

# **Bio-based preparation of nanocellulose and functionalization using polyelectrolytes**

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**Till Mio**



## Sammanfattning

Nanocellulosa, som kan utvinnas från skogsråvara, har de senaste åren fått mycket uppmärksamhet för sina intressanta egenskaper och breda användningsområde. Studierna i denna avhandling syftar till att vidga möjligheterna att använda nanocellulosa i olika applikationer. Detta har skett genom att utveckla en ny metod för att tillverka nanocellulosa och genom att studera möjligheten att adsorbera polyelektrolyter på material av nanocellulosa för att ändra hur bakterier interagerar med dessa. Nanocellulosan tillverkades genom att förbehandla pappersmassa med smält oxalsyra dihydrat. Reaktionsblandningen tvättades med etanol, aceton eller tetrahydrofuran innan den torkades och fibrillerades. Den resulterande nanocellulosan erhöles med högt utbyte, hade hög ytladdning (upp till  $1,4 \text{ mmol g}^{-1}$ ) och innehöll partiklar som både liknade nanofibriller och nanokristaller. Materialet visades kunna användas både för att tillverka Pickering emulsioner och tunna filmer med en styrka upp till 197 MPa, töjning upp till 5 %, E-modul upp till 10,6 GPa och syrepermeabilitet ner till  $0.31 \text{ cm}^3 \mu\text{m}^{-2} \text{ dag}^{-1} \text{ kPa}^{-1}$ .

Genom att adsorbera polyvinylamin och polyakrylsyra på material av nanocellulosa visades det vara möjligt att påverka mängden bakterier som fäster till materialet. Substraten bestod både av kompakta filmer och porösa aerogeler. Genom att variera ytladdningen på materialen, ytans struktur och antalet adsorberade lager av polymererna var det möjligt att tillverka material med både hög och låg bakterieadhesion. Detta gör det möjligt att anpassa material för användning antingen som kontaktaktivt- eller icke-adhesivt antibakteriellt material. Båda dessa kan vara miljövänliga alternativ till dagens antibakteriella material.

Nanocellulosa är ett material som inom snar framtid sannolikt kommer användas inom en mängd olika applikationer. För att öka mängden applikationer där nanocellulosa tillför ett stort värde är det nödvändigt att utveckla alternativa tillverkningsmetoder till dagens välkända, exempelvis, genom att använda den beskrivna oxaleringen som förbehandling. Förmågan att styra bakterieadhesionen på material av nanocellulosa ger därtill möjlighet att hitta nya användningsområden inom t.ex. hälso- och sjukvårdsbranschen.

Nyckelord: Cellulosa nanofibriller, cellulosa nanokristaller, oxalsyra, polyvinylamin, lager på lager, cellulosa oxalat, antibakteriell



## Abstract

Nanocellulose is a material which can be extracted from wood, and in recent years it has received great attention for its interesting properties and wide range of possible applications. With the aim of further expanding the applications of nanocellulose, this work has studied a new way to produce nanocellulose as well as the possibility of using polyelectrolyte adsorption to alter the interaction with bacteria of materials made from nanocellulose.

Nanocellulose was produced by a novel concurrent esterification and hydrolysis of wood pulp in molten oxalic acid dihydrate. The resulting mixture was washed using ethanol, acetone or tetrahydrofuran before the cellulose oxalate was dried and fibrillated. The nanocellulose obtained with a high yield had a high surface charge (up to  $1.4 \text{ mmol g}^{-1}$ ) and contained particles with a morphology similar to both cellulose nanocrystals and cellulose nanofibrils. The material was used to prepare both Pickering emulsions and thin films with a strength of up to 197 MPa, a strain at break of up to 5 %, a modulus of up to 10.6 GPa and an oxygen permeability as low as  $0.31 \text{ cm}^3 \mu\text{m m}^{-2} \text{ day}^{-1} \text{ kPa}^{-1}$ .

Polyelectrolyte adsorption of polyvinylamine and polyacrylic acid was used to modify materials made from nanocellulose. Materials in the form of films and aerogels were used as substrates. By altering the surface charge of the material, the surface structure and the number of layers of polyvinylamine/polyacrylic acid adsorbed, it was possible to prepare materials with both high and low bacterial adhesion. By changing the material properties it is possible to tailor materials with either contact-active or non-adhesive antibacterial properties, both of which are sustainable alternatives to the currently used antibacterial materials.

Nanocellulose is a material which in the near future will probably be used in many applications. In order to improve the suitability of nanocellulose in certain applications it will be necessary to use production methods which differ from the existing methods, for example by using oxalation as a pre-treatment. By modifying the bacterial adhesion to materials prepared from nanocellulose, new medical and health applications emerge.

**Keywords:** cellulose nanofibrils, cellulose nanocrystals, oxalic acid, polyvinylamine, layer by layer, cellulose oxalate, antibacterial



## List of appended papers

This thesis is based on the work presented in the following papers:

### Paper I

Esterification and hydrolysis of cellulose using oxalic acid dihydrate in a solvent-free reaction suitable for preparation of surface-functionalized cellulose nanocrystals with high yield

*Dongfang Li, Jonatan Henschen, Monica Ek*

*Green Chemistry* 19 (2017) 5564–5567

DOI: 10.1039/c7gc02489d

### Paper II

Preparation of cellulose nanomaterials via cellulose oxalates

*Jonatan Henschen, Dongfang Li, Monica Ek*

*Carbohydrate Polymers* 213 (2019) 208-216

DOI:10.1016/j.carbpol.2019.02.056

### Paper III

Bacterial adhesion to polyvinyl-amine-modified nanocellulose films

*Jonatan Henschen, Per A. Larsson, Josefin Illergård, Monica Ek, Lars Wågberg*

*Colloids and Surfaces B: Biointerfaces* 151 (2017) 224–231

DOI:10.1016/j.colsurfb.2016.12.018

### Paper IV

Contact-active antibacterial aerogels from cellulose nanofibrils

*Jonatan Henschen, Josefin Illergård, Per A. Larsson, Monica Ek, Lars Wågberg*

*Colloids and Surfaces B: Biointerfaces* 146 (2016) 415–422

DOI: 10.1016/j.colsurfb.2016.06.031

## **Contributions to the publications**

- Paper I** Planned and analysed the experimental work together with Dr. Li. Assisted in the experimental work and in writing the manuscript.
- Paper II** Planned, and performed the experimental work and analysed the results together with Dr. Li. Contributed by homogenizing all samples, performed FTIR, AFM, SEM, mechanical testing and oxygen permeability testing. Principal author of the manuscript.
- Paper III** Planned and performed the experimental work and analysed the results. Principal author of the manuscript.
- Paper IV** Planned and performed the experimental work and analysed the results. Principal author of the manuscript.

## Related work and contributions to conferences

Cellulose-based water purification using paper filters modified with polyelectrolyte multilayers to remove bacteria from water through electrostatic interactions

*Anna Ottenhall, Jonatan Henschen, Josefin Illergård and Monica Ek*  
Environmental Science: Water Research & Technology 4 (2018) 2070-2079

DOI: 10.1039/c8ew00514a

Antibacterial surface modification of nanocellulosic materials

*Jonatan Henschen, Josefin Illergård, Per Larsson, Monica Ek, Lars Wågberg*

249th ACS National Meeting, Denver, USA (2015)

Water purification using eco-friendly antibacterial fibres

*Anna Ottenhall, Jonatan Henschen, Josefin Illergård and Monica Ek*  
International Symposium on Wood Fibre and Pulping Chemistry, Vienna, Austria (2015)

Antibacterial aerogels from cellulose nanofibrils

*Jonatan Henschen, Josefin Illergård, Per Larsson, Monica Ek, Lars Wågberg*

251th ACS National Meeting, San Diego, USA (2015)

A quick and simple method to prepare functionalized cellulose nanocrystals with high yield

*Jonatan Henschen, Dongfang Li, Monica Ek*

7th Nordic Wood Biorefinery Conference, Stockholm, Sweden (2017)

Manufacture of nanocrystalline cellulose using oxalic acid dihydrate,

*Dongfang Li, Jonatan Henschen, Monica Ek*

Swedish patent no. SE539317 (2015)

Manufacture of hydrophobized nanocellulose intermediate as well as hydrophobized nanocellulose,

*Dongfang Li, Jonatan Henschen, Monica Ek*

Swedish patent application no. SE1750767 (2017)



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## Abbreviations

AFM	Atomic force microscopy
CFU	Colony forming units
CNC	Cellulose nanocrystal
CNF	Cellulose nanofibril
DMAc	Dimethylacetamide
DS	Degree of substitution
FCC	Free carboxyl content
GFP	Green fluorescent protein
Lbl	Layer-by-layer
LiCl	Lithium chloride
MCC	microcrystalline cellulose
NMR	Nuclear magnetic resonance
PAA	Polyacrylic acid
PDI	Polydispersity index
PVAm	Polyvinylamine
SDG	Sustainable Development Goal
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
TCC	Total carboxyl content
TEM	Transmission electron microscopy
TEMPO	2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
UN	United Nations

## Summary of samples

### Cellulose oxalate

DISS-ACE-35	Dissolving pulp, acetone wash, 35 min reaction
DISS-ACE-60	Dissolving pulp, acetone wash, 60 min reaction
DISS-ETOH-35	Dissolving pulp, ethanol wash, 35 min reaction
DISS-ETOH-60	Dissolving pulp, ethanol wash, 60 min reaction
DISS-THF-120	Dissolving pulp, THF wash, 120 min reaction
DISS-THF-15	Dissolving pulp, THF wash, 15 min reaction
DISS-THF-30	Dissolving pulp, THF wash, 30 min reaction
DISS-THF-60	Dissolving pulp, THF wash, 60 min reaction
KRAFT-ACE-35	Kraft pulp, acetone wash, 35 min reaction

KRAFT-ACE-60	Kraft pulp, acetone wash, 60 min reaction
KRAFT-ETOH-35	Kraft pulp, ethanol wash, 35 min reaction
KRAFT-ETOH-60	Kraft pulp, ethanol wash, 60 min reaction

### **Nanocellulose**

NDISS-ACE-35	Prepared from DISS-ACE-35 using microfluidizer
NDISS-ACE-60	Prepared from DISS-ACE-60 using microfluidizer
NDISS-ETOH-35	Prepared from DISS-ETOH-35 using microfluidizer
NDISS-ETOH-60	Prepared from DISS-ETOH-60 using microfluidizer
NDISS-THF-30	Prepared from DISS-THF-30 using ultra-sonication
NDISS-THF-60	Prepared from DISS-THF-60 using ultra-sonication
NKRAFT-ACE-35	Prepared from KRAFT-ACE-35 using microfluidizer
NKRAFT-ACE-60	Prepared from KRAFT-ACE-60 using microfluidizer
NKRAFT-ETOH-35	Prepared from KRAFT-ETOH-35 using microfluidizer
NKRAFT-ETOH-60	Prepared from KRAFT-ETOH-60 using microfluidizer

### **Films**

Crosslinked-40	40 $\mu\text{eq g}^{-1}$ charged fibrils, periodate treated.
Crosslinked-510	510 $\mu\text{eq g}^{-1}$ charged fibrils, periodate treated.
Crosslinked-620	620 $\mu\text{eq g}^{-1}$ charged fibrils, periodate treated
Plain-40	40 $\mu\text{eq g}^{-1}$ charged fibrils, membrane filtered and dried.
Plain-510	510 $\mu\text{eq g}^{-1}$ charged fibrils, membrane filtered and dried.
Plain-620	620 $\mu\text{eq g}^{-1}$ charged fibrils, membrane filtered and dried.
Structured-40	Fibrils with charge of 40 $\mu\text{eq g}^{-1}$ , dried between wire meshes at high pressure.

### **Aerogels**

Filt-1	Modified at 1 g L <sup>-1</sup> PVAm/PAA using filtration.
Filt-5	Modified at 5 g L <sup>-1</sup> PVAm/PAA using filtration.
Sol-1	Modified at 1 g L <sup>-1</sup> PVAm/PAA in excess solution.



## 1. Introduction

We are living in a time where human beings in large parts of the world severely pollute and deplete the resources from our planet, while others still struggle to achieve decent living conditions. The average Swede has a lifestyle that requires 4.2 earths to sustain.<sup>1</sup> At the same time, over 700 million people<sup>2</sup> are living in poverty without access to basic needs such as clean water. In 2016, all UN member states agreed on the Agenda 2030, containing 17 sustainable development goals (SDGs). The main purpose of Agenda 2030 is to provide basic living conditions for all, without further damaging our planet and the life we have here. The SDGs cover aspects ranging from eliminating poverty and providing clean water for all to responsible consumption and life on land.<sup>3</sup> In order to achieve these goals, it is necessary to thoroughly rethink how we produce, use and dispose of the things we consume, while at the same time looking further to develop new sustainable products which can continue to improve the global living conditions.

With over 2/3 of Sweden covered by forest, wood and the main component of wood, cellulose, is a resource which will play a key role in reaching the goals of Agenda 2030. Wood is a renewable resource that, with well managed forests, can be sustainably utilized to produce a wide range of chemicals, materials and fuels. These products can be both alternatives to fossil-based products and totally new products with new properties that solve today's problems.



## 2. Background

### The components of wood

Nature often has very smart ways to construct things and an excellent example of this is the wood in trees. Wood is constructed from different components that together form a hierarchical structure with structural elements ranging from a nanometer to a meter scale (Figure 1). This is similar to many high-performance materials that humans synthetically produce, for example carbon fibre composites. Trees are large organisms made up of different types of plant cells, some of which are also known as wood fibres. The wood fibres are surrounded by a protective cell wall which provides the tree with many of the characteristics typical for wood



Figure 1. The hierarchical structure of wood ranging from cellobiose, the repeating unit in cellulose, to the elementary fibril, fibril aggregate, wood fibre and tree trunk. (© Carl Moser)

such as strength. The cell wall is a layered structure consisting of three main components: cellulose, lignin and hemicellulose.<sup>4</sup>

The proportions of the wood components vary between different species, as well as with the location and environment in which the tree grows. Generally 40 – 50 % of the wood is composed of cellulose, a linear chain of 8 000 – 10 000 glucose units<sup>5</sup>, forming a polysaccharide. Each glucose unit in cellulose has three hydroxyl groups which are crucial in forming the structures making up wood. These hydroxyl groups make it possible to arrange the cellulose chains into sheets when placed next to each other. Through Van der Waal bonds, these sheets can in turn be stacked onto each other to produce long fibril structures.<sup>6</sup> The elementary fibrils in wood have a width between 3 and 5 nm and contain both regions which are crystalline and regions which are amorphous.<sup>6-8</sup> Multiple theories exist describing the structure of these elementary fibrils and the distribution of crystalline and amorphous regions. One theory presented by Larsson *et al.*<sup>9</sup> describes a crystalline core surrounded by less ordered (amorphous) cellulose (Figure 2). Fibrils have a tendency to aggregate to form fibril aggregates. These aggregates contain both crystalline and less ordered (amorphous) surfaces, but they also contain cellulose surfaces which are inaccessible and less ordered (amorphous) in between the fibrils (Figure 2).<sup>9</sup>

The fibril structures are stiff and provide strength to plants. In the cell

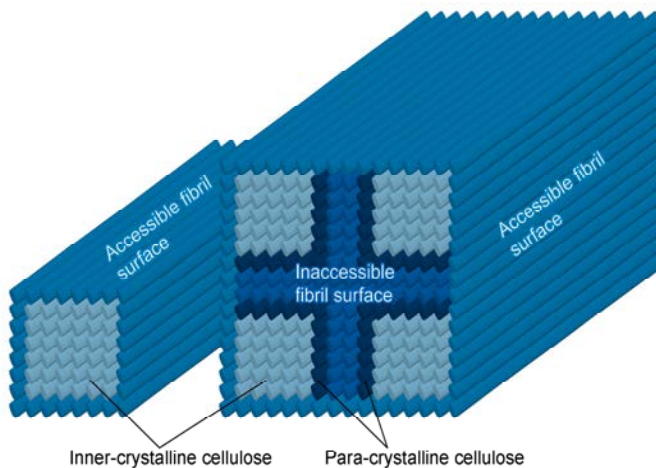


Figure 2. The structure of elementary fibrils (left) and fibril aggregates (right) as proposed by Larsson *et al.*<sup>9</sup> Adapted from Zuckerstätter *et al.*<sup>10</sup>

wall, fibrils are mixed with lignin and hemicelluloses which have important functions such as providing necessary flexibility to the tree and aiding water transportation. Hemicelluloses and lignin have interesting chemistries and high industrial relevance, but the focus of this thesis is on cellulose. The different chemical structures found in wood, many with interesting properties, make it a valuable resource with many uses.

## **Wood as a resource for materials**

Throughout history, wood has been an important resource, but its usage has varied greatly with time. Initially, wood was primarily the main source for heat and construction materials. As technology and understanding of the raw material has developed, so has the use of it. During the 20<sup>th</sup> and 21<sup>st</sup> centuries, wood has been an important resource for making textiles, chemicals and paper products. Many of the materials and chemicals which are currently produced from fossil resources can be produced from wood. In some cases it is even possible to produce products which are difficult, or impossible, to synthesize from fossil-based raw materials.

Paper products are produced through wood pulping; a process where wood chips are treated to separate the wood fibres from each other. This process often involves chemically removing the lignin and partly the hemicelluloses.<sup>11</sup> The processed pulp fibres are excellent for producing hygiene products, as well as printing paper and board products, due to properties such as high water absorbency, high porosity, and strong fibre-fibre interactions. Many of the products produced from pulp fibres are however sensitive to water and humidity, and are difficult to form into complex shapes. After pulping, fibres consist essentially of aggregated fibrils in the shape of a fibre. The large size of these aggregates makes it difficult to fully utilize the full potential, such as mechanical properties, of the individual fibrils. Going from fibres to fibre fragments and smaller components of the fibre, the material gains new properties.

The approach of making fibres into smaller fragments has for long been used to produce micro-crystalline cellulose (MCC).<sup>12</sup> MCC is produced by degrading the cellulose and removing some of the amorphous regions of the cellulose fibrils by, for example, acid-catalysed hydrolysis. This leads to new properties and uses of the cellulose, for example as a thickener in food or as a binder in pharmaceuticals. It is also possible to further reduce the size of the fibres by liberating the building

blocks of the fibre, the fibrils or crystals, producing nanocellulose. The smaller size leads to a greater surface area<sup>13</sup> which is vital for how it interacts with other materials<sup>14</sup> as well as with water. The strong interactions between nanocellulose and other materials make it possible to use wood for completely new applications such as reinforcing plastics<sup>15</sup> or as gas barriers<sup>16</sup>. Using sustainable raw materials from wood for these new applications, it may be possible to get one step closer to Agenda 2030. There are still, however, issues connected to producing nanocellulose, for example the high energy consumption. One of the goals of the work described in this thesis has been to investigate a new sustainable approach to the preparation of nanocellulose.

### **Nanocellulose**

Nanocellulose is a group of materials that consists of isolated cellulose fibrils, fibril aggregates or fragments from fibres which have a width less than 100 nm.<sup>17</sup> Nanocellulose has been highly praised for its properties such as high strength and stiffness<sup>18</sup>, low density<sup>19-20</sup>, insolubility, low thermal expansion coefficient<sup>21-22</sup> and high aspect ratio<sup>20</sup> making it possible to utilize the wood in new ways. Depending on the production process, it is possible to produce either cellulose nanofibrils (CNFs) or cellulose nanocrystals (CNCs) from pulp fibres. CNFs are the cellulose fibrils or fibril aggregates which have been isolated from cellulosic fibres. Essentially they can be produced by extensive milling and by applying high shear forces to the fibres.<sup>23-24</sup> Since this is a very energy demanding process, it is common to use a pre-treatment to reduce the energy required to liberate the fibrils<sup>25</sup>. Enzymatic methods can be used to cleave the cellulose chains into shorter fragments thereby decreasing the energy required to liberate the fibrils.<sup>26-27</sup> Another common chemical pre-treatment is to introduce charges in the cellulose structure which reduce the attractive forces holding the fibrils together, thereby lowering the energy input required to liberate the fibrils. Some of these methods are TEMPO-mediated oxidation<sup>28</sup>, carboxymethylation<sup>29</sup> or phosphorylation<sup>30</sup> of cellulose. The fibrils isolated by these methods are flexible, long (500 – 2 000 nm) and thin (4 – 20 nm)<sup>20</sup>. The hydrophilic nature of cellulose combined with the high surface area of the fibrils results in high affinity to water. Combining the high water affinity with the high aspect ratio results in a material that gives thick gels at low concentrations, as low as 0.4 g L<sup>-1</sup> for some types of CNF.<sup>31</sup>

CNCs on the other hand are produced using acid-catalysed hydrolysis to remove the amorphous regions of the cellulose fibres, leaving the crystalline regions.<sup>12</sup> This is similar to the procedure for producing MCC, but the hydrolysis is allowed to continue further, making it possible to separate the material into crystallites. After the hydrolysis, a highly crystalline (over 98 %) material is prepared, with particle lengths shorter than CNFs (50 – 500 nm) and smaller widths (3 – 5 nm).<sup>20</sup> As the particles consist mainly of crystals, they are rather stiff and non-flexible.

The demand for some of the traditional pulp and paper products has declined in recent years. In order to find new uses for the raw material, the industry has shown an increasing interest in nanocellulose. Many companies, for example Borregaard, Stora Enso and CelluForce, are today carrying out research and commercializing the production of CNCs and CNFs. A lot of these commercialization activities involve optimizing the production and chemical recovery of established procedures to suit large-scale operations. In academia, it is more common to work with new and alternative pathways to produce these types of materials. New production methods often result in changing the properties of the nanocellulose, which in some cases can be problematic and in other cases beneficial.

### **Materials from nanocellulose**

There are many similarities between CNCs and CNFs which sometimes makes it possible for them to provide similar benefits when added to a product. Both CNFs and CNCs are often produced in an aqueous system, making it common also to use the materials in different types of aqueous formulations. Some of the possible uses are in surface coatings<sup>32</sup>, personal care<sup>33-34</sup> and construction materials<sup>35</sup>. In these applications, the nanocellulose can act as a rheology modifier, suspension or emulsion stabilizer or strengthening agent. One issue when nanocellulose is used in this way is that the properties are dependent on the other components in the formulation. Both pH and ionic strength can cause aggregation of the fibrils, and this can greatly reduce the performance of the nanocellulose. Although the use of nanocellulose in aqueous formulations is interesting, it is possible to obtain completely different properties by changing the matrix. Depending both on the choice of matrix and on the preparation method, different properties and applications are obtained.

Nanocellulose is film-forming, which makes it easy to produce a film from a nanocellulose suspension. This can be done by using vacuum to remove the water over a membrane filter<sup>36-37</sup> or by allowing the water

evaporate and solvent-cast a film<sup>38</sup>. Filtration is faster than solvent casting but requires a membrane that easily allows water to pass through while retaining the nanocellulose. The size of the nanocellulose particles makes filtration non-trivial, as particles can clog the membrane pores and CNCs are often small enough to pass through membranes. Solvent casting is a slow and energy-demanding procedure, but the films produced are often of high quality, clear and thin. Films produced from nanocellulose are dense, making them strong, optically transparent and good oxygen barriers. This makes them interesting for a wide range of applications such as food packaging<sup>39</sup> and purification membranes<sup>40</sup>.

When water is removed from a nanocellulose suspension through filtration or evaporation, the fibril network structure collapses, but it is possible to retain the network structure by using freeze drying<sup>41</sup> or critical point drying<sup>13</sup>. These drying methods replace the water in a nanocellulose gel with air and the resulting materials are called aerogels. Aerogels are very lightweight and porous materials with a large surface area and low density,<sup>13</sup> and they are excellent substrates where a large surface area and low density are important, such as for energy storage<sup>42</sup> or thermal insulation<sup>43</sup>. If the aerogels are produced without any type of crosslinking they collapse when they come into contact with water, but the structure can be maintained in water by crosslinking the fibrils using, for example, a multifunctional acid.<sup>42</sup>

Working with nanosized materials always poses challenges and the same is true of nanocellulose. The small size and large surface area gives nanomaterials their interesting properties. In order to obtain their full potential, it is important to retain the size and prevent aggregation. Aggregation of nanocellulose is often prevented in aqueous solutions by ionic charges. Disrupting this stabilization by increasing the ionic strength or changing the pH can lead to aggregation. Charge stabilization also makes it difficult to disperse nanocellulose in less polar solvents. The size and surface chemistry of nanocellulose gives its high affinity to water, and it is time-consuming and energy-demanding to remove water from a nanocellulose suspension.

### **Functionalization of cellulosic materials using polyelectrolyte multilayers**

Cellulose has desirable properties making it a widely used material. Through the addition of new functional groups to cellulose, it is possible to further expand the uses of the material. Functionalization can be

achieved both by altering the chemical structure of the anhydrous glucose unit<sup>44</sup> or by adsorbing functional components onto the materials<sup>45</sup>. Chemical functionalization is often carried out by substituting cellulose hydroxyl groups with esters<sup>46-47</sup> or ethers<sup>48-49</sup>. Chemical functionalization is a good way to change the properties of cellulose and to make possible new applications, but it often requires high temperature, environmentally harmful reagents or solvents, and complex processes. If instead functionalization is achieved through the physical adsorption of a functional polymer, it is possible to achieve new properties in water-based systems at room temperature. Pulp fibres have an inherent negative surface charge originating mainly from residual hemicelluloses and lignin modified during the pulping process,<sup>50</sup> and nanocellulose often has these charges preserved unless a chemical treatment is adopted to increase it. The charge makes it possible to adsorb oppositely charged molecules and particles to the surface.<sup>51</sup> One group of such molecules is polyelectrolytes.

Polyelectrolytes are polymers containing chemical groups in its repeating unit which can be charged. They are excellent agents for functionalizing surfaces as they are adsorbed onto charged surfaces through irreversible entropy-driven mechanisms.<sup>52</sup> Polyelectrolyte adsorption can be used to prepare materials from cellulose fibres with specific mechanical properties<sup>53</sup>, electrical properties<sup>54-55</sup>, combustion properties<sup>56</sup> or bioactivity<sup>57</sup>. Polyelectrolytes can be adsorbed either as monolayers or as multilayers. Depending on the conditions, it is possible to adsorb the polyelectrolytes in different conformations. If the ionic strength of a polyelectrolyte solution is high it will be adsorbed in a coiled conformation, whereas low ion strength will lead to a flat conformation. The same effect can be achieved by changing the pH, or the degree of ionization; a high degree of ionization will lead to a flat conformation whereas a low will cause it to coil. The conformation controls the amount of polyelectrolyte it is possible onto adsorb on a surface. Many properties are dependent on the concentration of the active functional group, often making it feasible to adsorb polyelectrolytes as multilayers. This can be done using an approach called layer-by-layer (lbl).

Lbl, a method described by Decher<sup>51</sup>, is a procedure where layers of polyelectrolytes are adsorbed onto an oppositely charged surface producing a charge reversal on the surface. For example, when a cationic polyelectrolyte is adsorbed onto an anionic surface, it is possible to adsorb an excess of cationic charges, thereby reversing the charge of the

surface so that it becomes cationic. This change in surface charge enables the adsorption of an anionic polyelectrolyte and thereby the surface charge is again reversed. The alternating adsorption can be continued and multilayers can be created. Pulp fibres are often modified by this procedure by, for example, submerging them in different solutions followed by filtration. Suspended nanocellulose is too small to be easily recovered if added to different solutions and it has a tendency to aggregate in polyelectrolyte solutions. To adsorb polyelectrolytes onto nanocellulose is preferably done after producing a macroscopic material.

### **Antibacterial applications of cellulosic materials**

When new antibacterial materials are being developed it is vital to understand how it is possible to regulate bacterial interaction and to have good strategies to prevent bacteria growth if necessary. Three different mechanisms are used to prepare antibacterial materials. The first way is to create a surface to which bacteria do not adhere.<sup>58</sup> Non-adhesive materials prevent the formation of biofilms on surfaces but they do not kill bacteria. The second and easiest way to prepare an antibacterial material is by incorporating a biocide which is slowly leached out to the surface during the lifetime of the material.<sup>59-60</sup> This is an efficient way to prevent bacterial growth. The problem is that the leaching of biocides leads to the spreading of these antibacterial compounds in the environment, resulting in multiple issues. Exposing bacteria to a low concentration of biocides has been shown to promote the development of resistance, both to the specific biocide and also to other compounds such as antibiotics.<sup>61-62</sup> Many biocides have also been shown to affect aqueous organisms, and this may eventually lead to changes in the ecosystem, even at low concentrations.<sup>63-64</sup>

The third way to produce an antibacterial material is to create a contact-active antibacterial surface. Such a surface only kills bacteria when they are in contact with the surface, thereby eliminating the problems with leaching materials.<sup>65</sup> There are various types of antibacterial surfaces; some are, for example, based on specific antibacterial functional groups<sup>66</sup> or specific surface structures<sup>67-68</sup>. Polyvinylamine (PVAm) is a polyelectrolyte which has been shown to have antibacterial properties. PVAm can be adsorbed in multilayers by combining it with an anionic polyelectrolyte, and this makes it possible to produce antibacterial materials, which are contact-active using only aqueous solutions at room temperature.<sup>69</sup>

### **3. Objectives**

Nanocellulose has a great potential to be a sustainable alternative in many applications. Today however there are still problems with the commercial production of most types of nanocellulose and only a limited number of commercial applications have been presented. The production process can be improved by both optimization and differentiation. By differentiating the nanocellulose production it is probably possible to identify specific applications where the new nanocellulose has advantages over to others. In certain applications, it is necessary to surface functionalize materials made from nanocellulose to have a good fit, despite the initially good properties.

The studies described in this thesis aim at solving the above problems by evaluating a new production process for nanocellulose which differs largely from the established ones, and by functionalizing nanocellulosic materials using polyelectrolyte adsorption. The goal is to present a process which facilitates the production of nanocellulose and produces nanocellulose with desirable properties. By studying how polyelectrolytes are adsorbed onto materials from nanocellulose, it is possible to introduce a wide range of properties onto these materials, which will increase the suitable uses.



## 4. Preparation of cellulose oxalates

*Chapter 4-7 briefly describes the experimental work. For more detailed protocols, the reader is referred to the attached papers.*

Many everyday items, such as frames of eyeglasses, textiles and food additives, can be made from cellulose derivatives. By substituting some of the hydroxyl groups, the properties of cellulose can be completely changed making it, for example, water-soluble or thermoplastic. Derivatives can also be a starting point for further derivatization and new properties. In most cellulose derivatives, the hydroxyl group has been substituted with either an ether or an ester.

A common way to prepare esters is by reacting an alcohol with a carboxylic acid in a reaction called Fisher esterification. The reaction is reversible. To increase the yield it is common to have a large excess of the alcohol or to use it as both solvent and reagent. The hydroxyl groups on cellulose can be used for Fisher esterification, but as the concentration of hydroxyl groups is more or less fixed, it is necessary to have a high concentration of carboxylic acid groups in order to achieve a high degree of substitution (DS). There are many examples where cellulose and aqueous solutions of organic acids have reacted, leading mainly to acid hydrolysis of the cellulose and limited esterification. Other experiments using, for examples, acetic acid<sup>70</sup>, citric acid<sup>71</sup> and formic acid<sup>72</sup> have managed to produce different types of cellulose esters using Fisher esterification.

Gardea-Hernandez *et al.*<sup>73</sup> reported that it is possible to esterify wood chips using a melt of oxalic acid dihydrate. Since oxalic acid dihydrate melts at 101 °C, it is possible to utilize the organic acid in Fisher esterification, as both a liquid medium and reagent while having only 29 % water in the system. The low water content makes the simultaneous hydrolysis and esterification (Figure 3) of the hydroxyl groups possible. Wood is a complex material with many components that can take part in side reactions or inhibit the esterification of cellulose, but with wood pulp as starting material it is possible to maintain better control over the reaction and to produce more defined materials. Preparing cellulose

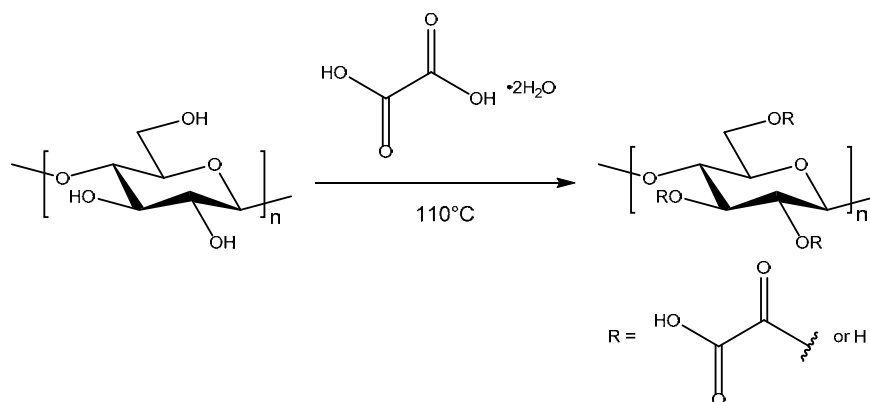


Figure 3. Esterification reaction between cellulose and oxalic acid dihydrate. Note: the acid-catalysed hydrolysis of cellulose is not shown.

oxalate through the reaction between molten oxalic acid dihydrate and wood pulp was first described in a Swedish patent<sup>74</sup> with the author of this thesis as one of the inventors. Later, the process was more thoroughly described in Papers I and II. Other procedures for producing cellulose oxalates using a low water content have been published by e.g. Chen *et al.*<sup>75</sup>, who reacted wood pulp with 70 % oxalic acid dehydrate diluted with 30% water and by Sirvio *et al.*<sup>76</sup>, who prepared a deep eutectic solvent by mixing oxalic acid dehydrate with choline chloride. Both these studies gave a material similar to the material presented in this thesis, but there are differences in the procedures which change the specific characteristics of the material produced.

## The production of cellulose oxalate

Cellulose oxalate was produced by mixing torn wood pulp sheets with oxalic acid dihydrate at a weight ratio of 1:3.8 and submerging them in an oil bath at 110 °C. The pulp and acid were continuously mixed for between 15 and 120 minutes with reflux before the mixture was allowed to cool while still being mixed. The first sign of a reaction is that the acid melts and wets the wood pulp and the pulp then gradually changes colour from white to brown. The acid solidifies again when the mixture is cooled down after the reaction. A large excess of acid is necessary to provide enough liquid to fully wet the fibres. To remove the excess acid, the mixture was washed using Tetrahydrofuran (THF), ethanol or acetone using either soxhlet extraction or solvent extraction followed by filtration.

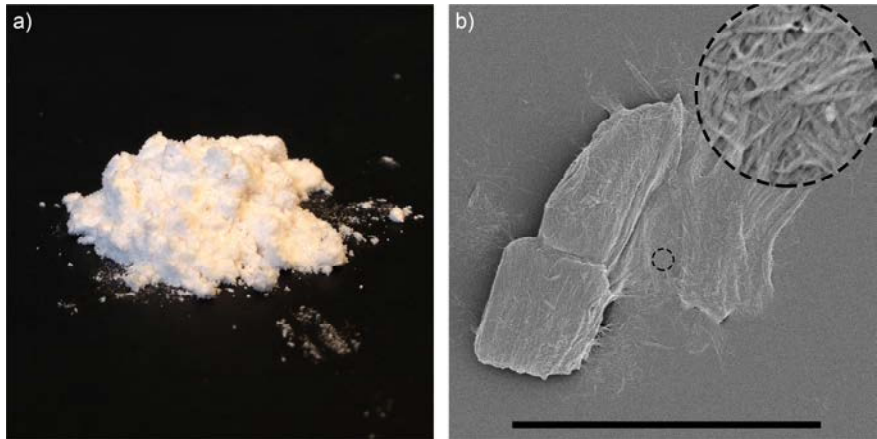


Figure 4. Cellulose oxalate after removing excess oxalic acid dihydrate and drying under ambient conditions, a) photograph and b) SEM micrograph. The inset in the micrograph shows a magnification of the circled area. The scale bar represents 10  $\mu\text{m}$  in the micrograph.

Eventually the remaining product was dried under ambient conditions, resulting in a fine powder (Figure 4). Samples were prepared using both softwood sulphite dissolving pulp and bleached softwood kraft pulp as raw material, as shown in Table 1. The dissolving pulp prepared from a mixture of spruce and pine was very pure with a cellulose content of  $\geq 96$  wt. %. This makes it suitable for derivatization as there are only low amounts of other components that may affect the reaction. The kraft pulp prepared from spruce has a higher content of hemicellulose, with the cellulose content being 84 wt. %. Kraft pulp is cheaper than dissolving pulp which means that it is desirable, if possible, to use kraft pulp in industrial processes.

Table 1. The cellulose oxalate samples produced.

	Reaction time [min]	Solvent and method used for washing
<b>Softwood dissolving pulp</b>		
DISS-THF-15	15	THF (soxhlet extraction)
DISS-THF-30	30	THF (soxhlet extraction)
DISS-ETOH-35	35	Ethanol (filtration)
DISS-ACE-35	35	Acetone (filtration)
DISS-THF-60	60	THF (soxhlet extraction)
DISS-ETOH-60	60	Ethanol (filtration)
DISS-ACE-60	60	Acetone (filtration)
DISS-THF-120	120	THF (soxhlet extraction)
<b>Bleached softwood kraft pulp</b>		
KRAFT-ETOH-35	35	Ethanol (filtration)
KRAFT-ACE-35	35	Acetone (filtration)
KRAFT-ETOH-60	60	Ethanol (filtration)
KRAFT-ACE-60	60	Acetone (filtration)

## Characterization of cellulose oxalate

The cellulose oxalates prepared forms a fine powder consisting of fibre fragments about 10  $\mu\text{m}$  in size (Figure 4). Micrographs of the powder also show that the treatment with oxalic acid dihydrate has partially liberated the fibrils in the fibre fragments. This is due to a combination of acid hydrolysis and esterification of the cellulose.

### Molecular weight

The severity of the acid-catalysed hydrolysis of cellulose was investigated by dissolving the samples in a solution of lithium chloride/dimethylacetamide (LiCl/DMAc) followed by size exclusion chromatography (SEC). The results show that the molecular weight dropped from almost 400 kDa to 40 kDa in the first 15 minutes (Figure 5), and that extending the time did not significantly affect the molecular weight. The polydispersity index (PDI) of the samples also decreased mainly during the first 15 minutes. A lower PDI indicates a more homogeneous material. A decrease in PDI combined with a decrease in molecular weight suggests that the hydrolysis occurs rapidly in the amorphous regions, leaving the less reactive crystalline regions intact.

### Esterification

One advantage of using highly concentrated oxalic acid is that it is possible to form cellulose oxalate esters. At low concentration, the solvent may hydrolyse the ester bond formed. There are both direct and indirect

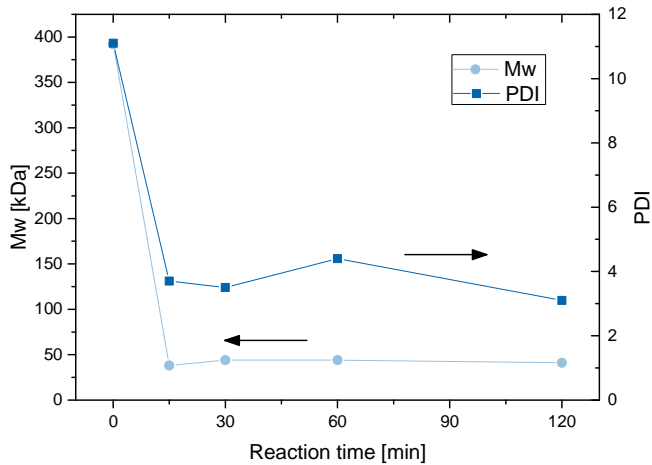


Figure 5. Molecular weight and PDI of samples prepared from softwood dissolving pulp, washed with THF. The samples were dissolved in LiCl/DMAc before SEC; pullulan standards were used for calibration.

ways to detect the result. In the work described in this thesis, a direct method was used to dissolve the product and analyse it using  $^{13}\text{C}$  NMR.<sup>77</sup> Oxalic acid has two symmetrical carbons, making it only possible to detect them as a single peak at 161 ppm using  $^{13}\text{C}$  NMR.<sup>78</sup> When the oxalic acid esterifies cellulose, one of these carbons changes shift as it changes from being a carboxylic acid to being an ester. As seen in Figure 6, the signals corresponding to the cellulose structure are found between 60 and 103 ppm. The carbon in the carboxylic acid of oxalic acid is detected at 174 ppm and the carboxylate ester at 157 ppm. The carboxylic acid is expected to be related to a higher shift than the carboxylate ester due to its lower electron density. In the structure of cellulose, there are three possible locations for esterification at C2-OH, C3-OH and C6-OH. From the NMR data it is not possible to tell where the ester is located on the cellulose, but it is most likely located at C6 which is more accessible and more reactive than the others.<sup>79</sup>

From the NMR data, it is not possible to accurately determine the degree of substitution of the cellulose. This can instead be done by using conductometric titration, a method where the charge of a material is calculated by monitoring the conductivity while titrating carboxylic acid groups with sodium hydroxide.<sup>80</sup> The change from the protonated to the sodium form of the carboxylic acid groups is recorded and makes it possible to calculate the free carboxylic content (FCC), or total charge of the material. As there is a potential for the oxalic acid to act as a cross-linker between cellulose chains, it is possible that the amount of ester is not equal to the amount of carboxylic acid and that the DS is underestimated. To determine the amount of crosslinking, all the esters were hydrolysed followed by a second conductometric titration.<sup>81</sup> This makes it possible to quantify the total carboxyl content (TCC) in the sample and calculate to the ratio of free carboxylic groups to total carboxylic groups. A system where all oxalates has one free acid group and one carboxyl ester bound to cellulose will give a ratio of 0.5 between the free groups and the total groups. A lower value indicates some degree

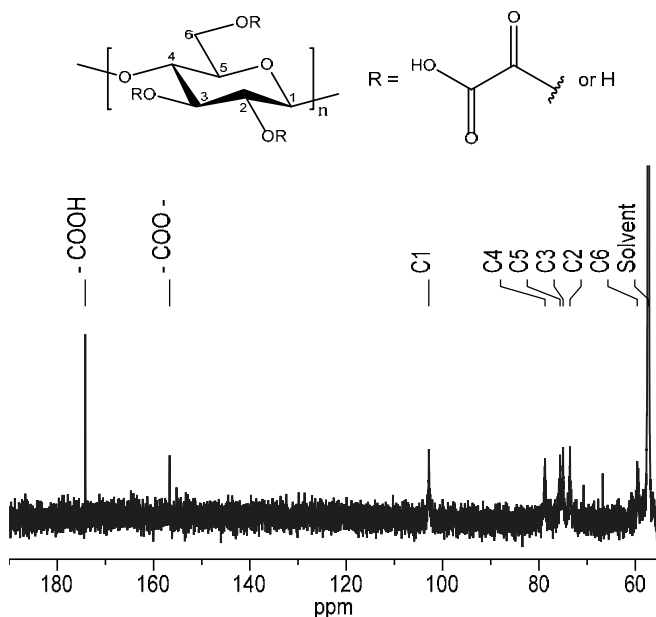


Figure 6. <sup>13</sup>C NMR spectra of cellulose oxalate (DISS-THF-15) dissolved in a solution of tetrabutylammonium fluoride trihydrate in DMSO-d<sub>6</sub>. The top image shows the structure and labelling of the carbons in cellulose oxalate.

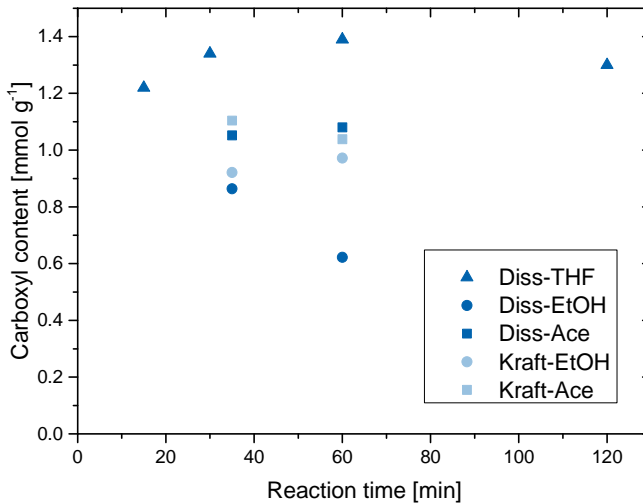


Figure 7. Free carboxyl content of cellulose oxalates determined by conductometric titration.

#### of crosslinking.

The FCC was determined for all the samples, and the TCC for the samples washed with THF (Figure 7). Samples washed with THF had the highest FCC; the highest sample being DISS-THF-60 at 1.4 mmol g<sup>-1</sup>. Samples washed with acetone and ethanol had a FCC between 0.85 and 1.1 mmol g<sup>-1</sup>, except for DISS-ACE-60 which appears to be an outlier at 0.62 mmol g<sup>-1</sup>. For the samples where the TCC was determined, the ratio after 15 minutes was 0.47 and this decreased to 0.42 as time continued. This means that between 84 % and 94 % of the available oxalates have a free carboxylic acid moiety which contributes to the FCC. A FCC of 1 mmol g<sup>-1</sup>, and 84 % free carboxyl groups corresponds to a DS of 0.17, indicates that there is on average one oxalate ester on approximately every 6<sup>th</sup> glucose unit. A surface charge of ca. 1 mmol g<sup>-1</sup> is comparable to the surface charge of many cellulose derivatives used to prepare nanocellulose. Carboxymethyl cellulose can be dispersed into nanocellulose at a surface charge of ca. 0.5 mmol g<sup>-1</sup>.<sup>29</sup> Nanocellulose prepared using TEMPO-mediated oxidation produces nanocellulose with a charge of roughly between 0.5 mmol g<sup>-1</sup> and 1.5 mmol g<sup>-1</sup>.<sup>82-83</sup>

According to the fibril model presented in the Background, approximately 5 % of the cellulose chains are accessible on the fibril aggregates.<sup>9, 84</sup> It is believed that the cellulose esterification occurs at only

C6-OH, so that a theoretical maximum DS, provided there is no swelling or other pre-treatment, is 0.05. As the DS of all the samples was considerably higher than 0.05, it appears that the oxalic acid is able to swell the aggregates and that esterification can occur on the amorphous but inaccessible surfaces between the fibrils, in which case the maximum theoretical DS is 0.25-0.5.

No clear trend regarding the FCC after the first 15 minutes could be observed, most of the samples had rather similar FCC values, which indicates that the esterification reaction is rapid and that prolonging the reaction does not lead to any significant increase in the degree of esterification.

One interesting trend which can be seen is that the samples washed with THF had a much higher FCC than the samples washed with acetone or ethanol. Samples washed with ethanol had a lower FCC than samples washed with acetone. Of these solvents, THF is the least polar and ethanol the most polar. When the cellulose oxalates are washed with polar solvents it is probable that short cellulose oxalate fragments with high surface charge may either dissolve in the solvent and be washed away or disperse into small particles, as seen in Figure 5, which either pass through the filter or are trapped on it, and are thereby removed from the product.

The FCC obtained in the cellulose oxalates is much higher than values reported by Chen *et al.*<sup>75</sup> and by Sirvio *et al.*<sup>76</sup> who reported a maximum of 0.39 mmol g<sup>-1</sup>. This could be because these authors added water to quench the reaction after the specified time. Adding water to a warm cellulose oxalate may hydrolyse the ester and thereby reduce the FCC. Depending on the application of cellulose oxalate, a high FCC may be desirable, for example if the material is to be used for the preparation of nanocellulose.

### **Gravimetric yield**

The gravimetric yield was calculated by dividing the mass of the product by the mass of the raw pulp added to the reaction. This way of calculating the yield, which is common in cellulose chemistry, makes it theoretically possible to have a yield over 100 % since esterification of the cellulose increases the mass of the pulp. The yield of the samples varied from 84 % to 99 % (Figure 8), where the samples washed with acetone and THF had a higher yield than the samples washed with ethanol. As mentioned

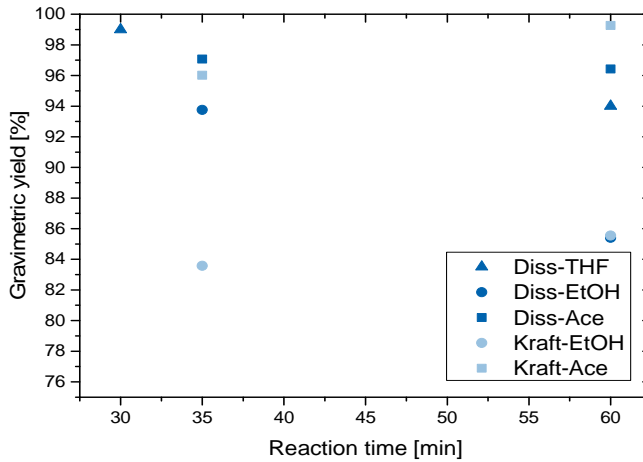


Figure 8. Gravimetric yield of the cellulose oxalates. No yield was obtained for DISS-THF-15 and DISS-THF-120.

above, this may be because ethanol is a more polar solvent and is able to remove highly charged and short cellulose oxalates.

Samples prepared from dissolving pulp had a tendency to decrease in yield with increasing time while, samples prepared from kraft pulp showed the opposite trend. Prolonging the reaction time is believed to increase the hydrolysis of dissolving pulp, making it possible for highly charged and short cellulose fragments to dissolve during washing. Removing these fragments decreases the yield. No clear difference can be observed regarding the yields of kraft pulp and dissolving pulp, despite their having different cellulose contents. Dissolving pulp contains more than 96 % cellulose and kraft pulp contains 84 % cellulose, and the rest is mainly hemicellulose. If only cellulose oxalate were present in the product, the yield of the kraft pulp would be lower, but since this is not observed, hemicellulose oxalates are probably also present in the product.

### Thermal properties

Some applications require materials that are capable of withstanding some degree of heat before they start to degrade. A common way to measure this is to slowly heat the sample while accurately recording the weight loss as it degrades by thermogravimetric analysis (TGA). This analysis can be used to determine both when the material starts degrading and the temperature at which the material degrades most rapidly. The initial degradation temperature for all the tested samples

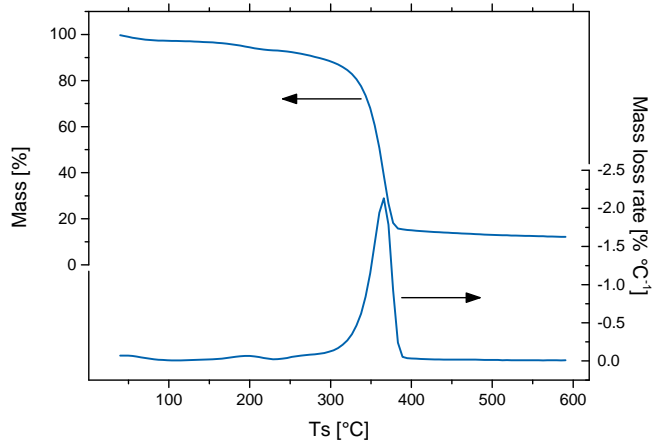


Figure 9. Thermal degradation of cellulose oxalate (DISS-ACE-60) determined by TGA. The left-hand axis shows the mass loss and the right-hand axis shows the derivative of the mass loss (i.e. the rate). All the samples had thermal decomposition profiles similar to that of the above sample.

was between 173 and 177 °C, and the samples showed three main regions with large weight losses (Figure 9). The first was between 30 and 130 °C, water adsorbed in the samples was removed.<sup>85</sup> In the second region, between 175 and 280 °C, the chemically attached oxalate groups degraded and the cellulose was dehydrated. The third and largest weight loss was between 280 and 375 °C, related to depolymerization of the cellulose.<sup>86</sup> The degradation of cellulose in the cellulose oxalate is rather similar to the degradation of pulp fibres<sup>87</sup> suggesting that oxalic acid does not catalyse the degradation of cellulose to any large extent, as can be observed in, for example, cellulose sulphate. This is presumably because the acid degrades before the cellulose.<sup>88</sup> The initial degradation point indicates the temperature range at which the material can be processed without any chemical change in the material. With a temperature of about 175 °C, it could be possible to process cellulose oxalate together with certain thermoplastics to prepare composites. Some thermoplastics with a low processing temperature are low density polyethylene, polystyrene, and polyvinylchloride.

## Summary of preparation of cellulose oxalates

By taking advantage of the low boiling point of oxalic acid dihydrate, it is possible to simultaneously hydrolyse and esterify cellulose into cellulose

oxalate in a bulk reaction. Due to the small amount of water present, it is possible to achieve a high degree of substitution and consequently a high content of carboxylic acid functionalities. The cellulose oxalate is prepared at a high yield and, as the reaction is performed with little water, it is not necessary to employ energy-intensive drying to remove excess water and obtain the product as a powder. Compared to similar methods, the product obtained has a higher surface charge, the method is simpler and the product is easily dried, which increases the flexibility of how to use it. The combination of high carboxyl content and the removal of the amorphous regions make the material a good candidate for the preparation of nanocellulose.



## 5. Nanocellulose from cellulose oxalate

Nanocellulose can be prepared in different qualities. The quality is often poor, for example, if it is prepared only by mechanical fibrillation, so that it contains a large amount of fibril aggregates and has a low transparency and low viscosity. High quality nanocellulose contains well dispersed fibrils and forms a clear suspension in water.<sup>89</sup> In order to produce well-dispersed CNFs or CNCs, it is advantageous to perform a chemical treatment which increases the charge of the cellulose before fibrillating the fibres. This also greatly reduces the energy requirement to produce CNFs or CNCs. Many different pre-treatments may be used in the preparation of CNF, for example TEMPO-mediated oxidation<sup>82</sup> or carboxymethylation<sup>29</sup>. Following the pre-treatment, the derivatized pulp fibres need to be homogenized using, for example, a microfluidizer which is an instrument where the material is pumped through narrow channels at high pressure creating high shear forces. These forces tear apart the fibril aggregates in the pulp fibres liberating the fibrils which are then stabilized by the electrostatic interactions introduced by the pre-treatment. It is possible to pass the material through a microfluidizer multiple times, and this increases the degree of fibrillation but also the energy consumption.<sup>27, 90</sup>

Well dispersed CNCs are often prepared using sulphuric acid<sup>12</sup> to hydrolyse the amorphous regions of cellulose, and this also leads to the formation of cellulose sulphate. The sulphate groups are anionic in aqueous conditions and aid in stabilizing and liberating the crystals.<sup>91</sup> After the chemical treatment, the material is often dispersed using ultrasonication, a method where high intensity sonic waves are used to create pressure variations in a liquid medium. These pressure variations disrupt the fibril aggregates and disperse CNCs. When the CNCs have been dispersed, they are stabilized by the ionic charge of the sulphate groups.

To the best of the author's knowledge, wood treated with oxalic acid dihydrate has before the work in this thesis only been used to prepare hydrophobic wood.<sup>73</sup> Cellulose oxalates are however excellent candidates for preparing nanocellulose. The cellulose derivative has been hydrolysed, reducing the molecular weight, and also has high surface charge, both of

which usually promote easy fibrillation of the nanocellulose. As seen in Figure 4, the fibre fragments start to fibrillate even before they have been exposed to a high shear force.

## Preparation of nanocellulose from cellulose oxalate

Nanocellulose was prepared by homogenizing cellulose oxalates. Except for DISS-THF-30 and DISS-THF-60, which were ultra-sonicated, all samples were prepared using microfluidization. Prior to sonicating or microfluidizing, the cellulose oxalates were dispersed in water and the pH was set to around 9.5 in order to deprotonate the carboxylic acids, which is crucial if it is to be possible to utilize the charge stabilization effect of the carboxylic acid group. After homogenization, the prefix “N” was added as a prefix to respective cellulose oxalate sample. Samples prepared through ultra-sonication were centrifuged to separate non-dispersed aggregates before the suspensions were evaluated.

Samples prepared through microfluidization were subjected to 1, 3 or 5 passes through the chambers. Unlike the ultra-sonicated samples, the microfluidized samples were not centrifuged prior to characterization. In order to calculate the degree of fibrillation of the microfluidized samples, two of them which had been passed 5 times through the homogenizer were centrifuged to remove non-dispersed material and the nanocellulose yield was calculated. The yield of the nanocellulose samples is presented in Table 2

Table 2. Nanocellulose yield determined by diluting the suspension obtained after microfluidization/ultra-sonication and comparing the amount of nanocellulose in suspension before and after centrifugation.

	<b>Nanocellulose yield</b>
NDISS-THF-30	59 %
NDISS-THF-60	86 %
NDISS-ACE-35	93 %
NKRAFT-ACE-35	98 %

## Characterization of nanocellulose

Differences in viscosity and transparency were observed when the samples that were microfluidized were compared with those that had been ultra-sonicated. Samples that were ultra-sonicated were rather clear and had a low viscosity, whereas samples that had been microfluidized were thick gels with varying transparency (Figure 10). The microfluidized



Figure 10. Nanocellulose oxalate after microfluidization at approximately 1.5 wt. %. The samples were homogenized with one pass through the microfluidizer (top) and three passes (bottom).

samples varied in colour, transparency and viscosity. Samples prepared from dissolving pulp had a lower transparency than the samples prepared from kraft pulp and they also had a slightly lower viscosity. Presumably this was due to the presence of a fraction of larger particles, which was also seen when the nanocellulose yields were compared. The yield when nanocellulose is produced by both microfluidization and ultra-sonication can be further increased by extending the processing time and optimizing the operation. The yield was higher for NKRAFT-ACE-35, prepared from kraft pulp, than for NDISS-ACE-35, prepared from dissolving pulp. This could be due to the presence of more hemicellulose in the kraft pulp. Hemicellulose has been said to facilitate the preparation of CNF using TEMPO-mediated oxidation by forming a sponge-like layer between the fibrils. This layer makes it easier for the TEMPO molecules to come in contact with the whole surface of the fibril.<sup>92</sup> Similar mechanisms may be present in the preparation of cellulose oxalates. A lack of hemicellulose may lead to a more uneven distribution of the surface charge and the formation of larger aggregates.

All the samples prepared from kraft pulp showed a brown discoloration, particularly in samples which had been reacted with oxalic acid for 60 minutes. The colour is believed to be due to degradation products from hemicelluloses. Acetone appears to be a better solvent of these degradation products, as the samples washed with acetone had less colour than those washed with ethanol. When the number of passes through the homogenizer was increased the turbidity decreased and the viscosity of all the samples increased, indicating that the fibrillation has increased.

Different microscopic techniques were used to image the individual nanocellulose particles. NDISS-THF-30 and NDISS-THF-60 prepared using ultra-sonication were imaged using a transmission electron microscope (TEM) (Figure 11). These images show mostly rod like crystallites with a width of 16 – 20 nm and a length of 150 – 220 nm, with few long fibrils. These dimensions and shape are similar to those reported for CNCs. The samples prepared using microfluidization were imaged with scanning electron microscopy (SEM) and atomic force microscopy (AFM) (Figure 12). The particles in these images appear to vary more than those prepared by ultra-sonication, with more long and flexible particles present. By measuring the size from the AFM images, NKRAFT-ETOH-35 with 3 passes was found to be on average 340 nm long and 3.2 nm wide, and NDISS-ETOH-60 with 5 passes was on average 370 nm long and 4.3 nm wide (Figure 13). The images contain particles which are longer than 1  $\mu\text{m}$  with a flexible appearance similar to

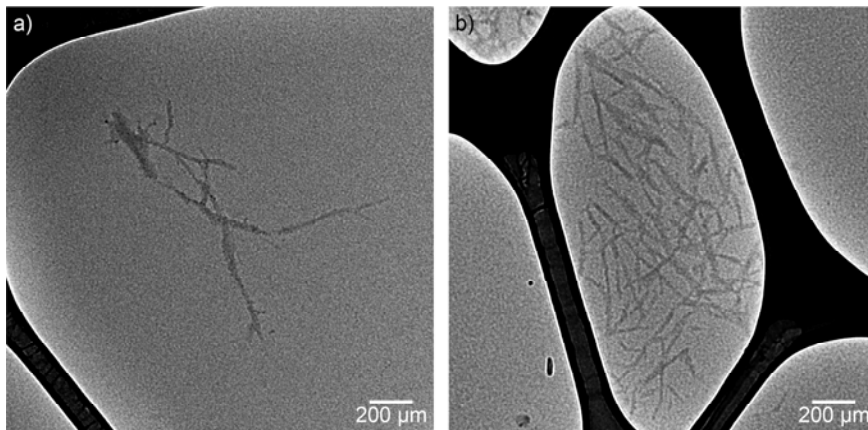


Figure 11. TEM micrograph of nanocellulose oxalate, a) NDISS-THF-30, b) NDISS-THF-60.

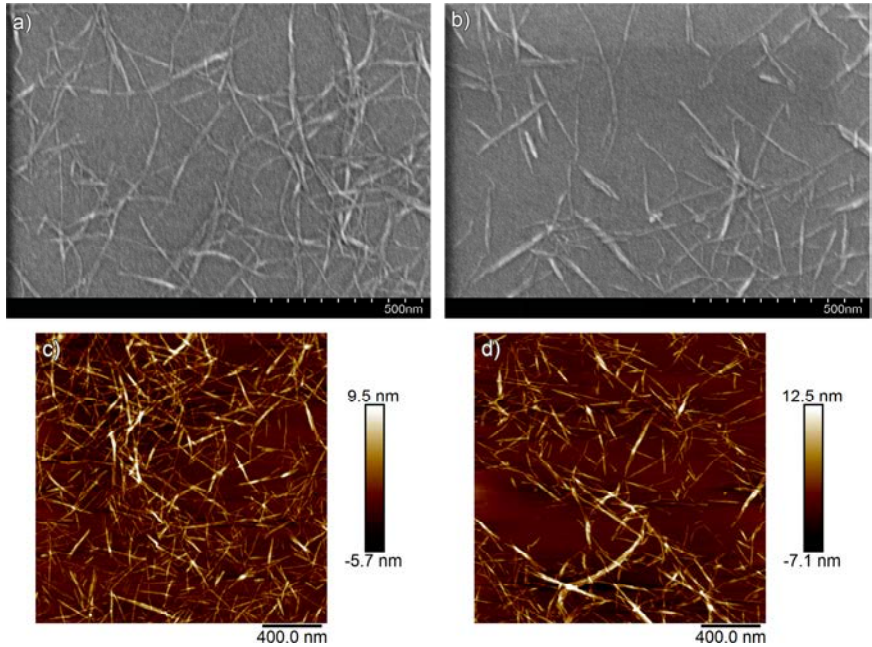


Figure 12. Representative SEM (top) and AFM (bottom) micrographs of nanocellulose adsorbed onto silica surfaces from suspensions of NKRAFT-ETOH-35 with 3 passes (left) and NDISS-ETOH-60 with 5 passes (right).

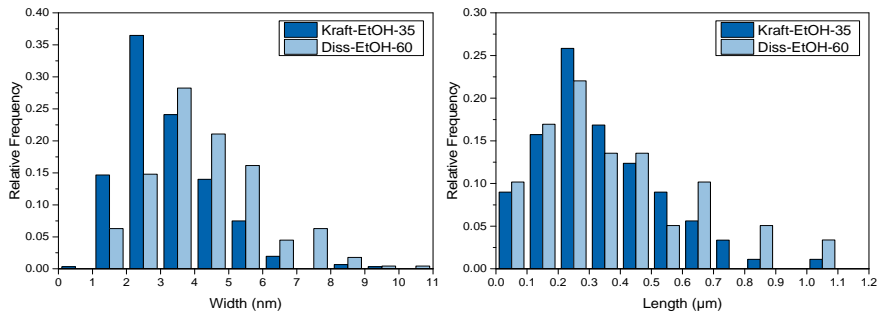


Figure 13. Width (left) and length (right) distribution of the nanocellulose adsorbed onto silica surfaces from suspensions of NDISS-ETOH-60 with 5 passes and NKRAFT-ETOH-35 with 3 passes measured using AFM.

that of CNF, and short and stiff particles similar to CNC. The difference in size between the particles prepared using ultra-sonication and microfluidization is probably due to the differences between the two fibrillation methods. Microfluidization and ultra-sonication operate using

different principles for liberating the fibrils. The ultra-sonicator used has a limited power, making it easier to disperse small particles than large ones. This can also be seen in the nanocellulose yield referred to earlier. The presence of particles resembling both CNFs and CNCs makes it is possible to combine the properties of these types of particles. Even though the uses of these materials are similar, there are applications where for example the viscosity of CNF is too high and the flexibility of CNC is too low. A hybrid material such as the nanocellulose from cellulose oxalate could solve this type of problem.

It is uncommon to find publications which report the production of nanocellulose by first pre-treating pulp fibres and then allowing them to dry before fibrillating the material. This is probably because it is regarded as unnecessary and energy-demanding to remove the water in which the pre-treated pulp is dispersed. There is also a risk of irreversible hornification of the fibres which will make fibrillation more difficult. In the present work, the cellulose oxalates were prepared and washed in non-aqueous systems which reduce the severity of hornification. After the oxalates had been dried from ethanol, they remained easily fibrillated. In commercial operations, it could be advantageous to use a dry cellulose derivative to prepare nanocellulose, allowing more flexible logistics and fibrillation. Fibrillation could, for example, be performed at different locations, using different methods for fibrillation from the same master batch of cellulose oxalate. Storing dry cellulose oxalate also has the advantage of minimizing the risk of bacterial growth which can occur when wet substances are stored.

## **Utilization of nanocellulose oxalate**

There are many applications where the properties and morphology of nanocellulose are of great advantage. In the following section, a selection of possible novel applications out of nanocellulose oxalate has been evaluated. As nanocellulose is often prepared as an aqueous dispersion, some of the easiest uses of nanocellulose are as a rheology modifier or as a stabilizer.

### **Emulsions**

Most oil and water mixtures are unstable and the liquid droplets quickly coalesce or flocculate to form two separated phases. Unstable oil/water mixtures can be stabilized using surfactants which decrease the interfacial

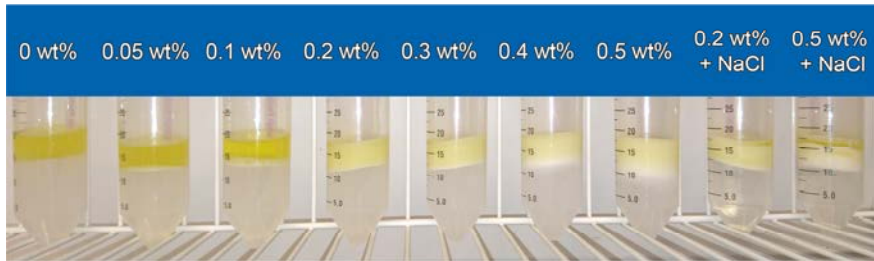


Figure 14. Oil/water emulsions after centrifugation prepared by mixing olive oil and water with different concentrations of NDISS-ACE-35 with 5 passes. The two emulsions on the right had 0.2 wt. % sodium chloride added. The white cream phase indicates a semi-stable emulsion.

energy between the two liquids and make them miscible, producing an emulsion. As CNF creates a continuous network in water, it is possible to increase the stability of an emulsion simply by locking the oil droplets in place and preventing them from coalescing or flocculating.<sup>93-94</sup> Another way to produce a stable emulsion without the use of surfactants is by preparing a Pickering emulsion,<sup>95-96</sup> which is stabilized by colloidal particles adsorbed onto the oil/water interface. These particles prevent the droplets from coming into contact with each other, and thereby coalescence.<sup>97-98</sup> The ability to form a Pickering emulsion is dependent on both the surface chemistry of the particles and their morphology. The chemical structure of cellulose where one side is more hydrophilic, combined with the small size of CNCs has been shown to be able to produce a Pickering emulsion.<sup>95</sup> Some studies have claimed that CNFs may also be able to form Pickering emulsions<sup>96</sup>, while others claim that CNFs are too long and can only stabilize emulsions by forming a network in the aqueous phase.<sup>95</sup>

By mixing 6 ml olive oil and 14 ml nanocellulose (NDISS-ACE-35 with 5 passes) dispersed in water with varying concentrations with an T18 UltraTurrax (IKA, Germany) at 16,000 RPM for 5 min, the mixture readily formed an emulsion even at 0.05 wt. % nanocellulose (results not shown). The stability of the emulsions formed was tested by centrifuging at 4,000 RCF for 5 min followed by an optical evaluation. After centrifugation, samples with less than 0.2 wt. % nanocellulose separated into an oil phase and a water phase, indicating that the stability of the emulsion droplets was too low to withstand the forces when the particles were forced together in the centrifuge (Figure 14). Emulsions with 0.2 wt. % nanocellulose or more also formed two phases, but the

emulsion droplets were stable enough to prevent coalescence when the droplets were forced together. Due to the difference in specific gravity between oil and water, the emulsified phase formed a redispersible cream phase during centrifugation. Some studies have shown that the addition of salt may stabilize a Pickering emulsion prepared from nanocellulose.<sup>99-100</sup> An increase in ionic strength decreases the colloidal stability of charged nanocellulose, possibly increasing the aggregation and particle size and thus reducing the emulsion stability.<sup>100</sup> The salt may also act to decrease the repulsion between the nanocellulose particles when they are on the droplet surface, thereby making it possible to increase the coverage of the surface and improve the emulsion stability. In the present study, the samples with 50 mM sodium chloride added showed a slightly lower stability, probably due to increased aggregation and particle size inhibiting the adsorption of nanocellulose by the interphase.

## Films

Another way to use nanocellulose is by making a film. The properties of films prepared from nanocellulose vary greatly, depending partly on how the films were prepared and on the type of nanocellulose. Literature data on films prepared from CNFs have reported good optical properties, mechanical strength and barrier properties (Table 3).<sup>16, 20, 32, 101-103</sup> Little data has been reported for self-supporting CNC films, as they are difficult to produce and test.

Table 3. Mechanical properties and oxygen permeability of NDISS-ACE-35 with 5 passes and NKRAFT-ACE-35 with 5 passes together with literature data for CNCs and CNFs

	Maximum tensile stress [MPa]	Tensile strain at maximum tensile stress [%]	Modulus [GPa]	Oxygen permeability [ $\text{cm}^3 \mu\text{m}^{-2} \text{day}^{-1} \text{kPa}^{-1}$ ] 50 % RH
NDISS-ACE-35 with 5 passes	142 (+-5)	3.0 (+-0.4)	10.6 (+-0.4)	0.54
NKRAFT-ACE-35 with 5 passes	197 (+-7)	5.0 (+-0.6)	10.2 (+-0.4)	0.31
Typical CNC films	70 <sup>101</sup>	0.6 <sup>101</sup>	6 - 14.9 <sup>20, 101</sup>	-
Typical CNF films	95-240 <sup>20</sup>	2-10 <sup>20</sup>	6-15 <sup>20</sup>	0.14-5.03 <sup>16, 32, 102-103</sup>

In the present work, films were prepared using both membrane filtration and solvent casting. Figure 15 shows a solvent-cast film from NDISS-THF-30 and membrane-filtered films from NDISS-ACE-35 with



Figure 15. Films from nanocellulose oxalate, a) NDISS-THF-30 prepared by solvent casting, b) i) NKRAFT-ACE-35 with 5 passes and ii) NDISS-ACE-35 with 5 passes prepared through filtration over a membrane.

5 passes and NKRAFT-ACE-35 with 5 passes. All the films are transparent, especially the solvent-cast film. The thickness of the films is approximately  $3\ \mu\text{m}$  for the solvent-cast film and  $30\ \mu\text{m}$  for the membrane-filtered film. Mechanical and oxygen permeability tests were only performed on the membrane-filtered film as the solvent-cast film was too thin and too fragile to handle.

The mechanical properties of NDISS-ACE-35 with 5 passes and NKRAFT-ACE-35 with 5 passes were generally comparable with those of other films prepared from CNF (Table 3). It appears that the film prepared from kraft pulp had both a higher maximum tensile stress and a greater tensile strain-at-break than the film from dissolving pulp. This is probably related to a difference in density as well as to the presence of larger particles in the samples prepared from dissolving pulp, which led to faults when producing a film.<sup>104</sup>

The oxygen permeability is a crucial property if the material is to be used in food packaging or other thin-film applications. A low oxygen permeability reduces the amount of oxygen which is able to penetrate the film, and this can increase the shelf life of food products. At 50 % relative humidity, the permeability of NDISS-ACE-35 with 5 passes and NKRAFT-ACE-35 with 5 passes was  $0.54$  and  $0.31\ \text{cm}^3\ \mu\text{m}\ \text{m}^{-2}\ \text{day}^{-1}\ \text{kPa}^{-1}$  respectively, which is comparable to other types of CNF. The permeability was lower for the sample prepared from kraft pulp, probably because of the higher degree of fibrillation in NKRAFT-ACE-35 with 5 passes.<sup>104</sup>

There are many ways in which the properties of the nanocellulose films can be altered, using both chemical and mechanical methods. As plain nanocellulose films are rather sensitive to water, it is sometimes desirable to cross link them, using for example periodate oxidation.<sup>105</sup> Crosslinking the film allows it to maintain most of its mechanical properties even in wet conditions. Depending on the drying method, it is possible to change the density and surface structure of the film. Films which have been compressed has better barrier properties and a higher wet strength than freely dried films.<sup>106</sup> By compressing the film between patterned surfaces it is also possible to change the surface structure, and this may in some cases be of interest.

### **Summary of nanocellulose from cellulose oxalate**

Cellulose oxalate is easily fibrillated using either microfluidization or ultra-sonication. The resulting nanocellulose is well dispersed and it produces gels at low concentration. The size of the particles obtained varies depending on the method for fibrillation. Ultra-sonication results in particles similar to CNCs and microfluidization results in particles with a large range of sizes. There are particles resembling both stiff and short CNCs and long and flexible CNFs. This may open up new uses for nanocellulose where neither CNCs nor CNFs can successfully be used.

The nanocellulose prepared from cellulose oxalate has been shown to be able to stabilize Pickering emulsions and form transparent films. During centrifugation, these emulsions cream but the oil droplets can easily be re-dispersed. Films of nanocellulose can be formed using both solvent drying and filtration over a membrane. The films have mechanical properties which are comparable to those of most other types of CNFs and have good oxygen-barrier properties.

## 6. Functionalizing nanocellulose materials

Producing a material for a specific application is not only about designing a material with a specific structure and morphology. It is also important to achieve a specific chemistry on the surface to make it behave as desired. An efficient and environmentally friendly method to modify the surface properties of a material is the lbl adsorption of polyelectrolyte multilayers.<sup>51</sup>

Antibacterial materials can be used in different types of applications either to prevent bacterial growth, for example in medical and food applications, or to remove bacteria from, for example, drinking water. Both leaching<sup>107</sup> and contact-active systems<sup>57</sup> have been used to make cellulosic materials antibacterial. Contact-active systems have the advantage of limiting the spread of antibacterial chemicals in the environment, but many contact-active systems require harsh conditions and chemicals to covalently bond antibacterial compounds onto the surface of the material. Multilayers with antibacterial polyelectrolytes provide a quick modification of cellulosic materials using mild conditions and aqueous solutions.<sup>51, 69</sup>

PVAm (Figure 16) is a cationic polymer which has been shown to have antibacterial effects.<sup>108</sup> Alternated with PAA (Figure 16), PVAm has been adsorbed on silica surfaces and pulp fibres to study bacterial interaction.<sup>57, 109</sup> By adsorbing PVAm/PAA in three layers onto pulp fibres, Illergård *et al.*<sup>57</sup> managed to prepare materials which were antibacterial using a non-leaching contact-active mechanism. In order to increase the polymer adsorption and antibacterial effect the pH during polymer adsorption and the addition of sodium chloride have been

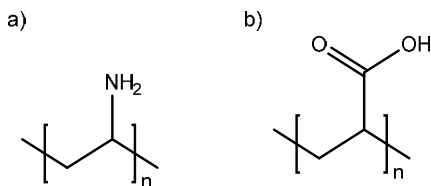


Figure 16. Chemical structures of a) polyvinylamine (PVAm) and b) polyacrylic acid (PAA).

studied<sup>110</sup>, as well as different types of pulp fibres<sup>111</sup>.

Even though nanocellulose and pulp fibres have similarities there are also many differences. When nanocellulose is being prepared it is common to introduce charged groups into the cellulose which change how it interacts with water. Chemical pulp fibres usually have a surface charge below  $100 \mu\text{eq g}^{-1}$ , whereas the surface charge of nanocellulose can be over  $1,500 \mu\text{eq g}^{-1}$ .<sup>82, 92</sup> The surface structure between the materials also differs greatly, and can vary between different types of nanocellulosic materials. Nanocellulose films, for example, can be produced using a wide range of procedures, all of which may result in different adsorption behaviours. For example, the swelling behaviour or density and surface structure may affect how the polyelectrolyte adsorbs.

This chapter describes the investigation of strategies to adsorb PVAm/PAA multilayers onto materials from nanocellulose. This has been done by investigating the adsorption onto differently charged nanocellulose films and different strategies to adsorb PVAm/PAA onto porous nanocellulose aerogels. In order to prepare these substrates, carboxymethylated CNF ( $\sim 600 \mu\text{eq g}^{-1}$ )<sup>29</sup> (RISE Bioeconomy) and CNF prepared without chemical pretreatment<sup>112</sup> (VTT) were used. These materials are examples of where the nanocellulose oxalate described in previous chapters can be used.

## Effect of charge and structure on the multilayer adsorption

The effect of substrate charge on the PVAm/PAA adsorption was investigated by preparing CNF films at different charge densities using a CNF suspension prepared without chemical pre-treatment as raw material. The raw material had a total charge of  $40 \mu\text{eq g}^{-1}$ . Charges were introduced into this CNF suspension using TEMPO-mediated oxidation<sup>83</sup> to produce two samples with a total charge of  $510 \mu\text{eq g}^{-1}$  and  $620 \mu\text{eq g}^{-1}$ . To achieve polyelectrolyte adsorption onto the CNF, films were prepared from the CNF before they were submerged in PVAm and PAA solutions. To study the effect of film swelling and structure on the multilayer adsorption, the films were prepared slightly differently. Plain films were prepared by membrane filtration followed by drying between two membranes at  $93^\circ\text{C}$  and a reduced pressure of  $95 \text{ kPa}$ . To prevent swelling, one set of films was oxidized using periodate oxidation.<sup>105</sup> This treatment crosslinks the film and prevents it from swelling in water.

Another film was pressed between two wire meshes under high pressure.<sup>106</sup> This decreases the swelling and increased the mechanical strength while forming a patterned surface from the mesh. The samples and their denotations are listed in Table 4.

Table 4. Films prepared from CNFs with different charge densities and preparation methods.

	Fibril charge [ $\mu\text{eq g}^{-1}$ ]		
	40	510	620
<b>Plain film</b>	Plain-40	Plain-510	Plain-620
<b>Crosslinked</b>	Crosslinked-40	Crosslinked-510	Crosslinked-620
<b>Structured</b>	Structured-40	-	-

Different charged CNF films are expected to exhibit differences in polyelectrolyte adsorption. More highly charged films swell more in water and should open up more surfaces on which adsorption can occur. It also means that more polyelectrolyte is required to associate with all the charges.<sup>113</sup> These two effects were expected to lead to higher polyelectrolyte adsorption on the more highly charged CNF films. The amount of adsorbed PVAm was assessed by utilizing the fact that PVAm is the only component in the multilayer system which contains nitrogen, so that the nitrogen content in the films can be used to determine the amount of PVAm adsorbed onto them. The expected trends were not however observed (Figure 17). The first layer of PVAm had the highest PVAm adsorption on the most highly charged film, but after the third layer had been deposited the lowest charged film had the highest PVAm adsorption. This may be due to a conformational effect of the adsorbed polymer in earlier layers. The higher charge is known to lead to a flatter conformation of the polyelectrolyte on the surface,<sup>114</sup> which leads to fewer excess charges adsorbed. The excess charges are important for the adsorption of the subsequent layers, and fewer excess charges lead to lower adsorption in the subsequent layers. The charge of the TEMPO-modified fibrils was about half the charge of the cellulose oxalates described earlier. This suggests that the cellulose oxalates may have too high a charge to be efficiently functionalized using lbl deposition. It has however been seen that it is possible to control the charge of the cellulose oxalates by hydrolysing the ester bond and removing the oxalate in water at an elevated temperature and thus to increase the likelihood that cellulose oxalates are good substrates for functionalization using the lbl procedure.

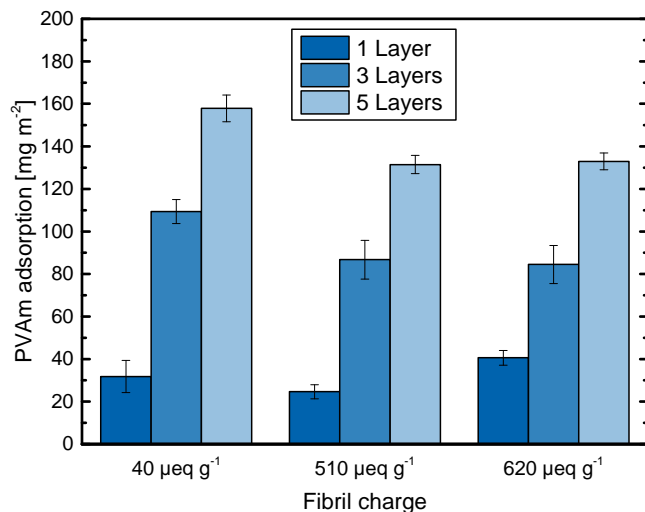


Figure 17. The PVAm adsorption on CNF films with different charge densities, determined from nitrogen analysis. Error bars represent standard deviations.

The manner of preparation of the films greatly influenced the PVAm adsorption (Figure 18). The crosslinked film had a much lower PVAm adsorption than the plain film, probably because the polyelectrolytes are able to be partially adsorbed on the inner surfaces of a swollen film. The pressed and structured film initially had a low PVAm adsorption which could be linked to the lower degree of swelling. As the film was only pressed and not covalently crosslinked, it is possible that it swelled after being submerged for a while, and that this led to a greater adsorption in the subsequent layers. The structuring of the film increases the surface area, and this may also partly explain the increased adsorption.

## Polyelectrolyte multilayer adsorption on aerogels

For some applications of nanocellulose, such as wound care or filters, it is more interesting to study at porous 3D structures rather than 2D films. Aerogels produced from nanocellulose have a high water absorbency, a high porosity and a large surface area, and this makes them interesting substrates for surface functionalization.

Aerogels were prepared from carboxymethylated CNFs which were freeze-dried and crosslinked, using 1,2,3,4-butanetetracarboxylic acid. This results in porous aerogels with a high charge,  $2,300 \mu\text{eq g}^{-1}$ .<sup>42</sup> Rapid

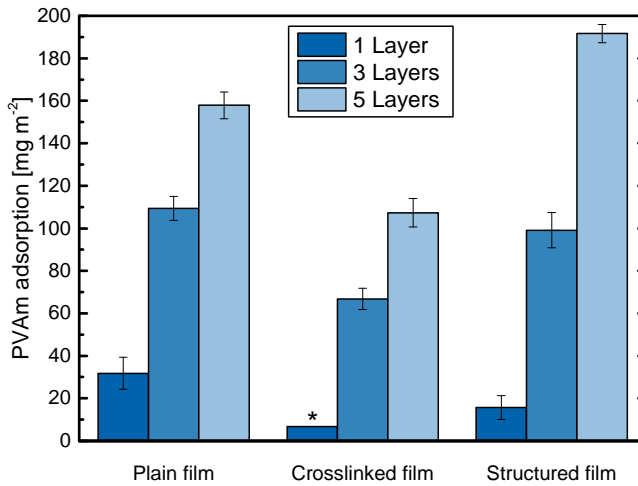


Figure 18. The PVAm adsorption of the plain, crosslinked and structured CNF films, all made from CNF with a fibril charge of  $40 \mu\text{eq g}^{-1}$ . \* The nitrogen content in the crosslinked film modified with 1 layer was outside the linear range of the calibration, and the plotted value represents the highest possible PVAm content. Error bars represent standard deviations.

and high water absorbency makes it easy to modify them with polyelectrolytes by immersing them in different solutions.

Different strategies can be employed to ensure that polyelectrolytes are adsorbed onto the aerogels. One strategy is simply to immerse an aerogel in a polymer solution. However, since the aerogel consists of a network of pores, it is difficult for the polymers to enter the aerogel and to be adsorbed on the inner surface of the aerogel, making it a slow process. An alternative strategy is to place an aerogel in a Büchner funnel and to filter polymer solution through the aerogel under vacuum. In practice, this was done by pipetting a small amount of polyelectrolyte solutions onto the aerogel to soak it, and to apply vacuum to drain it after a certain time.<sup>42</sup> The advantage of the vacuum strategy is that the polymers are forced through the aerogel making it possible for them to be adsorbed more quickly. The conditions used for the polymer adsorption on aerogels are listed in Table 5.

Table 5. Conditions for polyelectrolyte adsorption on aerogels.

Sample	Adsorption strategy	Polyelectrolyte concentration	Adsorption time	Polymer volume
Filt-1	Filtration	1 g L <sup>-1</sup>	5 min	4 ml
Filt-5	Filtration	5 g L <sup>-1</sup>	5 min	4 ml
Sol-1	Soaking in solution	1 g L <sup>-1</sup>	18 h	15 ml

The fast filtration strategy to adsorb PVAm onto the aerogels results in the same adsorption as the solution strategy, provided a higher (5 g L<sup>-1</sup>) concentration is used (Figure 19). Less PVAm will be adsorbed if only a moderate concentration (1 g L<sup>-1</sup>) is used when filtering. The different strategies resulted in different PVAm contents in the top and bottom of the aerogel. Filt-1 had a larger amount of PVAm on top, while the other samples had a higher PVAm content on the bottom of the aerogel. It is believed that two different effects cause this variation. In order to remove the water from the CNF while retaining the fibril network structure the wet structure is frozen in liquid nitrogen and freeze-dried. When they are being frozen, the samples freeze from the bottom. This is believed to cause an uneven freezing rate and crystallization leading to an uneven porosity and a variation in surface area of the top and bottom of the aerogel.<sup>115</sup> A greater surface area in the lower parts of the aerogel is then

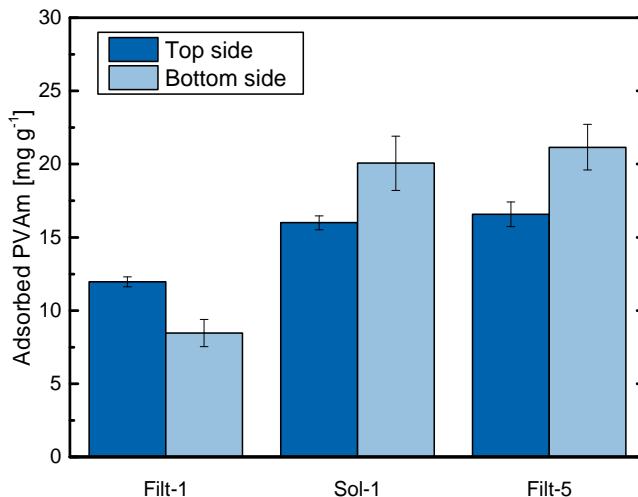


Figure 19. The PVAm adsorption resulting from different adsorption methods on CNF aerogels; having one layer PVAm deposited. The top side is the side facing upwards when the aerogels are being formed and when they are adsorbing the polyelectrolytes. Error bars represent standard deviations.

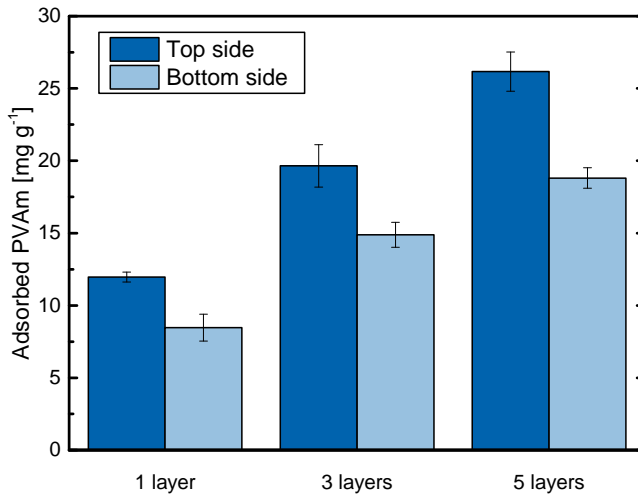


Figure 20. The PVAm adsorption after the deposition of 1, 3 and 5 layers of PVAm/PAA to CNF aerogels using filtration, the top side being the side facing up when the aerogels were produced and when the polyelectrolytes were adsorbed. Error bars represent standard deviations.

able to adsorb a larger amount of PVAm.

Filt-1 had a lower amount of PVAm adsorbed than the others, although an excess of PVAm was available. This is thought to be because the PVAm equilibrium concentration inside the aerogel drops below the equilibrium concentration necessary to fully saturate the surface. This also leads to a reversed relation between the amounts of PVAm on the top and bottom. This is presumably due to the addition of PVAm from the top of the aerogel, leading to a lower concentration at the bottom of the aerogel. Although Filt-1 had less PVAm than the other samples, it was possible to increase the adsorbed amount by adsorbing multilayers instead of only one layer of PVAm.

By alternating adsorption of PVAm and PAA in Filt-1 it was possible to have approximately the same amount of PVAm on Filt-1 after three layers as after one layer of Filt-5 or Sol-1 (Figure 20). When multilayers were deposited in Filt-1, the uneven polymer adsorption between the top and bottom was observed in all layers. The amount of PVAm adsorbed in each layer by Filt-1 decreased as the number of layers increase, due to it not being possible to fully saturate the surface with polyelectrolytes, shown in Figure 19.

## Summary of functionalization

Many aspects require consideration in order to achieve the desired adsorption of polyelectrolytes onto nanocellulosic materials. The adsorption of PVAm/PAA multilayers on a charged CNF film is dependent on the film's surface charge and structure. Following the first layer, a film with a high surface charge had lower polymer adsorption than a film with a low surface charge. This is believed to be due to conformational effects that occur when the charge of the substrate makes it difficult for the adsorbed polyelectrolytes to fully recharge it. By cross-linking CNF films, it was possible to reduce the swelling in water. This also prevented polyelectrolytes from being adsorbed on the inner surface of the film, thus reducing the amount of polymer adsorbed. Initially the same effect can be observed in press structure films, but this was later reversed and these films adsorbed large amounts of polyelectrolytes.

By forming porous aerogels from nanocellulose, it was possible to construct materials with a large surface area suitable for polyelectrolyte adsorption. The large surface area allows a high adsorption. Using vacuum-assisted drainage of the aerogel, it was possible to quickly adsorb polyelectrolyte on the inner surface of the material. The large surface area of the aerogel makes it necessary to use a high polymer concentration to be able to fully saturate the surfaces. If a high polymer concentration is undesired, it is possible to compensate for a low concentration by adsorbing multiple layers of polyelectrolyte instead. The aerogels produced were not perfectly homogenous, so it is difficult to achieve a material with a uniform concentration of polymer throughout the aerogel. This may now however be a problem when the material is being used.

## 7. Bacterial adsorption on polyelectrolyte-modified nanocellulose

Through evolution, bacteria have developed multiple strategies to increase their chances of survival. One of these is the tendency for bacteria to adhere to surfaces.<sup>116</sup> Many types of bacteria form biofilms, which often are problematic to humans. Depending on their use, it is desirable to design materials that interact with bacteria in different ways. Materials can either prevent or promote adhesion to a certain degree. Anti-adhesive materials can be used in for example medical devices where it is vital to minimize the possibility for bacteria to adhere to a material and proliferate.<sup>58</sup> Materials with a very high bacterial adhesion have, on the other hand, been shown to be effective as antibacterial materials through the high interaction and adhesion between the surface and the bacteria.<sup>117</sup>

Bacterial adhesion to a surface occurs in a two-step process.<sup>116, 118</sup> When a bacterium approaches a surface, it is subjected to both repulsive and attractive forces. If the attractive forces are larger, the bacterium will start to adhere to the surface.<sup>119</sup> Initially the adhesion is reversible and involves a weak adhesion between the surface and the bacterium. The bacterium behaves as if it is still in suspension and is affected by Brownian motion, van der Waals attraction forces and surface electrostatic effects. As the attraction increases, the process enters a second, irreversible, step, where the bacterium repositions and starts to synthesize adhesin in order to ensure a good adhesion.<sup>120</sup> This leads to molecular reactions between the surface and bacterium and the bacterium takes full advantage of being on a surface by changing its metabolism and forming a biofilm. By even further increase the bacterial adhesion, it is possible to go from an advantageous stage to a negative stage and the bacterium starts to experience stress induced-changes.<sup>116</sup> Under a high adhesive force, the cell envelope is affected and it is possible to kill bacteria by exerting a high attraction between the cell wall components and the surface.<sup>121</sup>

It is not fully understood what influences bacterial adhesion. Factors such as surface structure, surface roughness, hydrophobicity and surface chemistry are known to be important factors, but there is a poor understanding of the exact effects of them.<sup>119</sup> Topographical features in the micrometer size range for example, have a tendency to cause bacteria to adhere between the protruding structures.<sup>122</sup> This reduces the shear force experienced by the bacteria and protects the bacterium from being washed away. Smaller structures, and especially nanostructures, are more difficult to understand. Results of experiments show different affinities to nanostructured surfaces.<sup>120, 123</sup>

The cell envelope of a bacterium contains amino, carboxyl and phosphate groups which make the bacterium negatively charged on the outer surface at physiological pH. Cationic surfaces have been shown to increase bacterial adhesion<sup>124</sup>, even to the point where they may lead to attraction-induced stresses.<sup>116</sup> It has been suggested that a surface with a surface charge greater than  $10^{12} - 10^{16}$  charges  $\text{cm}^{-2}$ , depending on the strain and conditions, can act as an antibacterial surface through the high bacterial adhesion.<sup>125-126</sup> The surface charge can be increased by using polyelectrolyte multilayers to modify the surface characteristics.<sup>57</sup> Pulp fibres modified with PVAm/PAA have shown to be contact-active antibacterial, and this is a potentially sustainable and safe way to get rid of bacteria.

## Bacteria on films

The bacterial adhesion to the PVAm/PAA-modified CNF films was evaluated using microscopic imaging. The films were dipped in solutions containing *E. coli*, which had been modified to express green fluorescent protein (GFP), which makes it possible to easily image bacteria using fluorescence microscopy, as the bacteria emit green fluorescence and the intensity is related to the concentration of bacteria. By using GFP it is possible to overcome the difficulties of using stains to image bacteria on cellulose. These stains are often absorbed by cellulosic materials, making it difficult to obtain a high contrast between the background and the bacteria. The images were captured at low magnification and rather low sensitivity of the camera, making it easy to differentiate images with a large amount of bacteria, but difficult to differentiate images with a small amount of bacteria. This means that films which appear completely black

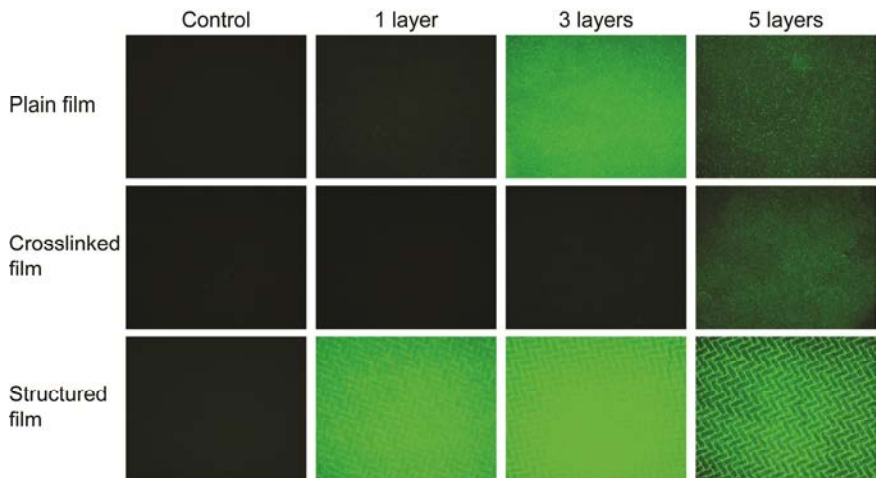


Figure 21. Bacteria on the CNF films prepared from fibrils with a charge of  $40 \mu\text{eq g}^{-1}$  made visible using fluorescence microscopy. The bacteria show a green colour when imaged. The micrographs are captured at a constant exposure so the light intensity is proportional to the bacterial concentration. Films with higher charge had no bacteria detected at these conditions.

can still have bacteria adhering to the surface. All the films where bacteria were detected are shown in Figure 21.

The micrographs showed that it was only those films which had not been modified using TEMPO-mediated oxidation which had a significant amount of bacteria on the surface. TEMPO-mediated oxidation increases the anionic charge of the substrate, but Figure 17 shows that the amount of PVAm adsorbed did not considerably change. PVAm is adsorbed on the surface in order to produce a material with a high cationic charge. As the amount of PVAm is constant while the substrate anionic charge increases, it is easy to imagine that the net positive charge of the material will be decreased, leading to lower bacteria adhesion.

Depositing more layers of PVAm/PAA onto the substrate should increase the bacterial adhesion as this will lead to a higher amount of PVAm, the active component, on the material. It is however apparent in Figure 21 that this is not always the case. Plain-40 with 5 layers has fewer bacteria on the surface than the film modified with 3 layers. The same is true of Structured-40 with 5 layers and 3 layers. A similar trend for the bacterial adhesion to reach a maximum at 3 layers has been observed earlier on silica surfaces.<sup>109</sup> Crosslinked-40 with 5 layers, however, had more bacteria on the surface than the material with 3 layers. Figure 18

indicates that there is only partial correlation between the bacteria adherence and the amount of PVAm adsorbed. No correlation is, for example, observed in Structured-40. One explanation of these findings could be that the adsorbed PVAm exceeding the charge stoichiometry is important to anticipate bacterial adhesion.

In order fully to understand why some layers have more bacteria than others, it is necessary to investigate the surface charge and surface structure of the samples, and the amount of PAA, the anionic component, in each layer. Further evidence of the importance of the PAA is the fact that Plain-40 with 3 layers and Crosslinked-40 with 5 layers have the same amount of PVAm, but different amounts of bacteria on the surface. This could be because Crosslinked-40 with 5 layers has an additional layer of PAA.

The structured samples were those which had most bacteria on the surface. In the micrographs, it is possible to see the pressed pattern on the film, but it is difficult to say from the fluorescence images whether the large amount of bacteria is due to a difference in bacteria adsorption or an effect on the surface area of the pattern. To further study this, the films were imaged using SEM.

The SEM micrographs show that Plain-40 is a flat and even film, whereas Structured-40 possesses ridges and valleys. The valleys in the structured film have been pressed and have a surface which is similar to that of the plain film, whereas the ridges have an uneven structure. Plain-40 has bacteria well distributed over the whole surface, but they are unevenly distributed on Structured-40 surface (Figure 22). In the valleys of Structured-40, the bacteria concentration is similar to that on the plain

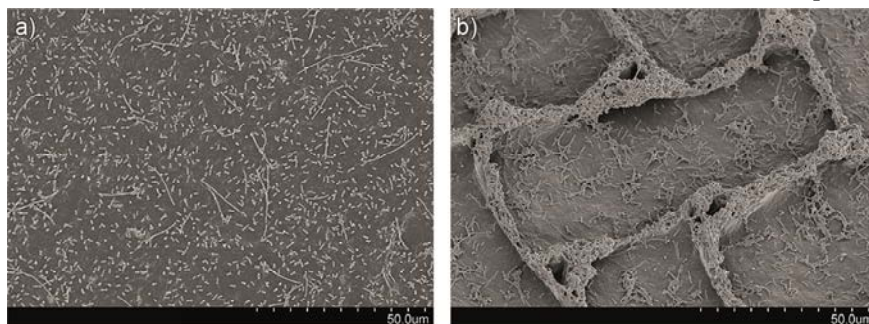


Figure 22. SEM micrographs of CNF films prepared from fibrils with a charge of  $40 \mu\text{eq g}^{-1}$  after incubation in bacterial solutions a) Plain-40 with 5 layers of PVAm/PAA, b) Structured-40 with 5 layers of PVAm/PAA.

film, but the ridges are crowded with bacteria. It is not known why the bacteria are so unevenly distributed on the film. The uneven surface may give rise to differences in the hydrodynamic forces acting on the surface and bacteria. It is possible that more bacteria are forced into contact with the ridges than the valleys and that this may give rise to an increase in adhesion. This theory does not however agree with other published reports which state that protrusions from surfaces often have fewer bacteria<sup>122, 127</sup>. Another possible explanation is that the process of producing the pattern leads to regions with different mechanical properties and structures. The pattern is produced by pressing the film between two woven meshes, and the pattern from the mesh is transferred by compressing certain regions in the films and leaving other more or less untouched. This means that the valleys will have been compressed and may have different mechanical properties, and a different porosity and micro-structure than the tops of the ridges. These differences can affect both how the bacteria interact with the surface<sup>122, 127-128</sup> and how PVAm is adsorbed on the surface<sup>129</sup>.

## **Bacteria on aerogels**

Bacterial adhesion to the aerogels was studied using confocal microscopy and by quantifying the amount of bacteria adhering to the material. Unlike the films, it is not possible to image the aerogels using fluorescence microscopy because the fluorescing bacteria are at different depths in the aerogel. Confocal microscopy uses optical sectioning and only displays the bacteria in a slice of the aerogel. Using microscopic techniques it is apparent that bacteria adhered preferentially to the aerogel which had been modified with PVAm/PAA (Figure 23). The adsorption of bacteria observed using confocal microscopy is uneven because the aerogels are porous materials. The dark areas are pores where bacteria cannot adhere to any surface. The uneven PVAm adsorption between the upper and lower parts of the aerogel previously observed was also observed in the amounts of bacteria on the surface. In Filt-1, there were more bacteria on the upper than the lower surface. Imaging the aerogels using SEM (Figure 24) shows bacteria adhering to the surface of the aerogel. Some bacteria adhered to the unmodified aerogels, but the modified aerogels had considerably more bacteria adhered. As in the case of the structured films, the bacteria appear to prefer to adhere to irregularities of the structures making up the aerogel.

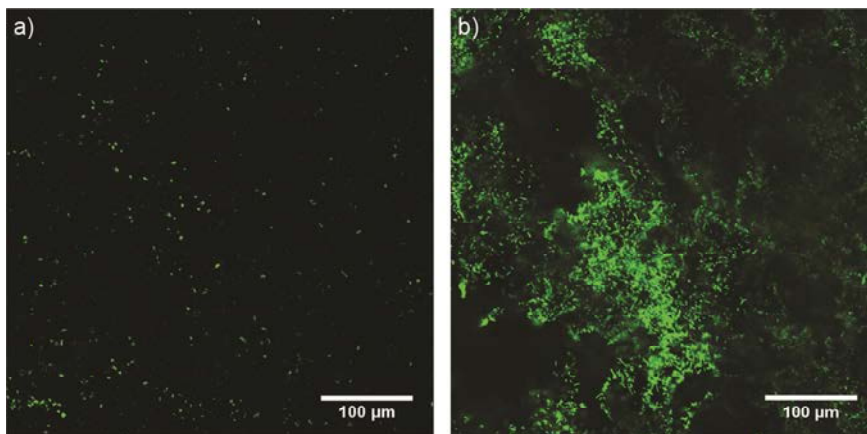


Figure 23. Confocal images of a) unmodified aerogel and b) AG3L after incubation with *E. coli*. The bacteria fluoresce in green when imaged. Both images are from the top part of the sample.

It is possible that the freezing-drying process leads to regions with a structure or properties that are more favourable for the bacteria, as in the structured films.

The amount of bacteria adhering to the surface of the contact-active antibacterial materials was measured by placing a sample of the material in a bacteria suspension of known concentration and then measuring the remaining number of bacteria after a defined time. It was then possible to see that the aerogels removed up to 99.97 % of the bacteria (Figure 25), the highest bacterial removal being observed in Filt-5 modified with 1 layer PVAm. Filt-1 modified with multilayers of PVAm/PAA showed an increase in bacterial removal with increasing number of multilayers, from

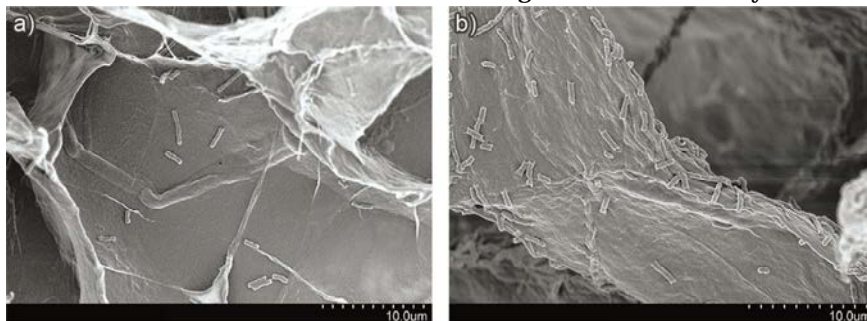


Figure 24. SEM micrographs of a) unmodified aerogel and b) AG3L after incubation with *E. coli*. Both images are of the top of the sample.

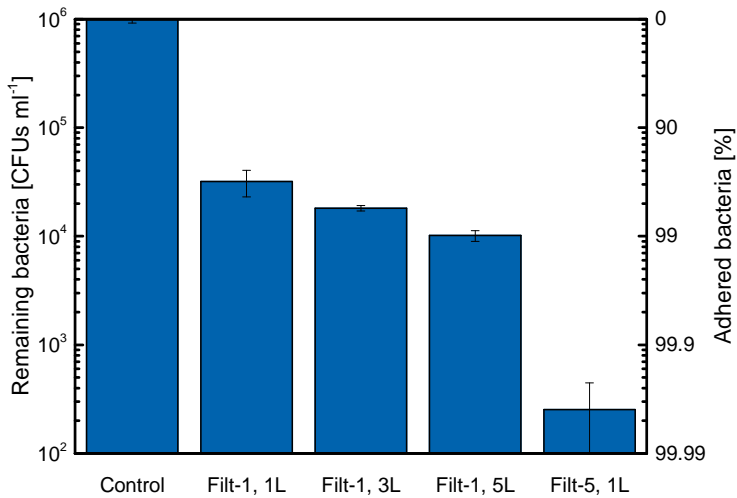


Figure 25. The amount of bacteria removed by aerogels with different numbers of PVAm/PAA layers and different adsorption strategies. 1L, 3L and 5L denote 1 layer PVAm, 3 layers PVAm/PAA and 5 layers PVAm/PAA respectively. Error bars represent standard deviations

97 % - 99 %. Filt-1 modified with 5 layers PVAm/PAA adsorbed more PVAm than Filt-5, but the bacterial adhesion was lower. This is probably due to the higher anionic contribution from the PAA present in Filt-5, leading to a decrease in the net charge of the system. It is believed that the charge contribution of PAA in the multilayers reduces the adhesion and antibacterial effect of contact-active antibacterial materials prepared by the multilayer adsorption of PVAm/PAA. This was observed in the confocal microscopy of the CNF films.

The aerogels are produced with a high surface charge with a high concentration of carboxylic acids. When added to an aqueous solution, the aerogels acidify the solution and the acidification is strong enough to make it difficult to buffer using a moderate buffer concentration. Most bacteria require a pH close to neutral to grow, so the aerogels are growth-inhibiting without the need for further modification.

## Summary of bacterial adsorption

It is possible to alter the properties of materials prepared from nanocellulose by the deposition of multilayers. The adsorption is however more complex on nanocellulose than on, for example, a silica surface or pulp fibres. PVAm/PAA multilayers are effective in altering the way in

which bacteria interact with the surfaces, and they can be an efficient way to prepare materials with a high bacterial adhesion.

As indicated in the previous chapter, it appears to be difficult to recharge the surface of a high charged CNF film by polyelectrolyte adsorption making few bacteria adhere to these materials. On the low charged films, the recharging is higher and a large number of bacteria adhere to these, with a maximum adhesion after 3 layers of PVAm/PAA. If multilayers are deposited on aerogels, they are able to remove up to 99.97 % of the bacteria from a solution. The effect was greatest with Filt-5, where a moderate amount of PVAm was adsorbed without any PAA. The bacteria adsorption observed in films with 3 layers of PVAm/PAA and in Filt-5 suggest that the anionic PAA can have a negative impact on bacterial adhesion. Both the modified aerogels and structured films had most of the bacteria adhered to the uneven edges rather than to the flat surfaces. This could be related to the microstructure and/or to hydrodynamic effects.

## 8. Conclusion

The development of new materials from safe and renewable resources is important in order to reach Agenda 2030, and nanocellulose may well be a part of this if we manage to find applications which benefit from it. Many types of nanocellulose have been described in the literature, and some types of nanocellulose are more suitable for certain applications than others. In order for nanocellulosic materials to be a success, it is important to adapt processes and properties that suit the specific use. By preparing nanocellulose from cellulose oxalate it is possible to achieve a material with distinctive properties. Nanocellulose produced via this route has the advantage of being prepared from a dry material, which increases the flexibility of nanocellulose production and fibrillation, and it also opens up new methods for incorporating nanocellulose in processes and materials. The resulting nanocellulose has a high carboxyl content which aids in fibrillating the material. The nanocellulose contains particles with a morphology similar to both CNF and CNCs, and this may also open up new application areas for the material.

We live with a constant interaction with bacteria. They are vital for our well-being such as the skin microbiome, but they can also cause great risks such as antibiotic-resistant bacterial infections or contamination of drinking water. The balance between promoting bacteria in our surrounding and preventing them is a sensitive point. Many of the goals in Agenda 2030 are linked to an interaction with bacteria, such as clean water and sanitation. These goals cover the need to prevent the spreading of bacteria while others, such as life below water, state that it is important to prevent harming other ecological systems at the same time.<sup>3</sup> By adsorbing polyelectrolytes on both dense films and porous aerogels of nanocellulose, it is possible to alter the bacterial adhesion on the material and thus to produce materials which can sustainably prevent the spread of bacteria. With cationic PVAm adsorbed onto the surfaces, the prepared materials were able to remove up to 99.97 % of the bacteria in an aqueous suspension. The adhesion was altered by changing the surface structure, the initial surface charge and the number of layers of PVAm/PAA. Tuning the bacterial adhesion to materials is important in developing sustainable

strategies to prevent the growth and spread of bacteria. High bacterial adhesion may be used to design non-leaching contact-active antibacterial surfaces, while low bacterial adhesion may be used for non-adhesive antibacterial surfaces. Both these strategies can offer strategies which are more sustainable than the current use of leaching biocides.

## 9. Future work

Looking forward, it is important to evaluate nanocellulose prepared from cellulose oxalate in specific applications. The choice of these applications should include areas where the unique properties of the nanocellulose and the production process can be fully appreciated. Numerous applications and processes could take advantage of starting with a dry cellulose oxalate dispersing the fibrils on site, preferably in current processes.

While simultaneously investigating applications of nanocellulose from cellulose oxalate it is important to study a number of unanswered scientific questions. Pulp fibres are not a homogenous material, and many variations exist, leading to multiple chemical reactions taking place when molten oxalic acid dihydrate is mixed with pulp fibres. In order to fully understand the process, it is necessary to characterize all the by-products from the reaction. It is also desirable to further characterize the derivatised cellulose, for example to study how the substituents are distributed in the material.

The adhesion of bacteria to surfaces is not fully understood. The current work illustrates that it is possible to use multilayers of PVAm/PAA to produce nanocellulosic materials which promote both high and low adhesion. It has been shown the structure, initial surface charge and PAA content can affect the adhesion. By studying these factors more closely it is probably possible to design sustainable materials with a high antibacterial capacity.



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