Structural and Electrochemical Properties of Functionalized Nanocellulose Materials and Their Biocompatibility
Abstract

Nanocellulose has received considerable interest during the last decade because it is renewable and biodegradable, and has excellent mechanical properties, nanoscale dimensions and wide functionalization possibilities. It is considered to be a unique and versatile platform on which new functional materials can be based.

This thesis focuses on nanocellulose from wood (NFC) and from Cladophora algae (CNC), functionalized with surface charges or coated with the conducting polymer polypyrrole (PPy), aiming to study the influence of synthesis processes on structural and electrochemical properties of such materials and assess their biocompatibility.

The most important results of the work demonstrated that 1) CNC was oxidized to the same extent using electrochemical TEMPO-mediated oxidation as with conventional TEMPO processes, which may facilitate easier reuse of the reaction medium; 2) NFC and CNC films with or without surface charges were non-cytotoxic as assessed by indirect in vitro testing. Anionic TEMPO-CNC films promoted fibroblast adhesion and proliferation in direct in vitro cytocompatibility testing, possibly due to its aligned fibril structure; 3) Rinsing of PPy-coated nanocellulose fibrils, which after drying into free-standing porous composites are applicable for energy storage and electrochemically controlled ion extraction, significantly degraded the PPy coating, unless acidic rinsing was employed. Only minor degradation was observed during long-term ambient storage; 4) Variations in the drying method as well as type and amount of nanocellulose offered ways of tailoring the porosities of nanocellulose/PPy composites between 30% and 98%, with increments of ~10%. Supercritical CO₂-drying generated composites with the largest specific surface area yet reported for nanocellulose/conducting polymer composites (246 m²/g). The electrochemical oxidation rate was found to be controlled by the composite porosity; 5) In blood compatibility assessments for potential hemodialysis applications, heparinization of CNC/PPy composites was required to obtain thrombogenic properties comparable to commercial hemodialysis membranes. The pro-inflammatory characteristics of non-heparinized and heparinized composites were, to some extent, superior to commercial membranes. The heparin coating did not affect the solute extraction capacity of the composite.

The presented results are deemed to be useful for tuning the properties of systems based on the studied materials in e.g. energy storage, ion exchange and biomaterial applications.

Keywords: Nanocellulose, nanofibrillated cellulose, Cladophora cellulose, polypyrrole, TEMPO-mediated oxidation, composite, porosity, cytocompatibility, blood compatibility


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To my family
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I  **Carlsson, D.O.,** Lindh, J., Nyholm, L., Strømme, M. & Mihranyan, A. Electrochemical TEMPO-mediated oxidation of highly crystalline nanocellulose in water. *In manuscript*


* Contributed equally

V  **Carlsson, D.O.,** Mihranyan, A., Strømme, M. & Nyholm, L. (2014) Tailoring porosities and electrochemical properties of composites composed of microfibrillated cellulose and polypyrrole. *RSC Advances, accepted for publication*


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Summary of my contribution to the papers included in the thesis:

**Paper I:** I participated in planning the study, performed all experimental work (except for XRD and CHN elemental analysis), analyzed data, wrote the initial manuscript and contributed to the continued writing process.

**Paper II:** I participated in planning the study, performed physicochemical characterization (except for XRD), analyzed data, wrote parts of the initial manuscript and contributed to the continued writing process. I did not perform any of the cytocompatibility experiments.

**Paper III:** I participated in planning the study, performed all experimental work (except for recording XPS spectra and ICP-AES), analyzed data, wrote the initial manuscript and contributed to the continued writing process.

**Paper IV:** I participated in planning the study, performed experimental work (except for viscosity measurements, freeze drying, supercritical CO2 drying, CHN elemental analysis and mechanical testing), analyzed data, wrote parts of the initial manuscript and contributed to the continued writing process.

**Paper V:** I participated in planning the study, performed all experimental work (except for CHN elemental analysis), analyzed data, wrote the initial manuscript and contributed to the continued writing process.

**Paper VI:** I participated in planning the study, performed physicochemical and electrochemical characterization, analyzed data, wrote parts of the initial manuscript and contributed to the continued writing process. I did not perform any of the blood compatibility experiments.
Also published

Journal articles


Conference contributions


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### Abbreviations

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<th>Description</th>
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<tbody>
<tr>
<td>BET</td>
<td>Brunauer-Emmet-Teller</td>
</tr>
<tr>
<td>CHN</td>
<td>Carbon, hydrogen and nitrogen</td>
</tr>
<tr>
<td>CNC</td>
<td>Cladophora nanocellulose</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic voltammetry</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>EPTMAC</td>
<td>Epoxypropyltrimethylammonium chloride</td>
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<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>FTIR-ATR</td>
<td>Fourier transform infrared – attenuated total reflectance spectroscopy</td>
</tr>
<tr>
<td>hDF</td>
<td>Human dermal fibroblasts</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>MFC</td>
<td>Microfibrillated cellulose</td>
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<tr>
<td>NFC</td>
<td>Nanofibrillated cellulose</td>
</tr>
<tr>
<td>PPy</td>
<td>Polypyrrole</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TCP</td>
<td>Tissue culture plate</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-tetramethylpiperidine-1-oxyl</td>
</tr>
<tr>
<td>TMX</td>
<td>Thermanox disc</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
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<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
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1. Introduction

Cellulose is the most common polymer on earth. The polymer chains form nanometer wide fibrils that provide mechanical support in the cell wall of e.g. trees and some algae. By extracting the fibrils, or parts thereof, through different chemical and mechanical treatments, one is left with what is known as nanocellulose.

Over the last decade the research on nanocellulose has increased dramatically and the field is expected to grow over the next 30 years.¹ Nanocellulose is renewable, biodegradable and has high strength and stiffness as well as nanoscale properties, such as large specific surface areas.¹⁻³ Some specific characteristics, such as surface charge and dimensions, vary depending on the preparation method and cellulose origin. Some applications of nanocellulose include tissue engineering scaffolds, wound dressings, reinforcement or scaffold material in composites, filtration media, rheology modifiers, drug delivery, solid phase for biocatalysis, paper and packaging.²

Although nanocellulose features many desired properties, new properties for specific applications can be added through functionalization. For example, this can include adding different groups to the surface of the nanocellulose through adsorption or chemical reactions, or coating the nanocellulose fibrils with other materials to form composites, in which the nanocellulose is used as a scaffold or template.

In broad terms, the work in this thesis concerns nanocellulose from wood and the green algae Cladophora sp. where the cellulose is functionalized with surface charges or coated with the conductive and electroactive polymer polypyrrole (PPy) to form nanocellulose and PPy composites (nanocellulose/PPy composites): The preparation of such nanocellulose-based materials and their structural and electrochemical properties as well as their biocompatibility are investigated.

The general outline of the thesis is as follows. In the next chapter, the overall and specific aims of the work are presented. Thereafter, short background information about cellulose, nanocellulose, nanocellulose functionalization, nanocellulose and polypyrrole composites, and biomaterials and biocompatibility is given. A brief description of the different nanocelluloses used in this work is then given, which is followed by results and discussion where the main findings are summarized. At the end, the work is summarized and some concluding remarks are given.
The overall aim of the present work was to study the influence of materials preparation processes on the structural and electrochemical properties of surface-charged nanocellulose and composites composed of nanocellulose and PPy, and assess the biocompatibility of such materials.

The specific aims of the papers were:

**Paper I**: To investigate the possibility of using electrochemical TEMPO-mediated oxidation for oxidation of *Cladophora* algae nanocellulose.

**Paper II**: To characterize the physicochemical properties of films composed of nanofibrillated cellulose from wood and *Cladophora* nanocellulose with different surface charges and assess their cytocompatibility.

**Paper III**: To study the stability of nanocellulose/PPy composites during rinsing in order to find an easy non-degrading rinsing procedure and to study the stability of the composites during storage.

**Paper IV**: To investigate the effects of different drying methods on the structural and electrochemical properties of nanocellulose/PPy composites.

**Paper V**: To study the effects of different types and amounts of nanocellulose on the porosity and electrochemical properties of nanocellulose/PPy composites.

**Paper VI**: To study the blood compatibility and ion exchange capability of nanocellulose/PPy composites intended for hemodialysis.
3. Background

3.1 Cellulose

In 1838 the French chemist Anselme Payen reported that he had isolated a fibrous carbohydrate material by subjecting various plant tissues to acid and ammonia treatments followed by water extraction. That material was named cellulose.

Today it is known that cellulose is a polymer with the primary role of providing structural support in the cell walls of land plants, bushes and trees as well as some algae. It is also produced by fungi, some bacteria and amoebas as well as tunicates (a sea creature). Cellulose is the most abundant polymer on earth and the annual worldwide production has been estimated to be $1.5 \times 10^{12}$ tons. It has traditionally been used for e.g. construction and as an energy source in the form of wood, as well as for producing paper and textiles.

Cellulose is a linear homopolymer composed of D-glucopyranose units linked through $\beta(1-4)$ glycosidic bonds, where each unit is twisted $180^\circ$ with respect to the neighboring units. Therefore the repeat unit is considered to be cellobiose, which consists of two D-glucopyranose units (Figure 1).

![Cellulose Chemical Structure](image)

*Figure 1. Chemical structure of cellulose with the carbon atoms numbered.*

The hydroxyl groups and oxygen atoms facilitate intra- and interchain hydrogen bonding. Intrachain bonding stabilizes the polymer chain, making the structure rather stiff and linear. The interchain hydrogen bonding promotes stacking and ordering of the chains, resulting in crystalline structures, as well as in less ordered chain structures. Due to the large number of hydroxyl groups and oxygen atoms on each cellobiose unit, there are several different crystal structures, or polymorphs. They differ in their hydrogen bonding pattern and, hence, stability. The crystalline form found in nature is called cellulose I and can, through different treatments, form cellulose II, III...
and IV. Cellulose II, for example, can be formed by two processes; regeneration, which involves solubilization and recrystallization; and mercerization, which involves strong alkali treatment. The work in this thesis has been focused entirely on cellulose composed of cellulose I.

Cellulose I can be further divided into cellulose Iα and Iβ, which differ in their hydrogen bonding pattern. Cellulose Iα (monoclinic) and Iβ (triclinic) coexist in different proportions depending on cellulose source. For example, cellulose from algae is dominated by Iα, while cellulose from trees is dominated by Iβ. Iβ is the more stable form and Iα can be converted, at least partly, to Iβ by annealing at 260-280°C in e.g. 0.1 M NaOH or organic solvents.

During biosynthesis, the cellulose chains are assembled into semicrystalline elementary fibrils that contain crystalline regions (cellulose Iα and Iβ) as well as non-crystalline regions. In wood, these fibrils are 3-5 nm in width and they aggregate to form microfibrils, or macrofibrils, which are up to 60 nm in width and several micrometers long. However, the distinction between the different fibrils in terms of size, and the organization and nature of the non-crystalline regions is still debated but it is generally assumed that the fibrils are composed of alternating crystalline and non-crystalline (amorphous or paracrystalline) regions. The key point, however, is that the smallest semicrystalline fibrils aggregate into larger fibril bundles which are embedded in matrix material, forming a hierarchically ordered natural composite material in the cell wall (Figure 2), where the fibrils constitute the main reinforcing component. In wood, the matrix consists of mainly hemicellulose (a group of amorphous and branched polysaccharides) and lignin (a complex hydrocarbon polymer), whereas the lignin content in algae typically is low.

Figure 2. Simplified illustration of the hierarchical organization of cellulose in the wood cell wall (after Lavoine et al. and modified according to Moon et al.).
The size and form of the individual crystalline regions (crystallites), as well as the fibrils, varies depending on the cellulose source and stems from differences in the biosynthesis processes. For example, the crystallites in wood are 3-5 nm in width whereas the crystallites in algae, e.g. Cladophora and Valonia, are significantly larger, ~20 nm.

3.2 Nanocellulose

Nanocellulose is a general descriptor of cellulose fibrils or crystallites with nanometer widths that has been extracted from its source and liberated from each other (to varying extent), and from matrix materials, by different mechanical and chemical treatments.

Nanocellulose, as a family of materials, possesses a number of appealing properties; good mechanical properties, wide functionalization possibilities, and nanoscale properties such as large surface areas and high aspect ratios (length to width ratios of the fibrils or crystallites), while also being renewable and biodegradable. In terms of mechanical properties, crystalline cellulose has been found to possess a tensile strength and axial elastic modulus comparable to, and in some cases higher than, common reinforcement materials such as Kevlar fibers and steel wires, while non-crystalline regions provide the fibrils with flexibility.

The most common source of nanocellulose is wood and in order to extract fibrils, the wood is first disintegrated chemically and/or mechanically in a pulping process, where cellulose fibers are liberated from the matrix materials. The fibers are then further disintegrated into fibrils. The pioneering works were done in the early 1980’s by Turbak et al. and Herrick et al., in which fibril aggregates up to 100 nm in width were extracted by subjecting wood pulp to high shear mechanical homogenization treatments many times. The resulting product was named microfibrillated cellulose (MFC). However, the process required large amounts of energy, which impeded a wide spread use of MFC.

Recently, pulp pretreatments have been introduced in order to reduce the number of homogenizer passes and thereby reducing the energy consumption of nanocellulose production. One pretreatment strategy involves subjecting the pulp to mild enzymatic hydrolysis, where endoglucanase enzymes degrade disordered cellulose. Another strategy is to functionalize the fibrils with anionic or cationic surface charges, leading to electrostatic repulsion between the fibrils, thereby easing the disintegration process during the subsequent mechanical homogenization treatment.

By employing pretreatments, individual fibrils or fibril aggregates that are semicrystalline and typically 3-20 nm in width and a few micrometers long are generated after homogenization. However, the dimensions of individual fibrils and fibril aggregates, and the degree of crystallinity (the fraction of
the material that is crystalline) depend on the preparation method and cellulose source. In general, pretreatments encompassing surface charges results in finer and more individualized fibrils than when enzymatic pretreatment is employed.

Today, the terms nanofibrillated cellulose (NFC) or nanofibrils have more and more replaced the term MFC, in order to emphasize the nanoscale nature of the fibrils and fibril aggregates and to distinguish them from the original MFC, which comprised larger fibril aggregates. The terms NFC or nanofibrils will be used throughout this thesis to describe nanocellulose prepared through pretreatment of wood pulp followed by mechanical homogenization.

Not only fibrils, but also the crystalline parts of the fibrils can be extracted. Cellulose crystallites are typically isolated from wood pulp using strong acid hydrolysis and sonication treatments. During the hydrolysis, non-crystalline regions are degraded faster than the crystalline regions, and the latter therefore remain after the treatment. This results in a highly crystalline form of nanocellulose, which is described as rod-like and typically is 3-5 nm in width and 100-300 nm in length. Nanocellulose extracted in this way is called cellulose whiskers, cellulose nanocrystals, or nanocrystalline cellulose.

As mentioned earlier, the size of the crystallites in nanocellulose varies depending on the cellulose source. When cellulose fibers from algae, e.g. Cladophora or Valonia, are subjected to acid hydrolysis, the resulting nanocellulose is also highly crystalline but does not have the rod-like appearance of wood nanocrystals, it is ~20 nm wide and has lengths comparable to nanofibrils from wood (> 1 µm). Thus, Cladophora nanocellulose can be considered as highly crystalline nanofibrils, and will for simplicity from here on be referred to as nanofibrils or fibrils, and be abbreviated as CNC (Cladophora nanocellulose).

After extraction, NFC is often stored in the form of hydrogels, where the solid content typically is 2-3% and the rest is water, to prevent irreversible fibril aggregation from occurring. When NFC hydrogels are dried in air, capillary forces act on the fibrils, pulling them closer together. This leads to an irreversible reaggregation phenomenon called hornification, where large numbers of hydrogen bonds are formed between the hydroxyl groups on adjacent fibrils. As a result, NFC hydrogels that are dried in air generally form cellulose sheets with low porosities and small specific surface areas (~1 m²/g). In contrast, the extent of hornification of CNC is limited compared to NFC and drying of CNC hydrogels into sheets results in a material of higher porosity and larger surface area (~100 m²/g).

In the NFC hydrogels, the fibrils form an open network structure that, as mentioned, collapses into a compact structure upon drying where the nanoscale features of the fibrils are lost. To overcome this, freeze-drying or supercritical drying of the hydrogel has recently been found to result in mechanically stable aerogels. Aerogels are porous nanostructures in which
the air volume exceeds 90% of the total volume, i.e. the porosity is >90%.\textsuperscript{29} In the aerogels, the open structure of the hydrogel, as well as nanomaterial-specific properties such as the large fibril surface area, are too a large extent retained. Specific surface areas as high as 484 m\textsuperscript{2}/g have, for example, been reported for nanocellulose aerogels.\textsuperscript{28}

### 3.3 Functionalization of nanocellulose

The properties of a substrate surface can be modified in several ways to facilitate a certain application by so called functionalization, including introduction of surface charges, attachment of specific surface groups as well as deposition of coatings on top of the substrate. The reactive hydroxyl groups, amply present on the nanocellulose surface, are often used as a starting point for the functionalization of nanocellulose. Extensive summaries of functionalization of nanocellulose can be found elsewhere.\textsuperscript{3, 5, 8, 30-33}

New functionalities can be introduced already during the nanocellulose preparation as mentioned above, where surface charges are introduced to further the disintegration process or to obtain stable nanocellulose dispersions. Surface charges may also be introduced after the nanocellulose has been extracted. One way to introduce surface charges is through etherification of the hydroxyl groups with monochloroacetic sodium salts, resulting in anionic carboxymethyl groups on the fibril surfaces (Figure 3a).\textsuperscript{22, 32, 34} Cationic surface charges can also be introduced through etherification, using epoxypropyltrimethylammonium chloride (EPTMAC), yielding quaternary ammonium (hydroxypropyltrimethylammonium) groups on the fibril surfaces (Figure 3b).\textsuperscript{21, 32, 35}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Cellulose functionalized with anionic carboxymethyl groups (a) and quaternary ammonium groups (b).\textsuperscript{32}}
\end{figure}

Another method of introducing anionic surface charges is through 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) mediated oxidation.\textsuperscript{19, 32, 36, 37} TEMPO is a radical and a weak oxidizing agent, but can be oxidized to an
oxoammonium cation, which is a much stronger oxidizing agent. Thus, in order to oxidize the TEMPO radical, additional oxidants, such as NaClO and NaBrO, are used to continuously regenerate the oxoammonium cation form of TEMPO. The oxoammonium ions then selectively oxidize the cellulose C6 hydroxyls to carboxylates via an aldehyde intermediate (Figure 4a). This oxidation system is abbreviated as TEMPO/NaBr/NaClO and another used oxidation system is TEMPO/NaClO/NaClO₂. Alternatively, the oxoammonium ions can be regenerated electrochemically at an electrode (Figure 4b). This approach is still relatively unexplored in the case of cellulose oxidation but will be discussed in more detail in Paper I.

Figure 4. TEMPO-mediated oxidation of cellulose C6 hydroxyl groups where oxoammonium cations are regenerated in the TEMPO/NaBr/NaClO system (a) or electrochemically at an electrode (b). Reprinted from Paper I.

Other types of functionalities than charged groups can also be introduced. For example, hydrophobic properties can be introduced through acetylation and silylation. Another example is polymer grafting, where a range of different polymers with various properties have been attached to the surface, either by grafting to or from the surface. Functionalization via non-covalent interactions, where surfactants or polyelectrolytes are adsorbed onto the surface, can also be applied, often with the purpose of improving the nanocellulose dispersibility or to control the assembly of individual fibrils into layered structures.

Another way of functionalizing nanocellulose is to coat the fibrils with other materials, thereby forming composite materials. Since the emergence a few years ago, nanocellulose aerogels have been employed as substrates to produce new functional materials with high porosities and large surface areas. For example TiO₂ and silanes have been deposited on aerogels to produce hydrophobic sponges for separation of oil from water. Actually, the TiO₂ coated aerogels could be photoswitched between a hydrophobic and...
hydrophilic state; when illuminated with UV-light, water corresponding to 16 times the weight of the aerogel itself could be absorbed.\textsuperscript{42} Furthermore, the 7 nm thick TiO$_2$ coating on the aerogel fibrils resulted in a significantly higher photocatalytic activity compared to a filter paper with a specific surface area of 1 m$^2$/g that had been coated using the same process.\textsuperscript{42} This was attributed to a larger amount of deposited TiO$_2$ on the aerogel due to its larger surface area.

Aerogels have also been used as templates for growth of ferromagnetic cobalt ferrite nanoparticles, resulting in magnetic aerogels that are envisioned to be useful in microfluidics devices and as electronic actuators.\textsuperscript{44} Nanofibrils have likewise been functionalized with silver nanoclusters to form a porous composite material with fluorescent properties and pronounced antibacterial activity.\textsuperscript{45}

Thin films of hyaluronic acid, carbon nanotubes and conducting polymers were recently coated onto aerogels through a rapid layer-by-layer process.\textsuperscript{46} A wide range of possible applications were suggested, including energy storage, biomedical devices and drug delivery. Furthermore, the conducting polymer polyaniline has been coated on an aerogel substrate.\textsuperscript{47} The coating corresponded to 4-7% of the total composite weight and the material displayed conductivities corresponding to $\sim$10 mS/cm. Porous and large surface area composites composed of nanocellulose and the conducting polymer polypyrrole have also been reported.\textsuperscript{48} Such composites have been studied in Papers III-VI and will be described in more detail below.

3.4 Polypyrrole

In 1862, Henry Letheby reported of aniline forming a conductive precipitate when oxidized, this compound was blue-green in color and could be turned colorless through reduction.\textsuperscript{49} This was the first report of an intrinsically conducting polymer, also called electronically conducting polymer or simply; conducting polymer, although it was not recognized as a polymer at the time.

During the following 100 years, the work with polyaniline was continued and other conducting polymers were identified, but the research field did not take off until 1977, when Alan J. Heeger, Alan G. MacDiarmid, Hideki Shirakawa and coworkers reported that the conductivity of polyacetylene was increased by 10 orders in magnitude when doped with iodine.\textsuperscript{50, 51} In 2000, Heeger, MacDiarmid and Shirakawa were awarded the Nobel prize in chemistry “for the discovery and development of conductive polymers”.\textsuperscript{52} Today, a large number of conducting polymers are known, and along with polyaniline and polythiophene, polypyrrole (PPy) is one of the most studied conducting polymers.\textsuperscript{52, 53} PPy can be synthesized through oxidative electrochemical or chemical polymerization of pyrrole in both aqueous and non-
aqueous solutions.\textsuperscript{52-54} In electrochemical polymerization, the monomers are oxidized at an electrode, resulting in a film deposited on the electrode surface. In chemical polymerization, the oxidation is carried out by oxidants, such as FeCl$_3$ or K$_2$S$_2$O$_8$ and a polymer powder is generally obtained.

The polymerization mechanism of pyrrole is still debated.\textsuperscript{54} One proposed mechanism is shown in Figure 5.\textsuperscript{54} It is conceived that in the initial step, pyrrole monomers are oxidized to radical cations and two radical cations subsequently dimerize and form an intermediate cation dimer, which subsequently releases two protons, resulting in an uncharged dimer. In the next step, the dimers are oxidized to radical cations that form an intermediate tetramer cation, which after proton release forms uncharged tetramers. In the same way, tetramers form octamers and so on. However, at high monomer concentrations, additional coupling may occur, resulting in trimers, which form hexamers and so on. In alternative mechanisms, the chain elongation proceeds via stepwise addition of radical cation monomers, oligomers or polymers to radical cation oligomers or polymers.\textsuperscript{53, 54} Furthermore, polymerization primarily occurs at the $\alpha$-position, but may occur in the $\beta$-position as well, resulting in branching and cross-linking of chains.

![Figure 5. Illustration of one proposed polymerization mechanism for the polymerization of pyrrole to PPy.\textsuperscript{54}](image)

After polymerization, the polymer is in an oxidized state, which means that it carries a positive (cationic) charge. The cationic charges of the polymer are compensated by counter ions carrying anionic charges, in order to maintain charge neutrality.\textsuperscript{52} The counter ions are present in the polymerization solution and the morphology, conductivity and electrochemical properties of the polymer are influenced by the choice of counter ions as well as by the solvent and polymerization method.\textsuperscript{52, 55-61}

In contrast to electrochemical polymerization, chemical polymerization can easily be used to produce bulk quantities of PPy and requires no conducting substrate and it is therefore preferred from an industrial point of view.\textsuperscript{52, 55} At the same time, impurities are introduced during chemical polymerization and the selection of counter ions and oxidizing agents is limited, as the oxidizing agent needs to be strong enough for polymerization to occur, while a too strong oxidizing agent will overoxidize (degrade) the material.\textsuperscript{52, 57}
PPy is electroactive, meaning it can be reversibly switched between a reduced (uncharged) state and an oxidized (cationically charged) state, by controlling the applied potential (Figure 6). It is conductive when it is in the oxidized state and non-conductive in its reduced state. The switching of oxidation states is tightly coupled to diffusion of counter ions into and out from the polymer to maintain charge neutrality. This means that PPy generally functions as an electrochemically controlled anion exchange material, although cation exchange properties can be introduced by employing bulky immobile anionic counter ions during polymerization. The switching of oxidation states and the movement of ions also results in swelling and shrinkage of the material; during oxidation, when anionic counter ions (typically) diffuse into the material, swelling occurs, while shrinkage occurs upon reduction, when the counter ions are expelled.

![Figure 6. Simplified illustration of PPy oxidation and reduction where counter ions are incorporated in the oxidized form to maintain charge neutrality.](image)

There are a number of different applications of PPy, and other conducting polymers as well, in various fields. Some examples are energy storage, biosensors, drug delivery, ion exchange, artificial nerves, and micro- and bioactuators.

3.5 Nanocellulose and polypyrrole composites

As touched upon in Section 3.3, composites consisting of nanocellulose and PPy can be produced, in which nanocellulose fibrils are coated with PPy, thereby adding new functions, i.e. conductivity and electroactivity, to the nanocellulose structure. At the same time, the fibrils provide a large surface area and mechanical support for the otherwise brittle PPy and facilitate the post-synthesis processing of PPy.

A composite consisting of CNC and PPy can be considered as an example of this type of material. In a typical composite synthesis procedure, pyrrole
is polymerized by FeCl$_3$ in an aqueous nanocellulose dispersion, resulting in PPy-coated fibrils (from here on referred to as composite fibers). This process is sometimes called \textit{in situ} polymerization. The composite fibers are then washed and dried in air into a black flexible paper sheet (Figure 7). The described coating process has been employed throughout the thesis work, but the subsequent steps (washing and drying) have been varied, as will be described in later sections.

![Figure 7. Photograph (left) and SEM micrograph (right) of a CNC/PPy composite.](image)

Returning to the example with the CNC/PPy composite, the composite fibers in the dry material form an entangled and porous network, as can be seen in the scanning electron microscopy (SEM) micrograph in Figure 7. The material has \(\sim80\%\) porosity and a specific surface area of up to \(80\ \text{m}^2/\text{g}\) (for comparison, a filter paper has approximately \(1\ \text{m}^2/\text{g}\))\cite{66, 67}. The thickness of the PPy coating is less than 50 nm and corresponds to \(\sim2/3\) of the total composite weight\cite{67}.

It should also be mentioned that PPy has been coated on other substrates than nanocellulose, e.g. silk fibers, filter papers and cellulose fibers\cite{55, 68-72}. However, nanocellulose fibrils provide a large surface area which means that higher mass loadings of PPy can be achieved while keeping the coating thin, as compared to substrates of much smaller surface area, e.g. filter papers\cite{72}. Keeping the coating thin is important for electrochemical applications as the counter ion diffusion in thick PPy films becomes rate limiting\cite{48}. Similar composites based on nanocellulose from wood\cite{73} as well as bacterial nanocellulose\cite{74} have also been reported.

Nanocellulose/PPy composites have been employed as electrodes in environmentally friendly energy storage devices\cite{67, 75} and as electrochemically controlled ion exchange materials for extraction of small inorganic and organic anions\cite{58, 76}, as well as for extraction of DNA of different sizes\cite{77-79}. In Papers III-VI, the development of nanocellulose/PPy composites is further described.
3.6 Biomaterials and biocompatibility

Biomaterials are materials that are intended to be used in biological systems, often as implants or devices for medical applications. The type of material used depends on the application, but generally ceramics, metals, natural materials, or polymers are used, where polymers comprise the largest class of biomaterials. Some well-known application examples are hip or knee joint replacements (e.g. titanium), artificial kidney treatments for patients with kidney failure (e.g. cellulose or polysulfone hemodialysis membranes) and contact lenses (e.g. silicone-acrylate).

Biomaterials are intimately linked to the concept of biocompatibility, which has been defined by Williams as follows:

Biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific situation.

This means that for biocompatibility to be achieved the interaction between the material and the host (patient) should not lead to complications and this is application specific. Complications can be either that the intended function of e.g. the device fails, or that the device triggers an unwanted response in the patient. The unwanted responses stem from the defense mechanisms in the body, which normally protect against pathogens and foreign materials, and heal injuries and wounds. When a material is brought into contact with the host, proteins adsorb non-specifically to the surface and a cascade of reactions follow. Although much is known about these processes, they are not completely understood as they are very complex and to a large extent inter-linked. General comprehensive descriptions of the defense mechanisms can be found elsewhere and will hence not be further discussed here.

In order to evaluate the biocompatibility with respect to the host response to a material, the interaction between the host and the material in terms of e.g. toxicity, blood-material interaction, inflammation, infection and tumorgenesis is investigated. The complexity of the host response is mirrored in the number of, and the variability in, material characteristics that can influence the response, some of which are listed in Table 1. From this follows that by changing one physicochemical property of a biomaterial, the biocompatibility has to be reassessed as the host response may have been altered.
Table 1. Examples of material characteristics that could influence the host response to a material.88

<table>
<thead>
<tr>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro- and nano-structure</td>
</tr>
<tr>
<td>Morphology</td>
</tr>
<tr>
<td>Crystallinity</td>
</tr>
<tr>
<td>Hydrophobicity/hydrophilicity</td>
</tr>
<tr>
<td>Macro-, micro-, and nano-porosity</td>
</tr>
<tr>
<td>Surface chemical composition</td>
</tr>
<tr>
<td>Surface topography</td>
</tr>
<tr>
<td>Surface charge</td>
</tr>
<tr>
<td>Leachables and contaminants and their toxicity</td>
</tr>
<tr>
<td>Material degradation products and their properties, including toxicity</td>
</tr>
</tbody>
</table>

The initial biocompatibility evaluation is based on in vitro testing of different responses to the material in model systems under controlled conditions, which, however, may not necessarily reflect the true response in the body and in vivo testing is therefore also required at later stages.84, 85 Usually the toxicity profile of a material is established before more application specific responses are investigated.

The cytocompatibility, or cytotoxicity, of a material can be assessed directly or indirectly with cultures of model cell lines, of e.g. fibroblasts, macrophages or stem cells, and the observed effects can vary depending on cell type.89-91 The two tests differ in the manner in which the test material is exposed to the cells. In direct tests, cells are in direct contact with the studied material, for example the material is used as cell culture substrate. In indirect tests, also called elution tests, cells are exposed to an extract solution of the material. The tests are evaluated through changes in cell proliferation, viability and morphology.

In order to assess blood compatibility, in vitro models are used where the material is brought in contact with blood or plasma under controlled conditions.92 Thereafter the events associated with the activation of the cascade systems in the blood are studied, such as protein adsorption, fibrin and thrombin formation, and platelet and leukocyte adhesion/activation, as well as complement system activation, by observing the material surface and by determining the levels of associated components in the blood.84, 93 The cytocompatibility of nanocellulose films comprising different surface charges and fibril structures was screened in Paper II, while the blood compatibility of nanocellulose/PPy composites was assessed in Paper VI.
4. Materials

The following brief section provides an overview of the nanocelluloses used in this thesis work. Nanocelluloses prepared from *Cladophora* sp. green algae (CNC) and from wood pulp (NFC) were prepared in different ways, as summarized in Table 2. Other experimental details are given in connection to the results and discussion. Full experimental descriptions can be found in the appended papers.

Table 2. Description of nanocelluloses used in the thesis work.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Paper(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC</td>
<td><em>Cladophora</em> nanocellulose, prepared through acid (HCl) hydrolysis. Provided as spray-dried powder by FMC Biopolymers (USA). Carboxyl group content: 0.04 mmol/g.</td>
<td>I, II, III, VI</td>
</tr>
<tr>
<td>TEMPO-CNC</td>
<td>Prepared through TEMPO-mediated oxidation of CNC in Papers I and II. Carboxyl group content in Paper II: 0.45 mmol/g.</td>
<td>I, II</td>
</tr>
<tr>
<td>EPTMAC-CNC</td>
<td>Prepared through EPTMAC quaternization of CNC in Paper II. Ammonium group content: 0.29 mmol/g.</td>
<td>II</td>
</tr>
<tr>
<td>ENZYME-NFC</td>
<td>Preparation included enzymatic pretreatment of bleached sulfate softwood pulp. Provided as never-dried hydrogel by collaborators at Innventia AB (Sweden). Carboxyl group content: 0.03 mmol/g.</td>
<td>II, V</td>
</tr>
<tr>
<td>CARBOXY-NFC</td>
<td>Preparation included carboxymethylation pretreatment of bleached sulfate softwood pulp. Provided as never-dried hydrogel by collaborators at Innventia AB (Sweden). Carboxyl group content: 0.53 mmol/g.</td>
<td>II, V</td>
</tr>
<tr>
<td>EPTMAC-NFC</td>
<td>Preparation included EPTMAC quaternization pretreatment of bleached sulfate softwood pulp. Provided as never-dried hydrogel by collaborators at Innventia AB (Sweden). Ammonium group content: 1.6 mmol/g.</td>
<td>II</td>
</tr>
<tr>
<td>TEMPO-NFC</td>
<td>Preparation included TEMPO-mediated oxidation pretreatment of sulfate softwood pulp. Provided as never-dried hydrogel by collaborators at KTH (Sweden). Carboxyl group content: 2.3 mmol/g.</td>
<td>IV</td>
</tr>
</tbody>
</table>
5. Results and discussion

5.1 Nanocellulose with surface charges

In this section, the results and discussions from Papers I and II are summarized. In Paper I, a method to introduce surface charges on CNC is demonstrated, while in Paper II nanocellulose films based on CNC and NFC with different surface charges were evaluated in terms of cytocompatibility.

5.1.1 Electrochemical TEMPO-mediated oxidation of Cladophora nanocellulose

TEMPO-mediated oxidation has become one of the most popular ways of introducing charges on nanocellulose fibrils, most often by employing the TEMPO/NaBr/NaClO system. Other systems, e.g. TEMPO/NaClO/NaClO₂, as well as electrochemical regeneration, has been employed, but have resulted in significantly lower degrees of oxidation, as compared to when the TEMPO/NaBr/NaClO system was employed. The ability of the oxoammonium ions to completely oxidize the surface of the fibrils has been questioned and it is conceived that the oxidation, to some extent, is carried out directly by NaBrO, NaClO and NaClO₂. Specifically, it has been postulated that TEMPO-species are sterically hindered from oxidizing all intermediate C6 aldehydes to carboxyls.

One drawback of TEMPO-mediated oxidation is the high cost of this compound and there hence is a need to develop methods in which TEMPO can be easily recovered and reused. From this perspective, electrochemical regeneration is appealing, as the same reaction medium, in principle, easily could be reused after the nanocellulose has been removed by e.g. filtration. In Paper I, the possibility of using electrochemical regeneration of oxoammonium ions for oxidation of CNC (Cladophora nanocellulose) was explored.

TEMPO was dissolved in carbonate buffer (pH 10) and the oxidations were carried out for 30 minutes up to 72 hours, with a ~12 cm² working electrode operating at +0.7 V vs. Ag/AgCl. The total charge over 72 hours, calculated from the measured current, was five times larger when cellulose was present as compared to in a control experiment without cellulose (Figure 8). This implies that the cellulose was oxidized, which was also confirmed through FTIR (Fourier transform infrared spectroscopy) analysis of the in-
soluble products. The FTIR data showed that the carboxyl groups were in their acidic forms and also indicated that the extent of oxidation was controlled by the electrolysis time. The maximum degree of oxidation appeared to be reached after four hours of oxidation (Figure 9).

![Figure 8. Chronoamperograms (left vertical axis) and chronocoulograms (right vertical axis) for electrochemical regeneration of oxoammonium ions during 72 hours in the presence and in the absence of CNC. Reprinted from Paper I.](image)

![Figure 9. FTIR spectra of oxidized CNC. The spectra have been normalized with respect to the C-H stretching vibration at 2897 cm$^{-1}$. Reprinted from Paper I.](image)
The amount of carboxylic acids, aldehydes and ketones obtained are shown in Table 3. The amount of carboxylic acids increased linearly up to 0.59 mmol/g during the first four hours of the oxidation and then remained constant for longer electrolysis times. The amount of aldehydes was 0.11 mmol/g for products oxidized between 30 minutes and three hours, and for longer electrolysis times no aldehydes were detected. The amount of ketones was below 0.071 mmol/g in all samples (but in many cases even lower) due to the selectivity of oxoammonium ions for the C6 hydroxyls.

Table 3. Carboxylic acid, total aldehyde and ketone, aldehyde, and ketone contents and degree of oxidation of CNC following oxidation.

<table>
<thead>
<tr>
<th>Oxidation time (h)</th>
<th>Carboxylic acid (µmol/g)a</th>
<th>Total aldehyde and ketone (µmol/g)b</th>
<th>Aldehyde (µmol/g)c</th>
<th>Ketone (µmol/g)d</th>
<th>Carboxylic acid (%)</th>
<th>Aldehyde (%)</th>
<th>D.O. (%)e</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>37 ± 2</td>
<td>&lt; 71</td>
<td>32 ± 12</td>
<td>&lt; 39</td>
<td>0.6</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>0.5</td>
<td>79 ± 7</td>
<td>107</td>
<td>95 ± 15</td>
<td>12</td>
<td>1.3</td>
<td>1.5</td>
<td>2.8</td>
</tr>
<tr>
<td>1</td>
<td>143 ± 13</td>
<td>107</td>
<td>110 ± 14</td>
<td>0</td>
<td>2.3</td>
<td>1.8</td>
<td>4.1</td>
</tr>
<tr>
<td>2</td>
<td>301 ± 9</td>
<td>107</td>
<td>93 ± 20</td>
<td>14</td>
<td>4.9</td>
<td>1.5</td>
<td>6.4</td>
</tr>
<tr>
<td>3</td>
<td>461 ± 10</td>
<td>114</td>
<td>88 ± 19</td>
<td>26</td>
<td>7.5</td>
<td>1.5</td>
<td>9.0</td>
</tr>
<tr>
<td>4</td>
<td>591 ± 10</td>
<td>&lt; 71</td>
<td>3 ± 10</td>
<td>&lt; 68</td>
<td>9.7</td>
<td>0</td>
<td>9.7</td>
</tr>
<tr>
<td>8</td>
<td>602 ± 7</td>
<td>&lt; 71</td>
<td>0</td>
<td>&lt; 71</td>
<td>9.8</td>
<td>0</td>
<td>9.8</td>
</tr>
<tr>
<td>24</td>
<td>595 ± 11</td>
<td>&lt; 71</td>
<td>0</td>
<td>&lt; 71</td>
<td>9.7</td>
<td>0</td>
<td>9.7</td>
</tr>
<tr>
<td>72</td>
<td>599 ± 21</td>
<td>&lt; 71</td>
<td>0</td>
<td>&lt; 71</td>
<td>9.8</td>
<td>0</td>
<td>9.8</td>
</tr>
</tbody>
</table>

a From conductometric titrations. The values represent the mean ± standard deviation (n=3).
b From CHN (carbon, hydrogen and nitrogen) elemental analysis of oximes after a Schiff base coupling reaction with hydroxylamine.
c The difference in carboxylic acid content before and after chlorite oxidation. The values represent the mean ± standard deviation (n=3).
d The difference between the total aldehyde and ketone content and the aldehyde content.
e The sum of the carboxylic acid and aldehyde content.

It has previously been shown that 0.52 mmol/g of carboxyls and 0 mmol/g of aldehydes, were achieved by using the TEMPO/NaBr/NaClO system, corresponding to a complete oxidation of the CNC fibril surface. As a slightly higher carboxyl content (0.59 mmol/g) and no aldehydes was found in the current work, it can be concluded that electrochemical regeneration of oxoammonium ions works just as well as using oxidants such as NaBrO and NaClO in order to completely oxidize the surface of the nanocellulose fibrils. Furthermore, this implies that there is no significant steric hindrance for the oxoammonium ions to oxidize the intermediate aldehyde groups, in contrast to earlier hypotheses. The results also show that the extent of oxidation is controlled by the oxidation time and that the maximum degree of oxidation is reached after four hours under the employed reaction conditions.

To evaluate if any depolymerization occurred during the oxidation, intrinsic viscosities were determined for all samples. Depolymerization may occur
due to the presence of aldehydes and ketones, which makes the glycosidic bonds susceptible to β-elimination under alkaline conditions.\textsuperscript{36, 101} Viscosities were determined before and after chlorite (ClO$_2^-$) oxidation (to oxidize aldehydes to carboxyls for the analysis) and thereby the possibility of depolymerization occurring during the course of dissolution and measurements could be dismissed, as no difference between the samples was observed (Figure 10).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure10.png}
\caption{Intrinsic viscosities determined for the insoluble products of TEMPO-oxidized CNC before and after chlorite oxidation. (n=5, error bars=1 standard deviation are hidden behind the symbols, left vertical axis), as well as the product recovery after electrolysis (right vertical axis). The lines are only intended as guides to the eye. Reprinted from Paper I.}
\end{figure}

During the first 3-4 hours, the intrinsic viscosity of the insoluble products decreased by ~20%, corresponding to a degree of polymerization decrease from ~740 to ~570.\textsuperscript{102} For samples electrolyzed for ≥ 4 h, an unexpected intrinsic viscosity increase was observed, while the product recovery decreased. As the intrinsic viscosity reflects some average value of the cellulose chain length distribution, it is conceived that the observed increase indicates that the distribution of the insoluble products shifts towards longer chains as the amount of soluble cellulose increases. However, from a practical point of view, there is no benefit in performing the oxidation for longer than four hours, in particular since the maximum degree of oxidation has already been reached and the product recovery has started to decrease.

A number of general characterization methods were subsequently employed to investigate if any other physicochemical properties were affected by different degrees of oxidation. SEM revealed no significant differences in morphology between any of the samples and the BET (Brunauer-Emmet-
specific surface area ranged between 116 and 132 m²/g for all samples. The crystallinity index, calculated from X-ray diffraction (XRD) data, varied randomly between 91% and 94% for all samples, showing that the fraction of crystalline material in the fibrils was unaffected by the oxidation. The water binding capacity was observed to increase as the degree of oxidation increased while the thermal stability decreased.

In summary, the results in Paper I showed that CNC can be oxidized to the same extent using electrochemical regeneration of oxoammonium ions as with the TEMPO/NaBr/NaClO system. This also showed that there is no steric hindrance for the oxoammonium ions to completely oxidize the fibril surface. The degree of oxidation could be controlled by the electrolysis time and the oxidation should not be carried out for longer than necessary as this will reduce the product recovery.

5.1.2 Cytocompatibility of nanocellulose films

Cellulose and derivatives thereof have been extensively studied for use in biomedical applications, e.g. for wound dressing and tissue engineering, and in particular it has been used for hemodialysis membranes and as pharmaceutical excipients. For nanocellulose, there are many in vitro and in vivo studies of the biocompatibility of bacterial nanocellulose for the potential use in artificial blood vessels and wound dressings. Wound dressings and tissue engineering have also been proposed as potential applications of nanocellulose from wood, as well as use as pharmaceutical excipients and in antimicrobial films. The cytocompatibility of NFC, and in particular for CNC, has, however, remained largely unexplored. The studies that have been reported have generally shown positive (cytocompatible) results with gel suspensions, hydrogels, aerogels or air-dried films based on different forms of NFC upon exposure to macrophages, liver, or fibroblast cell lines.

As described in Section 3.6, many parameters can affect the biocompatibility of materials and it cannot be assumed that nanocellulose shares the cytocompatibility of earlier cellulose-based materials. In addition, nanocellulose is a family of materials featuring e.g. different surface charges, fibril dimensions and film nanostructures, all of which could potentially affect the host response. The objective of Paper II was to evaluate the physicochemical properties of films prepared from different forms of nanocellulose and assess the in vitro cytocompatibility of the films, with the purpose of screening which types of films could be suitable for biomaterial applications in general.

Films were prepared from nanocellulose dispersions by employing reduced pressure filtration followed by drying in air. The different forms of nanocellulose used were CNC, TEMPO-CNC, EPTMAC-CNC, ENZYME-NFC, CARBOXY-NFC and EPTMAC-NFC. For notations see Table 2.
CNC and ENZYME-NFC fibrils do not carry significant numbers of surface charges, TEMPO-CNC and CARBOXY-NFC carry anionic surface charges at pH 7 due to carboxylate groups, while EPTMAC-CNC and EPTMAC-NFC have cationic surface charges due to quaternary ammonium groups. This was confirmed by determining the ζ-potential for each form of nanocellulose in dilute dispersions at pH 7 (Table 4). Depending on the nanocellulose used, the resulting films had different characteristics, as seen in Table 4.

Table 4. Characteristics of investigated nanocellulose films.

<table>
<thead>
<tr>
<th></th>
<th>CNC</th>
<th>TEMPO-CNC</th>
<th>EPTMAC-CNC</th>
<th>ENZYME-NFC</th>
<th>CARBOXY-NFC</th>
<th>EPTMAC-NFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET surface area (m²/g)</td>
<td>102</td>
<td>77</td>
<td>70</td>
<td>0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>ζ-potential at pH 7 (mV)</td>
<td>-12</td>
<td>-41</td>
<td>31</td>
<td>-7.5</td>
<td>-27</td>
<td>26</td>
</tr>
<tr>
<td>Water content at 100°C (wt%)</td>
<td>1.3</td>
<td>1.7</td>
<td>1.9</td>
<td>4.6</td>
<td>6.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Crystallinity index (%)</td>
<td>92</td>
<td>93</td>
<td>94</td>
<td>36</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

a From nitrogen adsorption isotherm data.
b From thermogravimetric analysis after equilibration at 42-43% relative humidity for ≥ 24 h.
c Estimated from XRD data using the method described by Segal et al.104

The nanostructure of the films varied significantly depending on the type of nanocellulose used (see Figure 11). All CNC-based films were more porous than the NFC-based films and films with significant surface charges (TEMPO-CNC, EPTMAC-CNC, CARBOXY-NFC and EPTMAC-NFC) were less porous than the corresponding film composed of CNC or ENZYME-NFC. These results were in good agreement with the specific surface areas (Table 3). Interestingly, only the fibrils of TEMPO-CNC were observed to form co-axially aligned fibril aggregates on the film surface (Figure 11b).
Figure 11. SEM micrographs of films composed of CNC (a), TEMPO-CNC (b) and EPTMAC-CNC (c) as well as ENZYME-NFC (d), CARBOXY-NFC (e) and EPTMAC-NFC (f) at ~75 kX magnification. The insets show the corresponding films at ~1.5 kX magnification. Reprinted from Paper II with permission from the publisher.

Indirect cytocompatibility tests were performed in compliance with the procedures outlined in the ISO-10993-5 guidelines. The films were extracted in culture medium for 24±2 h at 37°C and the medium was subsequently used to culture human dermal fibroblasts (hDF) in tissue culture plates (TCP). The cell viability was determined with respect to the negative control (viability determined for cells cultured in TCP extracted medium) and was for all films significantly above (95% confidence interval) the 70% limit set in the ISO-10993-5 guidelines. This means that no toxic effects due to leaching from any of the films could be detected.

These results were confirmed with light microscopy of the cells that had adhered to the culture plate. For the extracts of all films a great number of cells had adhered and displayed the typical elongated shape of hDF cells, in similarity to the negative control. This was distinctly dissimilar to the fewer and round-shaped cells of the positive control, where cells had been cultured in medium supplemented with 5% dimethyl sulfoxide (DMSO).

The direct in vitro cytocompatibility of the nanocellulose films was assessed by culturing hDF cells directly on the films and then determining the cell viability of the adhered cells (CNC samples) or the number of adherent cells (NFC samples). The alamar blue reagent interacted with the NFC samples in control experiments, whereas this was not observed with the CNC samples. Therefore another assay, an LDH (Lactate dehydrogenase) assay, had to be used for the NFC samples. As LDH is released into the culture medium by non-viable cells due to loss of membrane integrity during culturing, the medium was replaced with fresh medium prior to lysing the adherent
cells. Thus, the number of adherent cells determined through the LDH assay should correspond well to the number of viable cells on the NFC films.

For the CNC-based films (Figure 12a), only TEMPO-CNC, i.e. the only film featuring anionic and co-axially aligned fibril aggregates, possessed good cytocompatibility, i.e. comparable to the negative control (Thermanox disc, TMX). For the films composed of NFC (Figure 12b), only the EPTMAC-NFC film, i.e. the film comprising cationic fibrils, showed significantly higher number of adhered cells than the positive control (cells cultured on TMX in the presence of 5% DMSO), indicating that the EPTMAC-NFC film was more cytocompatible than the other NFC films.

Figure 12. Cell viability of hDF cells cultured on CNC, TEMPO-CNC and EPTMAC-CNC films (a), and number of adherent cells on ENZYME-NFC, CARBOXY-NFC and EPTMAC-NFC films (b). Corresponding values for cells cultured on TMX (negative control) and cells cultured on TMX in the presence of 5% DMSO (positive control) are shown in each panel. Data represent the mean ± standard error (n=5). Adapted from Paper II.

These results were confirmed by studying the number and morphology of adhered cells in SEM (Figure 13). While large numbers of spindle-shaped cells were spread over the TEMPO-CNC film (Figure 13b), in similarity to the negative control TMX (Figure 13d), few and mainly single round-shaped cells were observed on the other CNC-based films (Figure 13a and c). For the films composed of NFC, the cells on the EPTMAC-NFC film (Figure 13h) resembled the cells on the negative control TMX (Figure 13d) the most, whereas fewer and round-shaped cells were observed on the other NFC films.
Figure 13. SEM micrographs at ~1 kX magnification depicting hDF cells cultured on CNC (a), TEMPO-CNC (b) and EPTMAC-CNC (c) films, TMX (negative control, d), TMX in the presence of 5% DMSO (positive control, e), ENZYME-NFC (f), CARBOXY-NFC (g) and EPTMAC-NFC (h) films. Reprinted from Paper II with permission from the publisher.

Given the complex nature of cell-material interactions, one can only speculate on the background for the direct cytocompatibility testing results. However, it is known that the scale and ordering of the nanotopography can affect cell behavior in terms of e.g. adhesion and proliferation, and it is possible that the aggregation of the anionic fibrils into, essentially, larger fibrils and their coaxial alignment in the TEMPO-CNC films provide an ordered nanotopography that is favorable for hDF adhesion and growth. Furthermore, it has earlier been found that fibroblast cell proliferation was more efficient with aligned fibers, although the investigated fiber width in that case was significantly larger (0.97 µm).

In addition, it is expected that the adsorption and conformation of proteins on the surface of the fibrils are affected by the surface charges, thereby affecting the cell interaction. For example, stronger fibroblast adhesion and spreading was observed on carboxyl and amine terminated surfaces at biological pH, compared to OH-terminated surfaces, which was explained by that larger numbers of the adhesive proteins fibronectin and vitronectin had adsorbed on those surfaces. From this perspective it is surprising that the film composed of cationic Cladophora fibrils (EPTMAC-CNC) did not
promote hDF adhesion and spreading, but it could possibly be due to the lack of fibril aggregation and alignment.

The results from the direct in vitro cytocompatibility testing of films composed of NFC contrasted the results of the CNC based films; the cationic film (EPTMAC-NFC) displayed the greatest number of adhered cells among the NFC-based films. This further illustrates the complexity of the cell response to a material and the difficulty of assigning the observed effects to one specific material property. However, the water content determinations (Table 4) indicated that EPTMAC-NFC was the least hydrophilic of the three NFC films, which has been confirmed through contact angle measurements. It is possible that this may have contributed to the observed differences between NFC films as more hydrophobic surfaces tend to adsorb more proteins than hydrophilic ones.\textsuperscript{123}

In summary, no films were found to be cytotoxic when assessed through indirect testing. TEMPO-CNC promoted cell adhesion and proliferation, possibly due to the distinct aligned structure of the aggregated fibrils. Out of the NFC films, EPTMAC-NFC was found to be the most cytocompatible.

5.2 Nanocellulose and polypyrrole composites

In this section, results and discussions pertaining to composites composed of nanocellulose and PPy are summarized. In the first three papers (Papers III-V), the preparation and the resulting electrochemical properties are in focus. In the last paper, Paper VI, the blood compatibility and ion exchange capability of composites intended for hemodialysis are investigated.

5.2.1 Stability during rinsing

The preparation of the composites is straightforward; pyrrole is chemically polymerized by FeCl\textsubscript{3} in water in the presence of dispersed nanocellulose fibrils, resulting in PPy-coated fibrils. The composite fibers are then washed and dried into a paper-like material.

An often overlooked parameter in the preparation of PPy in general is the washing following synthesis. The washing step is carried out primarily to remove the remaining oxidizing agent (e.g. Fe\textsuperscript{3+}) and the corresponding reduced form (Fe\textsuperscript{2+}), but also any remaining pyrrole and non-adsorbed short PPy fragments. Typically, washing of PPy powders as well as composites has been carried out by rinsing the product with large amounts of water under reduced pressure, in the most extreme cases for several days.\textsuperscript{124-126} Furthermore, in order to produce non-cytotoxic composites, extensive rinsing is required.\textsuperscript{127} At the same time, it is known that PPy degrades in water,\textsuperscript{128} and therefore one could suspect that washing with water degrades the PPy layer of the composite. If so, the amount of charge that can be stored in PPy-based
energy storage devices or the number of ions that can be extracted in PPy-based ion-extraction membranes is reduced. In the first part of Paper III, the effects of rinsing with different volumes of different aqueous solutions were investigated, in order to find an easy rinsing method that did not degrade the PPy layer of the composite.

The nanocellulose used was CNC and pyrrole was polymerized using FeCl₃. After coating, the gel-like material was split into four parts, and the parts were rinsed with different amounts (2, 7, 12 or 17 liters) of the rinsing solution. Thereby the synthesis conditions had been identical for all samples rinsed with the same solution. The same procedure was repeated with other rinsing solutions. The rinsing solutions were water, 0.4 M NaCl (abbreviated NaCl) or 0.4 M HCl (abbreviated HCl). Additionally, in one series of samples, the pH during synthesis was decreased from ~2 to ~0.5 and the material was then rinsed with 0.4 M HCl (abbreviated HCl+HCl).

Following synthesis, washing and drying, the conductivity and electroactivity changes of the composite sheets were evaluated. The electroactivity of the composites was evaluated by calculating the specific charge capacity from cyclic voltammetry (CV) measurements at 5 mV/s and the results are shown in Figure 14. Both the conductivity and electroactivity followed the same general trend; water and 0.4 M NaCl rinsing resulted in significant rinsing volume-dependent conductivity and electroactivity losses, while the conductivities and electroactivities remained high after rinsing with 0.4 M HCl. This indicated that, in contrast to water or 0.4 M NaCl rinsing, rinsing with 0.4 M HCl did not degrade the PPy-layer of the composites.

![Figure 14](image)

**Figure 14.** Specific charge capacity for nanocellulose/PPy composites as a function of rinsing volume using different rinsing solutions. The presented values are average values and the error bars correspond to the absolute deviation (n=2). The error bar is hidden behind the symbol for most samples. The lines are only guides to the eyes. Adapted from Paper III.
The non-degrading effect of 0.4 M HCl rinsing, as well as the degrading effect of water or 0.4 M NaCl rinsing, was further evidenced using FTIR-ATR (Fourier transform infrared – attenuated total reflectance spectroscopy) and XPS (X-ray photoelectron spectroscopy). While no volume-dependent spectral changes could be observed as a result of 0.4 M HCl rinsing, several changes occurred due to rinsing with water or 0.4 M NaCl, as will be described below.

The generally accepted mechanism of PPy degradation is a nucleophilic attack by hydroxide ions on oxidized PPy (which is carrying positive charges), resulting in the formation of hydroxyl and carbonyl groups in the PPy backbone.\textsuperscript{125, 126, 129-142} This leads to a reduction of the PPy oxidation state and the conductivity and electroactivity are irreversibly reduced. In the present work, no significant numbers of carbonyl groups could be detected in any of the samples using XPS and FTIR-ATR analyses.

However, the shape of the 1030 cm\textsuperscript{-1} peak (previously assigned to a C-H bending vibration)\textsuperscript{143, 144} in the FTIR-ATR spectra was significantly affected by the extent of water or 0.4 M NaCl rinsing (Figure 15). In control experiments with samples that had been degraded by electrochemical cycling at pH 12, as well as samples degraded by cycling to high (overoxidative) potentials, significant changes in the FTIR-ATR peak shape were also observed. Both processes are known to generate hydroxylated PPy.\textsuperscript{135, 136} This indicated that the change in peak shape could be due to formation of increasing numbers of hydroxylated PPy. Thus, the gradually increased degradation following water or 0.4 M NaCl rinsing could be due to progressive formation of more hydroxyl groups in PPy. In contrast, the peak was not affected by 0.4 M HCl rinsing, confirming that no degradation occurred during the acidic rinsing.
The atomic ratio between chlorine (Cl⁻ counter ions) and nitrogen (N from PPy) was derived from XPS data in order to get an estimate of the number of counter ions in relation to PPy. While the ratio decreased from 0.20 to 0.10 or from 0.29 to 0.17 following 2 and 17 liters of rinsing with water or 0.4 M NaCl, respectively, the ratio remained constant at 0.27-0.29 for the samples rinsed with 2 or 17 liters of 0.4 M HCl. This further demonstrated the non-degrading effect of rinsing the composites with 0.4 M HCl. As the degradation is coupled to loss of positive charges in PPy, the number of counter ions was expected to decrease if degradation had occurred, as was the case after water or 0.4 M NaCl rinsing.

In addition to the non-degrading effect of 0.4 M HCl rinsing, another advantage of using such solution in the rinsing step of nanocellulose/PPy composite production is that a composite containing less iron species is obtained. While rinsing with water resulted in 0.15-0.19 wt% of iron species remaining in the final product, 0.016-0.021 wt% remained after rinsing with 17 liters of 0.4 M HCl.

Apart from showing that rinsing with acidic solutions is more effective for iron removal and does not cause any degradation, it was also demonstrat-
ed in Paper III that FTIR-ATR can be used as a quick analytical tool to evaluate the extent of PPy degradation. It was observed that most of the peaks, to varying extents, shifted in position as a result of degradation and it has previously been observed that changing the PPy oxidation state leads to peak shifts.\textsuperscript{143, 145} For one of the peaks, corresponding to a C-H bending vibration,\textsuperscript{143, 144} a linear correlation between the specific charge capacity and the peak wavenumber was found, shown in Figure 16. Thus, in future work this empirically derived correlation may be used to, in relative terms, evaluate the extent of degradation in samples.

![Figure 16](image)

*Figure 16. Correlation between the specific charge capacity and the FTIR-ATR wavenumber at \(\sim 1300\, \text{cm}^{-1}\) (a C-H bending vibration). The error bars represent the absolute deviation of two measurements and are in some cases hidden behind the symbol. Reprinted from Paper III with permission from the publisher.*

To conclude, rinsing with water or 0.4 M NaCl degrades the PPy coating, while acidic rinsing, i.e. 0.4 M HCl (aq) does not degrade the coating and more effectively removes iron species. The degradation was probably due to the formation of hydroxyl groups in the PPy backbone. A correlation between the amount of remaining electroactive material (proportional to the specific charge capacity) and the position of the C-H bending vibration peak around 1300 cm\(^{-1}\) in FTIR-ATR was observed.

5.2.2 Stability during aging in ambient air

In the second part of Paper III, the long-term stability of the composites in ambient air was investigated. It has been described earlier that PPy degrades in air, due to the presence of water and oxygen.\textsuperscript{131, 146-148} Indeed, in more inert atmospheres, PPy appears to be more stable.\textsuperscript{127, 131, 149} In addition, aging in air has been found to lead to formation of cytotoxic products.\textsuperscript{127}

The electroactivity was evaluated by calculating the specific charge capacities from CV measurements at 5 mV/s. As only acidic rinsing did not
degrade the PPy coating during rinsing, these samples are the most interesting. 4-14% of the initial specific charge capacity had been lost during ~200 days, corresponding to approximately 6-22 C/g (Figure 17). This indicated that some degradation had occurred but also that it was not extensive.

![Figure 17](image)

*Figure 17. Specific charge capacities for nanocellulose/PPy composites rinsed with different solutions and to different extents as a function of storage time in ambient air. The presented values are average values and the error bars correspond to the absolute deviation (n=2 at 2-80 days, n=3 at 210 days). The error bar is hidden behind the symbol for many samples. The lines are only intended as guides to the eyes. Reprinted from Paper III with permission from the publisher.*

The relationship established in Figure 16 between the extent of degradation and the FTIR-ATR peak wavenumber of the C-H bending vibration at ~1300 cm\(^{-1}\) was employed to investigate if the oxidation state of the composites had been reduced, which would indicate degradation. For the samples rinsed with 0.4 M HCl the peak shifted 5-7 cm\(^{-1}\) over ~200 days, which confirms that the oxidation state has been reduced and that long-term storage of the composites in air has a small degrading effect on the PPy coating.

5.2.3 Effects of different drying methods on structural properties

As described in Section 3.2, NFC hydrogels collapse into low-porosity structures upon ambient drying due to capillary forces exerted by water on the fibrils during drying in air. To maintain the open cellulose network of the hydrogel, freeze drying and supercritical drying have been employed, resulting in highly porous nanocellulose aerogel structures that after aerogel formation have been functionalized with e.g. magnetic particles and the conducting polymer polyaniline. In Paper IV it was explored if highly
porous aerogels could also be produced using nanocellulose fibrils already coated with PPy.

TEMPO-NFC fibrils were coated with PPy through \textit{in situ} polymerization of pyrrole using FeCl$_3$. After polymerization and rinsing, the composite gel was divided into four parts. Each part was dried differently; air drying, freeze drying from water, freeze drying from \textit{tert}-butanol; or supercritical CO$_2$ drying. The preparation process is illustrated in Figure 18 and the resulting dry composite materials were named as shown in the figure.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure18.png}
\caption{Illustration of the preparation of composites employing different drying methods. Reprinted from Paper IV with permission from the publisher.}
\end{figure}

The produced composites had very different structural and mechanical properties. Comp_Air was solid, hard and inflexible and could be broken into smaller pieces. The samples prepared by specialized drying were significantly more porous and could be divided into smaller pieces by using tweezers.

SEM micrographs of the composites produced using different drying procedures are shown in Figure 19. All samples displayed fibrous structures with single composite fiber widths of approximately 25-40 nm. With a TEMPO-NFC fibril diameter of ~3 nm, the thickness of the PPy-layer can be estimated to be up to ~20 nm. Comp_Air displayed the least porous structure with a high degree of fiber aggregation, due to capillary forces acting on the fibers during drying. Employing specialized forms of drying resulted in significantly more porous structures with less fiber aggregation, with Comp_CO$_2$ being the most porous sample.
Figure 19. SEM micrographs of TEMPO-NFC/PPy composites prepared through ambient drying (a and b), freeze drying from water (c and d), freeze drying from tert-butanol (e and f) and supercritical CO$_2$ drying (g and h). The scale bars correspond to 5 µm (a, c, e and g) and 200 nm (b, d, f and h), respectively. Reprinted from Paper IV with permission from the publisher.
The porosities of the samples were obtained from the measured true and bulk densities, and are shown in Table 5. It can be seen that while Comp_Air possessed 30-35% porosity, the samples prepared using specialized drying were found to have 92-98% porosity. It should be pointed out that it was difficult to obtain accurate bulk density values for these irregularly shaped samples, as the bulk density is calculated from the sample dimensions and weight. This could also explain why the variation between different batches in some cases was rather large, and why no significant difference was found between the Comp_Water, Comp_t-But and Comp_CO₂ although the SEM micrographs in Figure 19 indicated significant porosity differences. Still, it can be concluded that both freeze drying and supercritical CO₂ drying can be used to produce highly porous aerogel composites, whereas ambient drying result in a material with low porosity.

Table 5. Estimated porosities and BET specific surface areas for nanocellulose/PPy composites prepared using different drying methods.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Drying method</th>
<th>Porosity (%)&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>BET surface area (m²/g)&lt;sup&gt;b,c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comp_Air</td>
<td>Ambient drying</td>
<td>30-35</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>Freeze drying from water</td>
<td>95-96</td>
<td>37-106</td>
</tr>
<tr>
<td>Comp_Water</td>
<td>Freeze drying from tert-Butanol</td>
<td>97-98</td>
<td>147-162</td>
</tr>
<tr>
<td>Comp_CO₂</td>
<td>Supercritical CO2 drying</td>
<td>92-98</td>
<td>170-246</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from two separate batches.

<sup>b</sup> Calculated from bulk and true density measurements.

<sup>c</sup> Derived from N₂ adsorption isotherm data.

The BET specific surface areas, which were determined from nitrogen adsorption data, are also displayed in Table 5. Ambient drying resulted in a composite with very small surface area, < 1 m²/g, due to the significant aggregation of the composite fibers. Supercritical CO₂ drying, on the other hand, generated a composite with the largest surface area (246 m²/g) yet reported for a nanocellulose and conducting polymer composite.

As PPy was the only component in the composites containing nitrogen, the PPy content could be determined from CHN elemental analyses (Table 6). The composites contained ~70 wt% PPy and there was no significant difference between the samples, showing that no PPy was lost during any of the drying or solvent exchange steps. The amount of cellulose was estimated to 9 wt% by drying and weighing a complete synthesis batch.
Table 6. Nitrogen and PPy contents based on CHN elemental analyses.\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Nitrogen content\textsuperscript{b} (wt%)</th>
<th>PPy content (wt%)</th>
<th>PPy content (mmol/g composite)\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comp_Air</td>
<td>14.6±0.3</td>
<td>67.9±1.3</td>
<td>10.4±0.2</td>
</tr>
<tr>
<td>Comp_Water</td>
<td>14.9±0.3</td>
<td>69.0±1.3</td>
<td>10.6±0.2</td>
</tr>
<tr>
<td>Comp_t-But</td>
<td>15.1±0.3</td>
<td>70.1±1.3</td>
<td>10.8±0.2</td>
</tr>
<tr>
<td>Comp_CO\textsubscript{2}</td>
<td>15.4±0.3</td>
<td>71.7±1.3</td>
<td>11.0±0.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Values expressed as mean ± pooled standard deviation (n=10).
\textsuperscript{b}Values have been compensated for weight contributions from solvents and, in Comp_Water, also for impurities, see Paper IV for details.

Tensile testing of Comp_Air resulted in an average Young’s modulus of 0.51±0.12 GPa (standard deviation, n=4), an average maximum stress at break of 10.93±0.70 MPa (standard deviation, n=4) and a strain to failure of about 2.5%. These values were approximately the same as for previously published data for cellulose/PPy composites having significantly higher cellulose content, viz. 55.5 wt\%\textsuperscript{151} and 71.4wt\%\textsuperscript{152} respectively. The aerogel composites were brittle and were thus not characterized mechanically.

To summarize, composites composed of TEMPO-NFC and PPy can be produced with low porosity and small surface area or high porosity and large surface area by drying in air or employing specialized drying methods (freeze drying or supercritical CO\textsubscript{2} drying), respectively. The most compact samples were found to have a tensile strength comparable to similar materials with significantly higher cellulose content, while the high porosity composites were brittle, but mechanically stable.

5.2.4 Effects of different types and amounts of nanocellulose on structural properties

As a continuation of the work in Paper IV, the aim in Paper V was to produce composites that could fill the porosity gap between 35% and 82% (composites based on CNC have been found to have 82% porosity).\textsuperscript{66} In Paper II, it was observed that the porosities of the air-dried nanocellulose films varied depending on the type of nanocellulose used. In paper V it was investigated if such differences would be transferred to PPy-containing composites based on the different nanocelluloses. Furthermore, an observation made during work related to Paper IV was that if a higher amount of cellulose was used in the synthesis and the composite material was air-dried, the resulting material appeared denser in SEM than the sample with 30% porosity. That track was however not explored further in Paper IV, but was kept in mind for the work in Paper V. Therefore, two different concentrations of nanocellulose of two different types of nanocellulose were used.

In Paper V, 100 or 300 mg of ENZYME-NFC or CARBOXY-NFC were coated with PPy as earlier and the same amount of pyrrole and FeCl\textsubscript{3} was
used for all syntheses. After polymerization the composites were dried in air. The corresponding samples are named ENZYME-NFC_100, ENZYME-NFC_300, CARBOXY-NFC_100 and CARBOXY-NFC_300, depending on the type and amount (in mg) of nanocellulose used.

Surface and cross-section SEM micrographs were recorded to investigate the composite structures and estimate the relative porosities. Cross-section micrographs are shown in Figure 20. There was no observable difference compared to the surface SEM micrographs. Furthermore, the micrographs indicated that the composites had different porosities, depending on the amount and type of NFC used, with the porosities decreasing in the following order: ENZYME-NFC_100 > ENZYME-NFC_300 > CARBOXY-NFC_100 > CARBOXY-NFC_300. Although all samples were fibrous to some extent, a higher degree of composite fiber aggregation was observed when the pore volume decreased.

![Figure 20: Scanning electron micrographs depicting cross-sections of ENZYME-NFC_100 (a), ENZYME-NFC_300 (b), CARBOXY-NFC_100 (c) and CARBOXY-NFC_300 (d) samples at 200 kX magnification. Reprinted from Paper V.](image)

For both the ENZYME-NFC_100 and ENZYME-NFC_300 samples, the fiber widths estimated from the micrographs ranged from ~60 to 80 nm, while the fiber widths for the CARBOXY-NFC_100 and CARBOXY-NFC_300 samples ranged from ~40 to 60 nm. This difference is likely due to the differences in fibril widths inherent in these two NFC forms. Both ENZYME-NFC and CARBOXY-NFC feature fibrils and fibril aggregates ranging approximately from 5 to 20 nm. However, ENZYME-NFC is domi-
nated by fibril aggregates, whereas CARBOXY-NFC is composed of more individual fibrils, due to greater fibril repulsion resulting from the introduced surface charges. Based on ~20 nm fibril widths for ENZYME-NFC and ~5 nm widths for CARBOXY-NFC, the thickness of the PPy-layer can be estimated to ~20-30 nm.

To quantify the porosity differences seen in the micrographs, the total porosities of the samples were determined. As can be seen in Table 7, depending on the amount and type of nanocellulose, composite porosities ranging from 42% to 72% were obtained. Furthermore, the PPy-content, determined via CHN elemental analysis, corresponded to approximately 2/3 of the total composite weight (Table 7), with some variation between the samples.

Table 7. Porosities for ENZYME-NFC- and CARBOXY-NFC-based composites and the corresponding nitrogen and PPy contents.

<table>
<thead>
<tr>
<th></th>
<th>Porosity (%)a</th>
<th>Nitrogen content (wt%)b</th>
<th>PPy content (wt%)</th>
<th>PPy content (mmol/g composite)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENZYME-NFC_100</td>
<td>72.2</td>
<td>13.9</td>
<td>66.4</td>
<td>9.89</td>
</tr>
<tr>
<td>ENZYME-NFC_300</td>
<td>66.9</td>
<td>13.0</td>
<td>62.2</td>
<td>9.27</td>
</tr>
<tr>
<td>CARBOXY-NFC_100</td>
<td>51.7</td>
<td>15.2</td>
<td>72.8</td>
<td>10.8</td>
</tr>
<tr>
<td>CARBOXY-NFC_300</td>
<td>41.6</td>
<td>13.1</td>
<td>62.7</td>
<td>9.35</td>
</tr>
</tbody>
</table>

a Calculated from bulk and true density measurements.  
b From CHN elemental analyses.

Together with the composites prepared in Paper IV, which were based on TEMPO-NFC, and CNC-based composites with 82% porosity,66 these results show that the porosities of nanocellulose/PPy composites can be tailored between 30% and 98% with porosity increments corresponding to ~10%. While special forms of drying are required to reach porosities higher than 82%, porosities of 82% or lower can be achieved effortlessly by employing different forms and amounts of nanocellulose and letting the composite material dry in air.

It was reported in Paper II that the porosities of the nanocellulose films decreased in the order CNC > ENZYME-NFC > CARBOXY-NFC. As shown in the current study, the same trend in porosity could be observed for the corresponding composites. An explanation for the porosity trends could be that the different nanocellulose fibrils and fibril aggregates have different stiffness and thereby are affected by the capillary forces to different extents during drying. This has not been investigated, but it would, for example, be reasonable to assume that the thick and highly crystalline CNC fibrils are stiffer than e.g. the CARBOXY-NFC fibrils that are thinner and less crystalline.
5.2.5 Effect of porosity on the electrochemical properties

The electrochemical properties for the composites produced in Paper IV and Paper V were compared in Paper V in order to study how different porosities affect the electrochemical properties over an as large porosity interval as possible (30% to 98% porosity). The composites were analyzed with CV measurements in 2 M NaCl (aq) at potential scan rates ranging from 1 to 50 mV/s. Voltammograms are shown in Figure 21.

*Figure 21. Cyclic voltammograms for composites with different porosities produced in Paper IV and Paper V (the porosities are indicated in the figure legend in panel a) at potential scan rates ranging from 1 to 50 mV/s. The current has been normalized with respect to the PPy contents in the composites. Reprinted from Paper V.*
At 1 mV/s (Figure 21a), all samples with porosities above 42% displayed a well-defined PPy oxidation peak at approximately -0.4 V vs. Ag/AgCl. For the composite with 30% porosity a less distinct and broad oxidation peak is seen. At higher scan rates (panels b-f in Figure 21), the PPy oxidation peaks were shifted to more positive potentials with increasing scan rates and the oxidation peaks also became broader and less distinct as the scan rate was increased. This scan rate dependence of the shape of the voltammograms was more pronounced the lower the porosity was. In fact, for the least porous samples no clear oxidation peaks could be observed at sufficiently high scan rates. This shows that the oxidation rate decreases with decreased porosity.

The porosity effect on the PPy oxidation rate was further illustrated by calculating the specific oxidation charges from the voltammograms for the employed scan rates (Figure 22). At the lowest scan rate, 1 mV/s, all samples displayed the same specific charge capacity (300-320 C/g PPy), which corresponds to a doping degree of ~22%. These values are in good agreement with the capacity (~310 C/g PPy) found for CNC-based composites with 82% porosity.66 For the presently investigated samples, an increase in the scan rate resulted in a drop in the charge capacity for all samples, except for the composite with 98% porosity. The lower the porosity of the composite, the more pronounced was the capacity decrease. Chronoamperometric measurements at +0.3 V vs. Ag/AgCl confirmed that the oxidation rate of the composites was porosity dependent.

![Figure 22](image.png)
*Figure 22. Specific oxidation charges for composites with different porosities. The charges have been normalized with respect to the PPy amount in the composites. The error bars represent one standard deviation (n=3). The lines are only intended as guides to the eye. Reprinted from Paper V.*
As charge neutrality must be maintained for the oxidation to proceed, these results show that the rate of the counter ion diffusion depends on the porosity of the composite. The required amount of counter ions to maintain charge neutrality was calculated from the determined charge capacities at 1 mV/s scan rate and compared to the total amount of counter ions available in the composite pores (Table 8). The amount of counter ions present in the pores of the least porous samples was insufficient compared to the amount of counter ions required for the charge compensation. This means that a large number of counter ions had to diffuse from the bulk electrolyte solution outside the composite and through the composite pore network. In contrast, an excess of counter ions was found in the pores of the most porous sample, i.e. in the vicinity of the composite fibers, which lead to shorter diffusion lengths and less complex diffusion processes, and thereby rapid oxidation. Based on these results it can be concluded that the rate of oxidation decreases with decreased porosity due to the increased influence of diffusion caused by the decreasing amount of counter ions within these samples.

Table 8. The amount of counter ions required to reach the measured charge capacities and the amount of counter ions available in the composite pores.

<table>
<thead>
<tr>
<th>Porosity (%)</th>
<th>Charge capacity at 1 mV/s (C/g PPy)(^a)</th>
<th>Charge capacity at 1 mV/s (C/g composite)(^a)</th>
<th>Counter ions in oxidation (mmol/g composite)(^a)</th>
<th>Counter ions in composite pores (mmol/g composite)</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
<td>312 ± 10</td>
<td>224 ± 7</td>
<td>2.32 ± 0.08</td>
<td>68.14</td>
</tr>
<tr>
<td>72</td>
<td>296 ± 1</td>
<td>197 ± 1</td>
<td>2.04 ± 0.01</td>
<td>3.60</td>
</tr>
<tr>
<td>67</td>
<td>304 ± 1</td>
<td>189 ± 1</td>
<td>1.96 ± 0.01</td>
<td>2.77</td>
</tr>
<tr>
<td>52</td>
<td>307 ± 6</td>
<td>224 ± 5</td>
<td>2.32 ± 0.05</td>
<td>1.50</td>
</tr>
<tr>
<td>42</td>
<td>313 ± 7</td>
<td>196 ± 4</td>
<td>2.03 ± 0.04</td>
<td>0.97</td>
</tr>
<tr>
<td>30</td>
<td>317 ± 6</td>
<td>215 ± 4</td>
<td>2.24 ± 0.04</td>
<td>0.58</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as the mean ± standard deviation.

It has previously been proposed that the shape of the voltammetric oxidation peak for conducting polymers stems from a distribution of redox states within the polymer, where the number of redox states increases with chain length.\(^{54, 153}\) The results of the present work show that the oxidation behavior and peak shape of PPy also is influenced by the counter ion diffusion process.

The results in Papers IV and V likewise show that by tailoring the porosities of nanocellulose/PPy composites, the electrochemical properties can be modified and controlled. This provides new possibilities for the manufacturing of electrochemically controlled ion-extraction and energy storage devices with optimized performance. Furthermore, the synthesis conditions are identical in terms of the polymerization of pyrrole, which is appealing for fundamental studies of mass transport phenomena in conducting polymers in general, as additional variables other than the accessibility for counter ions are avoided. Thus, these types of materials may be interesting alternatives to
electrochemically synthesized conducting polymer films, where the synthesis conditions need to be altered in terms of e.g. polymerization charge\textsuperscript{154-157} or counter ions\textsuperscript{158} to generate films of different thicknesses and porosities.

To conclude, the porosity of the composites can be tailored between 30\% and 98\% with porosity increments corresponding to \(\sim\)10\%. Supercritical CO\(_2\) drying or freeze drying is required to reach the highest porosities, but porosities of 82\% or lower can be achieved by using different forms and amounts of nanocellulose. Thereby it is possible to control the electrochemical properties as the oxidation rate depends on the composite porosity, due to limitations in the counter ion diffusion process.

5.2.6 Blood compatibility

Patients with chronic kidney failure rely on hemodialysis for the removal of metabolic solutes, which are normally removed from the blood by healthy kidneys. The treatment relies on size-exclusion, diffusion and ultrafiltration principles, where sufficiently small solutes pass from the blood through a porous membrane into the dialysate. The treatments have saved many patients’ lives since first introduced in the middle of the 20\(^{th}\) century and has since been developed and refined. However, the treatments are time-consuming, cannot mimic other kidney functions (e.g. secretion and reabsorption), and the solute removal is not efficient enough, which leads to build-up of solutes in the body, which eventually reach toxic concentrations\textsuperscript{159-163}.

Recently, a membrane comprising an adsorbant (activated carbon) and a traditional polyethersulfone hemodialysis membrane was developed, where diffusion and adsorption was combined for solute removal, resulting in increased small-size model solutes removal, as compared to only diffusion\textsuperscript{164}. It is envisioned that nanocellulose/PPy composites could work in a similar way, by combining its porous structure for size-exclusion purification with its electrochemically controlled ion extraction capability. It can further be envisioned that the secretory functions of the kidney could be mimicked through electrochemically controlled release of desired solutes or even controlled release of drugs.

In Paper VI the blood compatibility of a composite based on CNC was investigated. Indirect \textit{in vitro} cytotoxicity testing as well as \textit{in vivo} cytotoxicity testing had been performed earlier in related work and the composites were found to be non-toxic\textsuperscript{127}. However, significant rinsing with water and incubation for 48 hours in a pH 7.4 biological buffer was found necessary in order to avoid toxic leachables. Extensive rinsing and/or incubation have also been required for other PPy materials to render them non-cytotoxic\textsuperscript{165-167}. Thus, the composites evaluated for blood compatibility were extensively rinsed with water and incubated in biological buffer, in accordance with the previous work\textsuperscript{127}.
However, the composite turned out to be highly thrombogenic, as will be shown below. Therefore it was tested to apply a heparin coating, as it is well-established that heparinization can improve the blood compatibility of materials.\textsuperscript{168, 169} An anionically charged heparin conjugate layer was applied to the cationically charged PPy-layer of the composite following synthesis. This was followed by a conditioning layer of a polyamine and then a second layer of the heparin conjugate. The coating was stable and could withstand repeated composite reduction and oxidation cycles (CV-cycling) as well as steam sterilization, while not affecting the porous and fibrous composite structure.

The thrombogenic and complement activation properties of the nanocellulose/PPy composites were determined and compared to those for three different reference dialysis membrane materials; regenerated cellulose, cellulose acetate and polysulfone, each with two different pore sizes (0.20 and 0.45 µm). The latter two materials, in particular polysulfone, are used in modern dialysis systems.

The thrombogenic properties of the membranes were evaluated by determining the reduction of platelet number in the blood after contact with the membranes and by observing the platelet adhesion on the membranes, as well as determining the extent of thrombin formation. Minimal platelet adhesion occurred on the heparinized composite membranes before and after CV-cycling (Figure 23a and b), indicating that these composites were not thrombogenic. In contrast, large numbers of platelets adhered on the non-heparinized membrane, along with a mesh of fibrin (Figure 23c).

These results were confirmed by a significantly larger reduction in platelet number in the blood following incubation of non-heparinized composites compared to heparinized ones. For the heparinized composites, no significant differences could be observed in relation to the reference materials. This was also true in terms of thrombin formation, as determined by measuring the levels of thrombin-antithrombin complex (TAT) in the blood plasma after whole blood contact with the materials (Figure 24). Thus, heparinized composites are no more thrombogenic than current hemodialysis membranes. In contrast, the non-heparinized composite was found significantly more thrombogenic, showing significantly elevated TAT levels (50-100 times) compared to the reference materials.
Figure 23. SEM micrographs showing extent of platelet adhesion after whole blood incubation with heparinized composites before (a) and after CV-cycling (b) and with non-heparinized composite (c). Reprinted from Paper VI with permission from the publisher.

Figure 24. Thrombin-antithrombin complex (TAT) levels in the blood after contact with heparinized composites or reference materials. The TAT level for the non-heparinized composite was omitted from the figure and corresponded to 10670 ± 1269 µg/l. The values represent the mean ± standard error of the mean with blood from 10 donors. (Legend: H –Heparinized composite; H Red-Ox – Heparinized composite after CV cycling; CA – Cellulose acetate; RC – Regenerated cellulose; PS – Polysulfone; 0.20 – 0.20 µm pore size; 0.45 – 0.45 µm pore size). Reprinted from Paper VI with permission from the publisher.

To evaluate the complement activation, which is involved in the inflammatory response, the levels of C3a and sC5b-9 in blood plasma were determined after the membranes had been in contact with whole blood. As can be seen in Figure 25, the heparinized as well as the non-heparinized composites caused significantly lower complement activation than the reference membranes.
Figure 25. C3a (a) and sC5b-9 (b) levels after whole blood-incubation. The values represent the mean ± standard error of the mean with blood from 10 donors. (Legend: H – Heparinized composite; H Red-Ox – Heparinized composite after CV cycling; NH – Non-heparinized composite; CA – Cellulose acetate; RC – Regenerated cellulose; PS – Polysulfone; 0.20 – 0.20 µm pore size; 0.45 – 0.45 µm pore size). Reprinted from Paper VI with permission from the publisher.

To investigate if the heparin coating affected the electrochemical properties of the composite and hence the extraction capacity of the composite, CV measurements with the model small size solutes phosphate or oxalate as electrolytes were performed. No significant difference could be observed between heparinized and non-heparinized composites with neither solute. However, these extraction experiments were performed with samples that had not been extensively rinsed, as this would have deteriorated the electrochemical properties, as shown in Paper III. The objective here was to show that the actual heparin coating and the heparin coating process did not affect the electrochemical properties of the composite.

Following this work and the work in Paper III, as well as the previously reported cytotoxicity testing, we tested to extensively rinse the composite with HCl with the aim of obtaining a highly electroactive and non-cytotoxic composite material. However, the cytotoxicity profile of the material was not improved.

In conclusion, the heparinized composite material possesses at least as good blood compatibility properties as presently used dialysis membranes in terms of platelet adhesion and thrombin generation. Furthermore, the results indicated that both non-heparinized and the heparinized composites may cause less inflammation than commercially available hemodialysis membranes. Also, the heparin coating did not affect the extraction capability of the composite.
6. Summary and concluding remarks

Nanocellulose is a versatile family of materials that, depending on origin and extraction method, have different characteristics in terms of e.g. dimensions, fraction of crystalline cellulose and surface charge. A variety of new properties for specific applications can be introduced through functionalization. The work in this thesis has been focused on nanofibrillated cellulose (NFC) from wood and nanocellulose from Cladophora sp. algae (CNC) that were functionalized with surface charges or coated with the conducting polymer polypyrrole (PPy). The overall ambition was to study the influence of materials preparation processes on structural and electrochemical properties of such materials and assess their biocompatibility. This aim is wide and includes many different aspects and, naturally, it was not possible to conduct a comprehensive study. Instead, specific details have been investigated. The results are summarized below and suggestions for future work are given.

Two studies relating to nanocellulose functionalized with surface charges were conducted. In the first, it was demonstrated that CNC could be oxidized to the same extent using electrochemical TEMPO-mediated oxidation as with the most commonly applied oxidation system, which includes TEMPO, NaBr and NaClO. This showed that the TEMPO species carrying out the actual oxidation (oxoammonium cations) are not sterically hindered from completely oxidizing the nanocellulose surface, in contrast to earlier hypotheses. Also, the degree of oxidation could be easily controlled in the electrochemical setup. A drawback of TEMPO-mediated oxidation is the high cost of the chemical, and an electrochemical setup is appealing as this could potentially facilitate easier reuse of the reaction medium. Another drawback, in particular in large scale preparations, is the need to wash the nanocellulose product following oxidation. One possible way of facilitating easy reuse of the medium and limit the need for washing, would be to immobilize TEMPO directly on an electrode. This concept has earlier been shown to work for soluble alcohols and could be an interesting track to explore for TEMPO-mediated oxidation of nanocellulose.

In the second study of nanocellulose functionalized with surface charges, the cytocompatibility of nanocellulose films composed of NFC or CNC with anionic, cationic or no significant surface charges was investigated. No toxic leachables could be detected in indirect in vitro cytocompatibility testing, showing that none of the materials were cytotoxic. Direct cytocompatibility testing of films composed of CNC indicated that only the films based on
TEMPO-oxidized CNC promoted fibroblast adhesion and proliferation. Only this film featured aggregated and aligned anionic fibrils on the surface. It is hypothesized that this distinct structure promotes cell adhesion and this could potentially be exploited for development of biomaterials that promote cell adhesion and proliferation of different cell types. In this respect, controlled nanocellulose fibril alignment by employing magnetic\textsuperscript{171} or electric\textsuperscript{172} fields could be very interesting. As for the NFC films, the film functionalized with cationic quaternary ammonium groups was found to be the most cytocompatible and might be used in tissue engineering applications.

In the second part of the thesis, composites composed of nanocellulose and the conducting polymer PPy were in focus. In the composites, nanocellulose fibrils are individually coated with PPy, which are dried into free-standing three-dimensional network structures with variable porosities and surface areas. These types of materials have been employed as electrodes in energy storage devices and as electrochemically controlled ion extraction membranes. The work presented in this thesis is relevant for both types of applications, as the primary objective was to gain control over the properties of the composites.

The first study on nanocellulose/PPy composites concerned the stability of the electroactive PPy layer of the composite during preparation and storage. It is important to avoid degradation as this decreases the amount of electroactive material, which leads to decreases of the amount of charge that can be stored in – or the number of ions that can be extracted with – the composite. Specifically, it was demonstrated that the most common way of washing PPy materials – water rinsing under reduced pressure – significantly degrades the material. Rinsing with 0.4 M hydrochloric acid, on the other hand, was observed not to degrade PPy and more effectively remove iron species stemming from the oxidizing agent (Fe\textsuperscript{3+}) used in the composite synthesis. An empirically derived correlation between the amount of remaining electroactive material and the position of a C-H bending vibration at \(\sim1300\) cm\(^{-1}\) in FTIR-ATR spectra was also demonstrated. This could in future work be used as a diagnostic tool to evaluate material degradation, in relative terms. It was further shown that storage in air over \(\sim200\) days has only a small degrading effect, corresponding to a 4-14% decrease of the amount of electroactive material.

In the subsequent studies it was shown that the porosity of the composites can be tailored between 30% and 98% with porosity increments corresponding to \(\sim10\)%. Supercritical CO\(_2\) drying or freeze drying were required to reach porosities higher than 82%. The largest specific surface area to date reported for a nanocellulose and conducting polymer composite (246 m\(^2\)/g) was obtained through supercritical CO\(_2\) drying.

Porosities between 30% and 82% could be obtained by proper selection of the type and amount of nanocellulose used, followed by drying in air. By employing composites with porosities covering the entire porosity range (30-
98%) it was demonstrated that the oxidation rate of the PPy coating decreased with decreased porosity due to an increased influence of counter ion diffusion. It has previously been proposed that the shape of the voltammetric oxidation peak of conducting polymers stems from a distribution of redox states within the polymer, where the number of redox states increases with chain length. The results of the present work show that the oxidation behavior and voltammetric peak shape for PPy also is significantly influenced by the counter ion diffusion process.

Altogether, the results showed that it is possible to control the electrochemical properties of nanocellulose/PPy composites by controlling the porosity, which to a large extent could be controlled by the choice of nanocellulose. This provides new possibilities for the manufacturing of electrochemically controlled ion extraction and energy storage devices with optimized performance. However, another important aspect in any application is the mechanical properties of the composite. Therefore, in future work the effect of porosity on the mechanical properties should be investigated.

One possible, new field of application for the investigated composite could be hemodialysis. The idea is that the porous structure and the electrochemically controlled ion extraction capability of the composite could allow for combined size exclusion purification and electrochemically controlled extraction of solutes in the blood, thereby potentially making the treatment more efficient. One of the first steps to assess the feasibility of the composite in such an application, was to investigate its blood compatibility. It was found that the composite was highly thrombogenic, but after applying a heparin coating on the composite surface, the thrombogenic properties were found to be comparable to commercial and currently used hemodialysis membranes. In addition, both heparinized and non-heparinized composites caused significantly lower complement activation, indicating that they may cause less inflammation than the reference hemodialysis membranes. The heparin coating was also found not to affect the solute extraction capacity of the composite.

The \textit{in vitro} and \textit{in vivo} cytocompatibility of the composite had been investigated earlier and the material was found to be non-cytotoxic if extensively rinsed with water. Thus, in terms of blood compatibility and cytocompatibility, heparinized nanocellulose/PPy composites are promising candidates for a new type of hemodialysis membranes. However, a number of issues remain to be investigated and resolved. For example, a preparation method that generates biocompatible and highly electroactive composites has to be identified, the pore-size distribution must be tailored in order to control the molecular weight cutoff for solutes, and potential electrode side-reactions during operation and their effect in terms of biocompatibility need to be investigated.
Cellulosa är ett välkänt material, inte minst i Sverige, och den vanligaste användningen är tillverkning av papper. Cellulosa är känt för att ha god böjbarhet och hög mekanisk styrka och utgör huvuddelen av cellväggen i träd, buskar och vissa alger, där den är den komponent som ger växterna dess mekaniska stabilitet. På molekylär nivå är cellulosa en polymer, dvs det är en lång molekylkedja som är uppbyggd av en och samma upprepande enhet, s k monomer. I cellulosas fall är monomeren en ringformad glukosmolekyl (en sockermolekyl) och kedjorna består av flera tusen glukosenheter. Kedjorna orienterar sig och binds ihop på olika sätt och ger upphov till både oordnade och ordnade strukturer i s k fibriller. Det är dessa fibriller eller delar av dem som gemensamt kallas för nanocellulosa efter att man har utvunnit dem från t ex pappersmassa.

Nanocellulosafibriller är generellt några få till några tiotal nanometer i diameter och upp till några mikrometer långa. Dock varierar dimensionerna en del beroende på cellulosans ursprung och använd utvinningsmetod. Andelarna av oordnat och ordnat material i fibrillerna kan också vara olika. Man kan på olika sätt tillföra nya önskade egenskaper till nanocellulosan, genom s k funktionalisering, antingen i samband med utvinningen eller efter. Detta kan t ex göras genom att tillföra ytladdningar eller belägga nanocellulosafibrillerna med ett annat material med andra egenskaper och därigenom skapa ett kompositmaterial. Goda funktionaliseringsmöjligheter är en av anledningarna till varför nanocellulosa är ett intressant material. Andra anledningar är att nanocellulosan är förnyelse- och nedbrytningsbar i naturen, har goda mekaniska egenskaper och är ett nanomaterial, vilket bl a innebär att det har en stor tillgänglig yta i förhållande till sin vikt.

I avhandlingen har nanocellulosa som utvunnits på olika sätt från algen grönslick (Cladophora sp.), förkortat CNC, och från barrträ, förkortat NFC, använts. I en del av avhandlingen har fokus varit på nanocellulosa med olika ytladdningar och i en annan del har nanocellulosa belagd med en elektriskt ledande polymer, polypyrrol (PPy), studerats. Det övergripande syftet har varit att undersöka hur olika framställningsmetoder påverkar materialens egenskaper. Särskilt har materialens strukturella och elektrokemiska egenskaper samt biokompatibilitet studerats. Med biokompatibilitet menas bl a ett materials förmåga att kunna fungera inuti kroppen eller i kontakt med blod utan att aktivera kroppens försvarsmekanismer. Exempel på icke-biokompatibla material är således ett implantat som orsakar inflammation,
ett dialysmembran som används vid behandling av patienter med kronisk njursvikt som orsakar blodkoagulation, eller ett material som utsöndrar giftiga ämnen.

I det första delarbetet studerades en elektrokemisk metod för att introducera negativa ytladdningar på CNC genom s k TEMPO-medierad oxidation. En elektrokemisk process skulle kunna underlätta återanvändning av reaktionslösningen jämfört med de vanligast förekommande TEMPO-processerna, vilket är intressant då kemikalen är dyr. I arbetet visades att denna elektrokemiska metod fungerade lika väl som de konventionella TEMPO-metoderna och det var också möjligt att styra graden av oxidering genom att kontrollera oxidationstiden.

Cellulosa och cellulosaföreningar har under många år studerats och används inom olika biomedicinska applikationer, t ex som dialysmembran och som förbandsmaterial. Nanocellulosa och dess applucerbarhet inom det biomedicinska området är relativt outforskat, men man tror att nanocellulosa skulle kunna vara användbart som t ex förbandsmaterial och substrat vid vävnadsodling. I det andra delarbetet undersökt cellkompatibiliteten hos NFC- och CNC-pappersark som hade negativa, positiva eller inga ytladdningar alls. Inga giftiga ämnen utsöndrades från något av arken, medan CNC med negativa laddningar främjade (fibroblast-) cellettillväxt på sin yta, möjligen tack vare den ordnade ytstruktur som arket uppvisade. NFC med positiva ytladdningar var den av de olika NFC-pappersarken som bäst främjade cellettillväxt och således skulle både negativt laddad CNC och positivt laddat NFC kunna vara användbara som substrat vid vävnadsodling.

Figur 1. Fotografi på ett böjbart kompositmaterial bestående av CNC och PPy (vänster). Samma material studerat med svepelekmikroskopi (höger).

I det första arbetet med kompositmaterialet studerades deras stabilitet under tvättning och lagring i luft. Motsatsen till att materialet är stabila är att de degraderas, vilket i det här fallet innebär att mindre laddning kan lagras eller att färre jonar kan extraheras. Det visade sig att den vanligaste metoden för att tvätta PPy, nämligen genom sköljning med vatten, degraderade kompositens PPy-lager. Ingen degradering skedde dock om en sur lösning (lösning med lågt pH) användes. Vidare noterades endast mycket lite degradering under lagring i luft under ~200 dygn.

I andra arbeten visades att porositeten, d v s andelen luft av hela materialets volym, hos kompositerna kunde kontrolleras mellan 30 och 98% genom att använda olika mängd och sorts nanocellulosa samt olika torkningsmetoder. Genom att använda en speciell torkningsmetod, som kallas superkritisk torkning, erhölls prover med 98% porositet och en ytarea på ~250 m²/g, d v s ett gram av kompositen har en yta som är lika stor som en tennisbana. Det visades också att de elektrokemiska egenskaperna hos kompositerna till hög grad påverkades av deras porositet på så sätt att oxidationen av materialet går snabbare ju högre porositeten är. Detta beror på att transporten av motjoner inte är begränsad vid hög porositet, medan lägre porositeter gör att transporten begränsas i allt större utsträckning.

Slutligen undersöktes möjligheten att kunna använda kompositmaterialet som en ny typ av dialysembran vid behandling av njursvikt för att avlägsna slaggprodukter. Den bakomliggande tanken är att man ska kunna kombinera kompositernas porösa struktur med elektrokemiskt kontrollerad extraktion av skadliga ämnen från blodet för att på så sätt effektivisera behandlingen. En sådan applikation ligger långt fram i tiden. Ett viktigt krav är dock att materialet är biokompatibla. Det visade sig att materialet var trombogena, d v s orsakade aktivering av de system som leder till blodkoagulering, såvida inte ett heparinlager deponerades på kompositerna. Med hepariniserade kompositer erhölls liknande resultat som för kommersiella dialysembran vad gäller trombin-bildning och inbindning av blodplättar, vilka var de komponenter av blodkoagulationssystemet som undersöktes. Vidare tydde resul-
kunna vara mindre inflammationsframkallande än de kommersiella membranen, samtidigt som heparinlagret inte påverkade kompositernas förmåga att extrahera joner från modelllösningar.

Sammantaget visar avhandlingen att många olika egenskaper hos material baserade på nanocellulosa kan påverkas och anpassas till behov inom olika användningsområden, bl a energilagring, jonextraktion och biomaterialtillämpningar.
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9. References


A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology.