii) films in which the portion of regenerated amorphous cellulose was predominant (Figure 35). The presented set of cellulose films allowed for the systematic analysis of the role of the crystalline/amorphous ratio on water vapour sorption without the possible behavioural interferences arising from chemical heterogeneities.

The cellulose films were prepared by first spin coating a layer of CNCs onto the surface of a silica substrate and then coating the network with a layer of TMSC, which was then regenerated back into amorphous cellulose. The crystalline/amorphous ratio was controlled by varying the proportions of each component in the films. AFM images, where the distinct morphology of the underlying CNC film disappeared upon the addition of a thicker film of TMSC, revealed that far from existing as two separate layers, the TMSC penetrated into the morphology of the underlying CNC network to form a fully interspersed thin film structure (Figure 36).
The films with a similar crystalline/amorphous ratio as the plant cell wall had underlying CNC layers of areal masses of 0.3, 1.2, or 1.9 μg cm\(^{-2}\) (from 5, 10 and 20 g l\(^{-1}\) CNC solutions, respectively) with a regenerated amorphous cellulose film of 2.4 μg cm\(^{-2}\) (from a 10 g l\(^{-1}\) TMSC solution) on top whereas the predominantly amorphous films had underlying CNC layers of 0.3, 1.2, or 1.9 μg cm\(^{-2}\) underneath an 8.4 μg cm\(^{-2}\) regenerated amorphous cellulose film (from a 20 g l\(^{-1}\) TMSC solution). Although the TMSC was embedded into the underlying CNC network upon spin coating, a portion of it remained ‘free standing’ on top of the layer of CNCs. The thickness of this portion of regenerated amorphous cellulose varied which each sample due to the variations in the densities and structures of the underlying CNC films (Table 7). The thickness of this layer was determined by subtracting the initial thickness of the underlying CNC films from the initial thickness of the entire composite film as determined by SE at 0 %RH.

Table 7. Thickness of the CNC/regenerated amorphous cellulose composite films and their individual components (as measured by SE at 0 %RH). (Paper IV)

<table>
<thead>
<tr>
<th>CNC/regenerated amorphous cellulose</th>
<th>Initial thickness of film nm</th>
<th>Initial thickness of underlying CNC nm</th>
<th>Thickness of overlying amorphous layer nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 / 2.4</td>
<td>34</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>1.2 / 2.4</td>
<td>36</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>1.9 / 2.4</td>
<td>43</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>0.3 / 8.4</td>
<td>64</td>
<td>10</td>
<td>54</td>
</tr>
<tr>
<td>1.2 / 8.4</td>
<td>70</td>
<td>15</td>
<td>55</td>
</tr>
<tr>
<td>1.9 / 8.4</td>
<td>83</td>
<td>26</td>
<td>57</td>
</tr>
</tbody>
</table>
Figure 36. 3 × 3 μm² AFM height images of each two layered cellulose systems, a) 0.3, b) 1.2 and c) 1.9 μg cm⁻² CNC films all underneath a 2.4 μg cm⁻² regenerated amorphous cellulose film and d) 0.3, e) 1.2 and f) 1.9 μg cm⁻² CNC films all underneath an 8.4 μg cm⁻² regenerated amorphous cellulose film. (Paper IV)

As can be seen from the SE results in Figure 37, the swelling behaviours of the two layered cellulose systems were prominently governed by their crystalline/amorphous ratios. For the plant cell wall –like films, an increase in the fraction of the CNCs in the film promoted the swelling of the systems (Figure 37a-c). In these three composite films, the swelling ratios when hydrated – the ratio between the ratio between the hydrated thickness and the initial (dry) thickness of the film – were either equivalent to or greater than that of the 2.4 μg cm⁻² amorphous reference film (with a swelling ratio of 1.5).

In contrast, when the crystalline portion of the predominantly amorphous systems increased and the crystalline/amorphous ratio became sufficiently large, the presence of the underlying CNC film seemed to inhibit the swelling of the film (Figure 37 f). Nonetheless, inhibition only occurred in the two layered film with the thickest underlying CNC layer as swelling of the films with a 0.3 and 1.2 μg cm⁻² CNC film exhibited the same swelling ratio as the 8.4 μg cm⁻² regenerated amorphous cellulose reference film (with a swelling ratio of 1.7) (Figure 37 d-e).
Based on the results attained from the SE measurements, it would seem that the simple addition of a crystalline phase to an amorphous thin film introduces an unexpected complexity to the system. The opposite trends in the two types of systems as a result of varying crystalline/amorphous prevent any true comparability between them and more significantly the analysis of the trends occurring within each of the two systems.

To gain a better understanding of the water vapour adsorption behaviour of the sample categories, RH measurements were also carried out using QCM-D (Figure 38). As with the pure CNC films, the QCM-D data was used to calculate the mass of water vapour absorbed by the thin films, however, the mass in this case was compared to the total mass of cellulose (both crystalline and amorphous) in each system.
Interestingly, although the swelling ratios of the plant cell wall–like samples were either equivalent to or slightly greater than that of the 2.4 µg cm⁻² reference film, each of the samples systematically adsorbed less water vapour upon hydration. The water vapour uptake capabilities of the films with a 0.3, 1.2 or 1.9 µg cm⁻² CNC layer underneath a 2.4 µg cm⁻² regenerated amorphous cellulose film sample were 0.31, 0.33 and 0.42 mg H₂O/mg cellulose, respectively, whereas the corresponding value for the reference film was 0.45 mg H₂O/mg cellulose. The results show that the addition of a CNC network underneath the 2.4 µg cm⁻² amorphous layer provides a flexibility to the system, allowing for a larger change in thickness upon hydration with a smaller water vapour mass uptake. This would suggest that water vapour adsorption in these plant cell wall–like films likely occurs, as it did with the fully crystalline CNC films, at the surface of the CNCs.

Figure 38. Δf of each two layered cellulose system as a function of time (and) RH monitored by QCM-D measurements, a) 0.3, b) 1.2 and c) 1.9 µg cm⁻² CNC films all underneath a 2.4 µg cm⁻² regenerated amorphous cellulose film and d) 0.3, e) 1.2 and f) 1.9 µg cm⁻² CNC films all underneath an 8.4 µg cm⁻² regenerated amorphous cellulose film. (Paper IV)
The combined results from the SE and QCM-D measurements for the plant cell wall–like samples correlate well with a previous report in which model systems consisting of a crystalline and amorphous phase similar to the structure of the plant cell wall, showed that an excess of water vapour is adsorbed at the crystalline/amorphous interface (Kulasinski et al. 2015). This phenomenon explains the promotion of swelling, with less water vapour mass uptake, as a function of the thickness of the underlying CNC layer – when more CNCs are present in the film, a higher number of crystalline/amorphous interfaces exist and the film exhibits a more pronounced flexibility.

In addition to this though, as was the case with the swelling ratios, the mass of water vapour adsorption in the samples where the crystalline/amorphous ratio of the plant cell wall is considerably exceeded, the addition of the CNC layer prohibited water vapour uptake into the system. Each sample adsorbed a smaller mass of water vapour upon hydration than their corresponding regenerated amorphous cellulose reference films. The masses of water vapour adsorbed were 0.39, 0.37 and 0.33 mg H₂O/mg cellulose for the 0.3, 1.2 and 1.9 μg cm⁻² CNC film underneath 8.4 μg cm⁻² regenerated amorphous cellulose whereas for the 8.4 μg cm⁻² regenerated amorphous cellulose reference film it was 0.39 mg H₂O/mg cellulose.

A qualitative analysis of the mass of water vapour uptake per mass of cellulose as a function of the normalized surface area of CNCs (or number of crystalline/amorphous interfaces) of each sample suggests that there exists a minimum the water vapour uptake capability at hydration (Figure 39). As mentioned, in the plant cell wall–like systems, as the number of interfaces increases so does the mass of water vapour adsorbed upon film at hydration. However, in the predominantly amorphous composite films a decrease in interfaces coincides with an increase in water vapour adsorption. The occurrence of this minimum has two possible explanations, either i) a decrease in the number of crystalline/amorphous interfaces or ii) an increase in the thickness of the overlying amorphous cellulose significantly shifts the swelling behaviour of the films away from that of the plant cell wall–like systems.

As a result, it would seem that swelling of these composite films is not only governed by the crystalline/amorphous ratio of the system but also the thickness of the overlying, ‘free standing’ layer of regenerated amorphous cellulose on top of the CNC films or the interfacial contact between the amorphous or crystalline phases (or both) (Figure 40).
Figure 39. Mass of water vapour adsorbed by composited film at hydration as a function of the number of crystalline/amorphous interfaces (or normalized surface area and CNCs in each sample). (Paper IV)

In comparison to the purely crystalline CNC films in which substrate surface coverage was complete, the mass of water vapour adsorbed by all of the two layered systems was systematically larger, as could be expected. The masses of water vapour adsorbed by the purely crystalline films were 1.8, 0.22 and 0.28 mg H₂O/mg cellulose for the 10 (0.3 μg cm⁻²), 15 (1.2 μg cm⁻²) and 25 nm (1.9 μg cm⁻²) CNC films, respectively. It should be noted that the mass of water vapour adsorbed by the 5 nm CNC film is unrealistic as the film did not provide the hydrophilic silica with full coverage. Interestingly however, when examining the fully covering films, the inherent water vapour adsorption capability of the individual CNC and amorphous films was found to be noticeable less when compared to that of the combined two layered systems – further underlining the complexity of the combination of two significantly different phases to form a composite film and ultimately the plant cell wall.
Figure 40. Schematic representation of the water vapour adsorption of an ultrathin cellulose composite film with a) crystalline/amorphous ratio similar to that of the woody plant cell wall and b) predominantly amorphous composition. (Paper IV)
5. Concluding Remarks

The objective of this thesis was to provide a thorough investigation into the two dimensional assembly of cellulose-based polysaccharides and the response of two dimensional structure to external stimuli, namely water and water vapour. This investigation was carried out by examining the external parameters effecting the two dimensional assembly of monomolecular ultrathin films of cellulose derivatives and by monitoring the behaviour of these derivatives at the air/water interface. The spectrum of materials was broadened by the introduction of native crystalline cellulose whose interactions with water vapour were explored in detail in the two dimensional realm of ultrathin films.

It was found that the transfer behaviours of monomolecular films of cellulose derivatives from the air/water interface were significantly affected by the total surface free energy of solid substrates onto which they were transferred. Interestingly, with the highly amphiphilic derivative, TMSC, dewetting of the transferred ultrathin film progressed as a function of decreasing surface energy of the substrates. On substrates of high surface energy TMSC was able to form smooth homogeneous films representative of ones typical for the polymer. In contrast, on the surface of hydrophobic substrates with a lower surface energy the homogeneity of the film was disrupted resulting in distinct nanostructures. As a result of appreciable dewetting on the substrate of lowest surface energy, TMSC formed a dendritic fractal pattern, a morphology previously unreported for cellulose-based polysaccharides. Tuning of these fractal structures was found to be possible via a simple TMSC regeneration and consecutive immersion in water. To complement these findings, a systematic analysis of the transfer behaviour onto the same substrates was carried out for monomolecular films of cellulose ester thin films with varying acetate and residual hydroxyl group content. The self-assembly on the surface of the solid substrates of the cellulose esters, CTA, CAP and CAB, was markedly different from that of the TMSC due to the reduced amphiphilicity of the esters. The thermodynamic reasoning behind the dewetting of the TMSC films was not applicable to the cellulose ester films though nevertheless, interesting morphologies of the
monomolecular films were detected. A thorough investigation into the spreading capabilities of the three cellulose esters revealed that interactions occurring at the air/water interface are predominantly determined by the residual hydroxyl groups of the anhydroglucose unit rather than by the degree or size of the ester substituent.

The complexities of the interactions between cellulose-based polysaccharides and water were further studied by the hydration of purely crystalline CNC films via exposure to water vapour. It was found that the mechanism by which CNC thin films become hydrated occurs somewhat counterintuitively. Instead of wetting the bulk of this film and saturating it, water vapour adsorbs at the CNC/air interface and envelopes each individual crystallite in three monolayers of water. These findings, although speculative and based on several assumptions, concur well with those reported previously in the literature. In order to improve the proposed hydration model, further studies would be required in which water vapour adsorption could be monitored more precisely, for example with the aid of small-angle x-ray scattering.

In order to further investigate the adsorption of water vapour of native cellulose, two double layered cellulose systems with varying crystallinities were compared. The first of these systems was designed to have a crystalline/amorphous ratio similar to that of the woody plant cell wall and the second had a crystalline/amorphous ratio considerably less than that of the plant cell wall. It was noted that the addition of a crystalline phase to a purely amorphous system resulted in an unexpected complexity with regard to water vapour adsorption. Interestingly, when the water vapour adsorption behaviours of the two cellulose systems were compared the trends present within each were diametrically opposite. Where the degree of swelling was promoted by an increased portion of crystalline cellulose in the plant cell wall -like films, it was inhibited in the predominantly amorphous films. The intricate dependence on water vapour adsorption on the crystalline/amorphous ratio of the films provides evidence that evolution may have controlled the ratio of crystalline and amorphous components in the plant cell wall in order to optimize the integrity and swelling response of the plant cell.

Overall, the findings within this thesis not only provide new insight into the self-assemblies of cellulose-based materials as a result of several parameter variations but also confirm those which have been reported previously. Although purely fundamental, work of the kind presented here is an essential part in the development of new materials based on cellulose.


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