Jonna Ojala

FUNCTIONALIZED CELLULOSE NANOPARTICLES IN THE STABILIZATION OF OIL-IN-WATER EMULSIONS

BIO-BASED APPROACH TO CHEMICAL OIL SPILL RESPONSE
JONNA OJALA

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Bio-based approach to chemical oil spill response

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Abstract

Nanocellulose is a renewable, biodegradable, and easily available material that is considered as an attractive resource for many different value-added applications in the emerging bio-based economy. Its outstanding properties, such as strength, lightness, transparency, and good thermal insulation, have inspired research and product development around nanocellulose. The potential of nanocellulose to replace synthetic chemicals made from non-renewable sources, for example, is considered to be very promising. Chemical functionalization, that is, the modification of the cellulosic surface properties, is seen to be beneficial in applications such as those in which higher hydrophobicity is needed.

In this thesis, the ability of cellulose nanoparticles to stabilize oil droplets in oil-in-water emulsions was studied. The aim of the study was to explore the possibility of developing a new type of "green" oil spill chemical from cellulose. Therefore, the cellulose was chemically modified in an aquatic environment with a sequential periodate oxidation and chlorite oxidation followed by reductive amination reaction, which increased the hydrophobicity of the produced nanocellulose. In addition, the use of deep-eutectic solvents in the preparation of modified (succinylated and carboxylated) and non-modified cellulose nanoparticles was studied. Chemical (kraft) pulp, dissolving pulp, and semi-chemical fine fibers were used as raw materials in this research.

The results demonstrated that chemically modified cellulose nanoparticles work well as stabilizers for oil-water emulsions resulting in small, stable oil droplets and impeding creaming, which is a typical phenomenon for particle stabilized emulsions. The modification of cellulose nanoparticles improved their ability to partition at the oil-water interface, which enabled efficient and irreversible adsorption. It was found that because of their small size, the cellulose nanocrystals can be compressed more tightly onto the surface of the oil droplet, while longer and more flexible cellulose nanofibrils formed a web structure between the oil droplets. All cellulose nanoparticle-stabilized emulsions were stable against droplet coalescence, and even at low temperatures, they retained their droplet size and stability. Salinity, on the other hand, improved stability when CNCs from chemical pulp were used, but it negatively affected stability when nanocrystals from semichemical pulp were used.

Keywords: cellulose, cellulose nanocrystal, cellulose nanoparticle, chemical oil-spill response, deep eutectic solvent, emulsion stabilization, oil-in-water emulsion, periodate-chlorite oxidation
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Tiivistelmä


Asiasanat: emulsion stabilointi, kemiallinen öljyntorjunta, perjodaati-kloriittihapetus, selluloosa, selluloosananokide, selluloosananokuitu, selluloosananopartikkel, syväeutektinen liuotin, öljy-vesiemulsio
Persistence and determination are always rewarded
Christine Rice
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Oulu, 2019

Jonna Ojala
### Abbreviations and symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AGU</td>
<td>Anhydroglucose unit</td>
</tr>
<tr>
<td>CNC</td>
<td>Cellulose nanocrystal</td>
</tr>
<tr>
<td>CNF</td>
<td>Cellulose nanofibril</td>
</tr>
<tr>
<td>CP</td>
<td>Chemical pulp</td>
</tr>
<tr>
<td>CryoSEM</td>
<td>Scanning electron cryomicroscopy</td>
</tr>
<tr>
<td>CrI</td>
<td>Crystallinity index of cellulose</td>
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<tr>
<td>DAC</td>
<td>Dialdehyde cellulose</td>
</tr>
<tr>
<td>DCC</td>
<td>Dicarboxylic acid cellulose</td>
</tr>
<tr>
<td>DES</td>
<td>Deep eutectic solvent</td>
</tr>
<tr>
<td>DIS</td>
<td>Dissolving pulp</td>
</tr>
<tr>
<td>FESEM</td>
<td>Field emission scanning electron microscopy</td>
</tr>
<tr>
<td>O/W</td>
<td>Oil-in-water (emulsion)</td>
</tr>
<tr>
<td>PO-DAC</td>
<td>Partially oxidized dialdehyde cellulose</td>
</tr>
<tr>
<td>SCP</td>
<td>Semichemical pulp</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-tetramethyl-1-piperidinylxy</td>
</tr>
<tr>
<td>WAXD</td>
<td>Wide-angle X-ray diffraction</td>
</tr>
<tr>
<td>WCA</td>
<td>Water contact angle</td>
</tr>
<tr>
<td>W/O</td>
<td>Water-in-oil (emulsion)</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
</tbody>
</table>

### Greek symbols

- $\gamma$  Surface tension (mN/m)
- $\rho$   Density (kg/m$^3$)
- $\theta$ Water contact angle (°)
Original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


The author’s contributions to Papers I-IV were as follows:

The author of this thesis was the primary author of Papers I, II and IV and a co-author of Paper III. The responsibilities as a primary author included the experimental design, experimental laboratory work, data analysis and reporting the results in the first draft of publication. All the initial manuscripts I, II and IV were written by the author of this thesis, and co-authors participated by providing valuable comments and corrections. Co-authoring in the Paper III included participation in experimental design, writing and reviewing of the publication.
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1 Introduction and aims

1.1 Background

1.1.1 Marine oil spills in last decades

Fossil oil continues to hold a strong position as one of the top necessities in everyday life, and although people are extremely aware of diminishing oil resources and the negative impacts of fossil oil use on air, water, and land (Fingas, 2011a), there are no signs of significant reductions in oil exploitation in the near future. Renewable energy is the fastest growing type of energy, but despite the fast development of alternative energy sources, their share will not exceed 5.5% before 2040, and it is assumed that fossil fuels will remain the main energy source for decades. According to OPEC (OPEC, 2017), the use of fossil fuels will account for approximately 74% of all energy alternatives in 2040. In addition to energy production, oil is used in various products of everyday life, including bitumen for road surfaces, lubricants, waxes, and multiple uses in the chemical industry. Therefore, the demand for oil and its production rate will probably increase in the near future, which will keep the role of fossil oil important in our society. (Kontorovich, 2009; Nashawi, Malallah & Al-Bisharah, 2010).

A large amount of fossil oil resources remain unfound, especially those located in northern areas. It has been estimated that undiscovered oil reservoirs contain approximately a billion cubic meters (m³) of oil beyond the Arctic Circle, corresponding to 13% of all unfound oil resources (Gautier et al., 2009). These enormous oil resources together with about 30% of undiscovered natural gas resources attract the attention of stakeholders who are greatly interested in pursuing new business in the Arctic. These activities will, in turn, increase oil and gas drilling, their exploitation, as well as the marine transportation in northern locations, and, consequently, increase the risk of oil accidents (Fingas, 2011a).

The total volume of spilled oil from tank vessels in 2017 was around 7,000 tons, indicating that oil accidents are still a notable environmental problem. However, the number of large oil spillages has drastically decreased since the late 1960s. Presently, the dominant sources of oil spills are mostly related to shipping. Thus, commonly occurring oil spills are relatively small, causing mainly local problems. Small- and medium-sized spills (< 700 tons) comprise 95% of all reported oil accidents. These accidents are often caused by loading and discharging
operations in ports and terminals near coastlines (ITOPF, 2017). In addition, consumer-related operations attributed to heating oil tanks, tanker trucks, and smaller vessels are causing smaller spills (Schmidt-Etkin, 2011). Put simply, the two main reasons for oil spills are suggested to be the urge for a higher economic value in the oil industry, and on the other side, a lack of concern for marine safety, perhaps due to unawareness of the severity of possible ramifications to the ecosystem (Mei & Yin, 2009).

1.1.2 Environmental implications of oil accidents

Even though the frequency of serious marine oil spills is low, the total volumes of spilled oils are typically large. The 1920s saw nearly 200 large (> 700 tons) oil spills, causing widespread environmental and social problems (ITOPF, 2017). An oil spill, as a form of pollution, has variable fates and effects on nature. In the case of large aquatic oil spills, such as Exxon Valdez in 1989 and the British Petroleum spill in the Gulf of Mexico in 2010, ecosystems suffer from serious damage for years after the accidents. The hydrophobic oil floats on the surface of the water, preventing sunlight from reaching the deeper water layers. In addition, oil can cover the marine plants and leaves, reducing the level of natural photosynthesis reactions (Gordon & Prouse, 1973; Pulich, Winters & Van Baalen, 1974) and causing changes in oxygen release. These actions can, in turn, lead to losses in biodiversity. Oil also tends to accumulate in living organisms, and some more soluble compounds of oil dissolve further in time, causing even more problems. Those effects can only be detected several years after an oil accident (Mei & Yin, 2009).

The possible consequences of an oil accident may be detrimental to a marine ecosystem even in a once-off accident. Furthermore, the social impacts can be severe and long lasting through economic losses. In addition, oil spills carry direct health risks for people living near coastal areas and workers performing the spill clean-up activities. However, only a few studies have addressed the direct human health problems caused by oil spills and the following clean-up processes (Laffon, Pásaro & Valdiglesias, 2016).

1.1.3 The current preparedness in oil recovery in marine environment

The preparedness for the possible marine oil accidents is mainly based on three different response technologies. These include physical (i.e., mechanical) methods,
such as booms and sorbents, chemical methods, such as oil dispersants and oil herding agents, and in-situ burning, or a combination of these methods. The choice of method depends on the conditions and the oil characteristics.

The complexity of choosing a proper oil spill clean-up method is associated with the heterogeneous composition of oils and the large number of physical processes that oils expose in the marine environment. Sunlight affects the photochemical reactions, and wind and waves speed up the spreading and evaporation of oil. It is difficult to predict the probable effects of marine chemicals and microbes on the oil hydrocarbons. They might degrade quickly or remain there for quite a long time (Farrington, 2014). The next sections will describe the main characteristics of the different response technologies but will concentrate on the features and the possibilities of oil dispersants.

**Physical countermeasures**

When a marine oil spill occurs, a fast response is needed to minimize the damage to nature and humans. Containment equipment is typically used first to prevent the spillage from spreading. Moreover, the containment enables the further treatment of the spill by recovering, burning, or dispersal. The most preferred oil response technique is to collect oil by mechanical countermeasures that include oil booms (floating, barrier-like equipment), sorbents (materials that recover oil through adsorption or absorption), and skimmers (mechanical devices for recovery of oil from the water surface). Most booms perform well in seas with smooth waves. Harsh climates and wind are likely to break the booms and cause leakages. Skimmers, however, are even more susceptible to weather conditions. When the sea is rough, skimmers tend to recover more water than oil. Still, in good conditions, skimmers offer many advantages and are effective either used alone or combined with another response method (Fingas, 2011b; ITOPF, 2014).

Sorbents are materials that are able to soak up the oil in the recovery processes by adsorption. The sorbents derived from natural (both organic and inorganic) or synthetic materials are typically used as a backup for other methods, such as cleaning the traces of oil or solely in small spills (Fingas, 2011b). The benefits of sorbents are that the adsorbed oil is fully removed from the marine environment, and the sorbents can be used in areas where skimmers and booms are unsuitable. Moreover, sorbents may be reusable, but the sorption capacity can decrease after recovery (ITOPF, 2012; Korhonen, Kettunen, Ras, & Ikkala, 2011; Liu et al., 2013).
**In-situ burning**

If direct oil removal is not possible, in-situ burning (or dispersion by chemical dispersants) is the alternative method to be applied. In-situ burning removes the oil from water (or land) by incinerating the oil in place. In-situ burning is applicable in harsh conditions (e.g., ice-infested waters) and is suitable for any oil type and slick size. The prerequisite for burning is that the oil slick is thick enough (3–8 mm) to be ignited (van Gelderen, Fritt-Rasmussen & Jomaas, 2017). Therefore, chemical oil herding agents or containment equipment are used together with in-situ burning to thicken the slick (Buist, Potter, Nedwed, & Mullin, 2011). The drawbacks associated with in-situ burning are the possible pollution from burn residues and the plumes of smoke (Mullin & Champ, 2003).

**Chemical countermeasures**

The spilled oil starts to disperse slowly into the water through the weathering processes soon after the oil spill occurs (Fig. 1). The oil slick breaks due to the wind and wave actions and small droplets intrude in the water column. Droplets < 50 µm in size tend to remain suspended in the water column and, thus, become widely dispersed (Fingas, 2015). As a result, the dispersed oil is more easily exposed to the natural degradation processes, for example, by microbial degradation. However, some oil re-coalesces and rises up to the water surface or eventually sinks and sediments on the bottom of the sea/lake. The latter typically occurs in shallow waters when oil gets close to the shore.

Chemical countermeasures are typically used in situations when quick oil spill responses are needed or the mechanical response and oil collecting are too difficult to carry out. Those actions might be used in near-coast accidents to prevent the oil from spreading to vulnerable coastal areas. The oil dispersants are mixtures of soluble surfactants, and the main aim of using dispersing agents is to accelerate the natural biodegradation of oil by breaking down the oil slick into smaller droplets. By accumulating onto oil droplet surfaces and allowing them to sink into deeper waters, dispersants make the oil more available to hydrocarbon degrading bacteria and other assisting organisms that naturally remove oil from the environment (Lessard & DeMarco, 2000; Prince, 2015).
Fig. 1. Weathering processes of oil in water including natural dispersion. (Adapted after ITOPF, 2018)

Commercially available oil dispersants are typically amphiphilic hydrocarbons that have two main components: a solvent and a surfactant. The solvent aids the transport and distribution of the surfactant. The surfactant is chemically similar to soaps and detergents; that is, they have a hydrophilic part with an affinity toward water and a hydrophobic part, sometimes referred to as an “oleophilic” part, which has an affinity to oil (Fig. 2). For example, one commercial oil dispersant from the Nalco Holding Company mainly consists of 2-butoxyethanol and 1,2-propanediol as solvents and organic sulfonic acid salts and aliphatic acids of sorbitol esters as surfactants (Nalco, 2016). The surfactant possesses the ability to reduce the surface tension and break the oil into smaller droplets (Prince, 2015). In practice, aircraft can apply the dispersants, which reduces the response time. This can prevent the oil from reaching the shallow waters and reduce the oiling of sensitive habitats, such as coastal wetlands, beaches or mangroves (National Research Council (U.S.), 2013). Another major benefit of using oil spill dispersants is their effectiveness. However, their performance is affected by oil composition (e.g., viscosity), wind and waves, oil weathering, the type and dosage of the dispersant, the water temperature, and the salinity of the water (Chandrasekar, Sorial & Weaver, 2006).
The use of oil dispersants is typically restricted in cases where the collecting of oil is somehow difficult or even impossible. Moreover, some countries have banned the use of synthetic dispersants and have questioned their safety (Wang, 2010), even though the dispersants may be very effective and fast to use, especially in deep waters and near-coast spills. For example in Finland, their use is under permission and typically considered as a last response option (SEA consulting group, 2013). This is because many of the current available synthetic dispersants have been linked to negative impacts concerning human health and the environment (Solomon, 2010). Consequently, there is a clear need for effective, non-hazardous, and sustainable oil response chemicals. For example, in the Arctic region, mechanical containment is difficult to implement due to the windy and icy weather conditions, and chemical response agents can be a more effective option. In addition, in the case of large spills, mechanical methods are too slow to respond, and the spread of oil to the shores cannot be prevented (Nevalainen, Helle & Vanhatalo, 2017).

1.2 Approach and aims of the thesis

The main aim of the thesis was to develop nanocellulose-based biochemicals for oil dispersants, using different chemical pretreatments and cellulosic raw materials from different sources. Especially, the objective was to establish new information on the fabrication and performance of nanocellulose-based dispersants as stabilizers in diesel oil-in-water (o/w) emulsions.
The more specific objectives are defined as follows:

i. To develop suitable chemical pretreatments for producing modified nanocelluloses for o/w emulsion stabilization.

ii. To address the performance of a nanocellulose-based biochemical as a dispersing agent in a model o/w emulsion system based on diesel oil-in-water in a lab-scale.

iii. To gain a more specific understanding of the possibilities of the surface functionalized nanocelluloses as oil dispersants and stabilizers.

In Papers I and II, fabrication of functionalized nanocelluloses for o/w emulsification using oxidative modification was investigated. Non-derivatizing deep eutectic solvents (DESs) were used as the reaction media for the pretreatment of cellulose as well as in the cellulose functionalization in Papers III and IV to produce green, nanocellulose-based dispersants. Different cellulose raw materials were used for nanocellulose production, including chemically bleached hardwood cellulose (Paper I), softwood dissolving cellulose (Papers III and IV), and semi-chemical, lignin-rich pulp (Paper II). Moreover, commercial cellulose nanocrystals from softwood were used as a reference material for emulsion stability and particle size evaluation (Papers III and IV). The effects of background electrolytes and cold environments on diesel o/w emulsion stabilization were studied in Papers II and III.

1.3 Outline of the thesis

This thesis is organized into five chapters: an introduction on the approaches for oil spill response, a review of the literature related to functionalized nanocelluloses and o/w emulsions, a description of experimental setups and materials, a discussion about the results, and the concluding remarks.

- Chapter 1 provides the general background of oil spills in the marine environment and describes the current technologies on oil spill preparedness and response. This chapter also describes the motivation for the whole research and states the main aims of the work.
- Chapter 2 reviews the cellulose structure, production of nanocelluloses using chemical pretreatments, and finally focuses on o/w emulsions. Especially, an overview of how nanoparticles, both organic and inorganic, can be utilized in o/w emulsification and emulsion stabilization is given.
Chapter 3 presents all the materials and analytics used and gives details of laboratory experiments.

Chapter 4 reports the results related to the fabrication and use of nanocelluloses in o/w emulsion stabilization and discusses the findings.

Chapter 5 concludes the results from this research.
2 Review of the literature

2.1 Nanocelluloses

2.1.1 Structure of the cellulose

Cellulose is an organic biopolymer that can be found in large quantities in plant cells (wood, cotton, hemp, grasses), and it also exists in fungi, bacteria, and even some animals (marine tunicates; Klemm et al., 2005). Cellulose is a linear polysaccharide, consisting of repeating anhydroglucose units (AGUs, a six-atom cyclic form with five carbon atoms and one oxygen atom) bound together with β-(1,4)-glycosidic bonds (Sixta, 2006; French, 2017). The chemical structure of the cellulose is presented in Fig. 3. Native wood cellulose molecules are composed of (degree of polymerization) approximately 8,000–10,000 AGUs. Each AGU has three reactive hydroxyl groups at positions C2, C3, and C6 that enable the packing of cellulose structures (i.e., fibrillar and semicrystalline) by intermolecular hydrogen bonding between adjacent chains. That configuration is also responsible for the physical properties of these materials (Nishiyama, 2009). The intramolecular hydrogen bonds are formed between the OH group at C3 and the oxygen atom at C5 as well as between the OH group at C2 and oxygen at C6 (Dufresne, 2012). The intramolecular hydrogen bonding forms the two-fold screw structure of the cellulose molecule, creating a very stiff structure (Moon, Martini, Nairn, Simonsen & Youngblood, 2011).

Cellulose is the dominant constituent of wood with a share of 45–50 wt.-% while hemicelluloses and lignin comprise 20–30 wt.-% and 15–40 wt.-%, respectively, depending on the tree species. Cellulose works as a main structural component that gives the mechanical strength to cell walls in plants. Lignin acts as a glue-like substance, embedding the cellulose and hemicellulose. It also provides natural hydrophobicity in plants (Rowell, 2005). Because of its abundance and biodegradability, cellulose is an appealing building block in different applications, including traditional paper and board products but also several sustainable, value-added applications (Habibi, Lucia & Rojas, 2010). At the moment, wood is the most important industrial cellulose source (Siró & Plackett, 2010).
2.1.2 Nano- and microsized cellulose

Native cellulose fibers (Fig. 4) consist of microfibrils that are held tightly together by hydrogen bonds in a matrix of lignin, pectin, and hemicellulose. They are typically 5–50 nm in width and several micrometers in length, and form the core of the secondary layer (S2) of plant cell walls. The microfibrils are further structured of nanofibrils with widths of 2–5 nm. They consist of less-ordered regions (commonly referred as amorphous regions) and ordered (crystalline) regions with highly uniform structure (Dufresne, 2012).

Cellulose nanomaterials (micro- and nanosized cellulose) can be divided into categories: microcrystalline cellulose (MCC), cellulose nanocrystals (CNCs), cellulose nanofibers (or nanofibrils; CNFs), tunicate CNCs (t-CNCs), algal cellulose (AC), and bacterial cellulose (BC) (Foster et al., 2018; Klemm et al., 2011). Since this thesis concentrates on CNCs and CNFs, the manufacture and properties of those cellulose nanoparticles are described in more detail in the next section. Generally, the term nanoparticle can refer to any particle that has at least
one nanoscale dimension. In this thesis, both “nanocellulose” and “nanoparticle” refer to CNCs and CNFs produced from cellulose.

![Diagram of hierarchical structure of cellulose](image)

Fig. 4. Schematic illustration of the hierarchical structure of cellulose (From Kekäläinen (2016), reprinted by permission from Acta Universitatis Ouluensis © 2016).

Each cellulose nanoparticle type has its own characteristic morphology (i.e., width and length distribution); thus, the aspect ratio can vary significantly. Moreover, alterations in the crystallinity and crystal structure may occur, depending on the source and the technique used in nanocellulose production (Moon et al., 2011). In the production of CNFs, both less-ordered and crystalline regions remain in the structure, resulting in flexible and fibrous nanofibrils with typical lengths of several micrometers. CNCs, in turn, are shorter and stiffer with highly crystalline structures and without less-ordered regions. The different production methods of cellulose nanoparticles are discussed more thoroughly in the following section.

### 2.2 The production of nanocelluloses

Cellulose nano- and microfibrils can be liberated from cellulose fibers with both mechanical and chemical methods, while the production of CNCs is based solely on chemical treatments or combining them with milder mechanical treatments.
Today, the chemical methods used for CNC production cover a variety of hydrolysis reactions where different acids (both organic and inorganic) are used. The chemical modifications are also commonly utilized in CNF production as pretreatments to loosen the fiber structure by weakening the strong interfibrillar hydrogen bonding, thus improving the mechanical disintegration. In addition to improving the quality of nanocellulose, chemical pretreatments decrease the energy consumption in mechanical-fiber processing.

2.2.1 Chemical pretreatment of cellulose for nanocellulose production

Nanocelluloses can be fabricated through several different production methods. Traditional mineral (inorganic) acid hydrolysis has been utilized to fabricate CNCs since 1949 (Rånby, Banderet & Sillén, 1949). Since then, alternative methods based on e.g. enzyme-catalyzed hydrolysis or concentrated organic acid hydrolysis have also been developed (Chen, Zhu, Baez, Kitin & Elder, 2016).

Different oxidative methods to produce nanocelluloses employ a wide range of oxidizing agents. TEMPO (2,2,6,6-tetramethylpiperidine-1-yl)oxyl oxidation, introduced by Saito, Nishiyama, Pataux, Vignon & Isogai (2006), is probably the most widely studied production method. Another method uses sequential periodate-chlorite oxidation for cellulose fibers, and it has been originally introduced by Sirviö, Hyväkkö, Liimatainen, Niinimäki & Hormi (2011). Since then, it has been studied by others, such as van de Ven and co-workers (Yang, Alam & van de Ven, 2013), and it was also used in this thesis. Periodate-chlorite oxidation produces a very selective reaction with hydroxyl groups at carbons 2 and 3, converting them to aldehydes (Sirviö et al. 2011). In the second reaction, aldehydes are further converted into carboxyls. In sequential periodate-chlorite oxidation, two carboxyl groups are formed in one AGU, resulting in higher carboxyl contents compared to the groups formed using the TEMPO oxidation method. The studies of Wågberg’s research group include periodate oxidations of different wood cellulosics following reduction reactions. (Hollerz, López Durán, Larsson & Wågberg, 2017; López Durán, Larsson & Wågberg, 2016). In addition, the combination of TEMPO and periodate oxidation has been investigated to better understand the fundamentals of the effects of chemical modifications on cellulose in paper products (López Durán, Larsson & Wågberg, 2018). Leung et al. (2011) suggested the preparation of carboxyalted CNCs through ammonium persulfate (APS) oxidation. This novel
procedure to produce CNCs from various biomasses using a chemical with low toxicity and short reaction times enables easy scale-up.

Chemical oxidative reactions, such as TEMPO oxidation (Saito et al., 2006; Saito, Kimura, Nishiyama & Isogai, 2007; Isogai, Saito & Fukuzumi, 2011), sequential periodate-chlorite oxidation (Sirviö et al., 2011; Liimatainen, Visanko, Sirviö, Hormi & Niinimäki, 2012) and carboxymethylation (Fall, Lindström, Sundman, Ödberg & Wågberg, 2011), increase the surface charge density of cellulose and simultaneously promote nanofibrillation. The basic idea of this novel pretreatment route is to introduce aldehyde groups into the cellulose pulp by oxidizing the reactive hydroxyl groups at carbons 2 and 3 with sodium periodate, according to the chemical reaction shown in Fig. 5. The resulting dialdehyde cellulose (DAC) can be further oxidized to dicarboxyl cellulose (DCC).

Fig. 5. Periodate and chlorite oxidation of cellulose.

Nanocellulose contains an abundance of reactive hydroxyl groups that enable chemical surface modification. Cationization, etherification via carboxylation, silylation, esterification, and polymer grafting, for example, have been reported to improve the structure of nanocellulose, thus, increasing its potential for diverse applications (Habibi, 2014; Peng, Dhar, Liu & Tam, 2011).

Natural CNFs and CNCs are inherently mainly hydrophilic, which makes them viable for forming o/w emulsions as such, but they are often chemically modified to better interact with the oil phase (Cunha, Mougel, Cathala, Berglund & Capron, 2014; Tang, Sisler, Grishkewich & Tam, 2017). Studies by Rowan and co-workers reported the surface modification of CNCs by introducing different alkylamine groups to gain a more amphiphilic nature (Zhang, Karimkhani, Makowski, Samaranayake & Rowan, 2017). Fig. 6 describes schematically one possible fabrication route for amphiphilic nanoparticles from wood cellulose. Here, the wood cellulose is used as a raw material for synthesizing bifunctionalized cellulose nanoparticles through oxidation and amination reactions, and those cellulose nanoparticles are predicted to slow down the creaming phenomenon, potentially
making the naturally occurring biodegradation of oil in the marine environment more efficient.

**Fig. 6.** One possible fabrication route for cellulose nanoparticles for oil spill response.

### 2.2.2 Mechanical fibrillation of cellulose

The first attempts to produce cellulose nanofibril (or microfibril) -like material from wood were published by Turbak, Snyder & Sandberg (1983) and Herrick Casebier, Hamilton & Sandberg (1983) in the early 1980s. They treated cellulose pulp mechanically by subjecting the fibers to shear and impact forces in a high-pressure homogenizer and obtained microfibrillated celluloses (MFCs), that is, larger aggregates of individual nanofibrils. Although this was a remarkable achievement at that time, larger interest in the possibilities of nanocelluloses did not arise until 20 years later.

Mechanical fibrillation methods for the CNF production include homogenization at high pressure (Liimatainen et al., 2012; Turbak et al., 1983), microfluidization (Taipale, Österberg, Nykänen, Ruokolainen & Laine, 2010; Zimmermann, Pöhler & Geiger, 2004), grinding with, for example, a Masuko masscolloider (Taniguchi & Okamura, 1998), and ultrasonic methods (Saito, Kuramae, Wohlerl, Berglund & Isogai, 2013; Wang & Cheng, 2009). Most typically, chemical (alkaline or oxidative treatments) or enzymatic methods are combined
with mechanical treatments because of high energy consumption and, thus, the high production costs of mechanical procedures alone (Spence, Venditti, Rojas, Habibi & Pawlak, 2011). The use of pretreatments also results in a more homogeneous quality of CNF, which can be monitored, for example, by analyzing the morphology, crystallinity, degree of polymerization, chemical composition, or rheology of CNF suspensions (Moser, Lindström & Henriksson, 2015).

The microfluidization, which was used as a mechanical treatment in this thesis, is a high-intensity method designed for cell disruption, emulsion, and liposome generation as well as particle size reduction to nano-size. The disintegration is based on a high shear and impact forces. The cellulose suspension is put into the inlet reservoir and pumped through the auxiliary processing module (APM) followed by the interaction chamber (IXC). Inside the specially designed Z-shaped interaction channels, the cellulose suspension is exposed to intense forces that cause the effective fibrillation (Kargarzadeh, Ioelovich, Ahmad, Thomas & Dufresne, 2017; Microfluidics, 2018). The increase in the number of passes through the device or the decrease in the internal diameter of the chambers improve the quality (homogeneity) of the product, but this may start to decrease the fibril length or lead to agglomeration due to the increased surface area (Kargarzadeh et al., 2017).

2.2.3 Deep eutectic solvents as pretreatment and reaction media for nanocellulose production

Deep eutectic solvents (DESs) are chemical complexes formed between a hydrogen bond donor (e.g., urea, glycerol, or glucose) and hydrogen bond acceptor (e.g., a halide salt of quaternary ammonium salt such as choline chloride). These eutectic mixtures are created through phase changes from two (or more) substances at the eutectic point, which is the minimum melting temperature for the combined system. DESs can be derived from biodegradable and bulk chemicals, which can originate from renewable sources. More importantly, production processes are cheap and simple enough to be used on an industrial scale (Alonso et al., 2016).

DESs are becoming one of the highly favored solvents in different applications over “traditional” solvents, such as water, super-critical fluids (e.g., CO₂), ethanol, glycerol, or ionic liquids. (Alonso et al., 2016). This is because of the many advantages of DESs, such as negligible vapor pressure, biocompatibility (e.g., with enzymes), low toxicity, and low flammability. Moreover, compatibility with
different lignocellulosic biomasses makes DES appealing alternatives due to their sustainable modification and fractionation.

DESs have recently been studied for utilization as a “green media” for pretreatment of cellulosic biomass (Procentese et al., 2015; Xia, Baker, Li, Ravula & Zhao, 2014; Xu, Ding, Han, Dong & Ni, 2016). They have been used, for example, to enhance enzymatic saccharification for further fermentation. Some DES combinations are also able to remove selective lignin from lignocellulose biomasses and dissolve the hemicellulose, thereby replacing costly enzymatic pretreatments (Zhang, Xia & Ma, 2016). Moreover, DES have also been utilized for cellulose modification, such as cationic acetylation (Abbott, Bell, Handa & Stoddart, 2005) or anionic functionalization (Selkälä, Sirviö, Lorite & Liimatainen, 2016).

2.3 Emulsion formation and stability

2.3.1 Physical properties of emulsions

Emulsion is a mixture of two or more liquids where one phase is present as dispersed droplets in another phase, which is often referred to as the continuous phase (Tadros, 2016). Those two phases are immiscible and need external aid to mix. The emulsion is a thermodynamically unstable system, which means that the system is constantly trying to reduce its interfacial energy. Therefore, the emulsion is prone to phase separation and needs to be stabilized with an emulsifier (Lyklema, 2000; Tadros, 2016). An emulsifier is a substance that promotes proper emulsion formation and stabilization as it prevents the dispersed phase from coalescing. Efficient emulsifiers and their sub-class, surfactants are used as a third component in emulsion formation to prevent de-emulsification (e.g., coalescence) and phase separation i.e. creaming. Emulsifiers are typically either non-ionic or a mixture of non-ionic and ionic surfactants (Jafari, Assadpoor, He & Bhandari, 2008; Tadros, 2013a).

Emulsions are typically classified into three different categories, namely oil-in-water (o/w), water-in-oil (w/o), and oil-in-oil (o/o), but combinations of these also exist (Binks, 1995). Emulsification (or homogenization) is the process of mixing two immiscible liquids together to form an emulsion. Typically, mechanical aids, such as a high-speed mechanical mixer or ultrasonic homogenizer are used to bring external energy to the system, thereby changing the balance of its free internal
energy. In 1913, Bancroft formulated a rule that explains why every type of emulsion forms a continuous phase from a liquid with surfactants. In practice, this means that the emulsion type depends more on the emulsifying agent than the ratios of different phases (oil, water) present. Typically, water-soluble surfactants form o/w emulsions whereas oil-soluble surfactants form w/o emulsions. (Bancroft, 1915; Bancroft, 1913; Ruckenstein 1996). In nanoparticle-stabilized emulsions, the increased hydrophobicity improves the attachment at interfaces; however, the nanoparticle is still in the water phase.

Based on the size of the emulsion droplets, either macroemulsion, miniemulsion, or microemulsion is obtained. The o/w and w/o macroemulsions have a size > 400 nm and are the most common types of emulsions (Anderson & Daniels, 2003). Miniemulsions are in a size range of 50–400 nm, and the only thermodynamically stable emulsion type—microemulsion—usually has a size range of 5–50 nm (Binks, 1995; Tadros, Izquierdo, Esquena & Solans, 2004). Microemulsions are formed spontaneously (without any external input) in correct conditions (i.e., at least 20% surfactant concentration) and are visually transparent, unlike mini- and macroemulsions (Tadros et al., 2004). Therefore, microemulsions are often categorized separately from “true” emulsions (Shinoda & Lindman, 1987).

2.3.2 Emulsion stability

The stability of an emulsion refers to its ability to remain unchanged over a period of time. Emulsion stability depends on various factors. Due to the increase of free energy in dispersion, an emulsion is a thermodynamically unstable system. Despite their thermodynamic instability, emulsions can be kinetically stable. Kinetic stability of emulsion refers to all processes that change the emulsion properties (i.e., the many instability phenomena that occur either simultaneously or alone and in any order). Fig. 7 illustrates schematically the most important mechanisms of emulsion instability, including sedimentation, creaming, flocculation, and coalescence (Dickinson, 2009).

Ostwald ripening (or disproportionation) in an emulsion is a thermodynamically driven process where small droplets dissolve and finally disappear from the emulsion by diffusing into larger droplets. This process changes the total droplet size distribution of an emulsion (Tadros, 2013b). Ostwald ripening differs from coalescence in that in Ostwald ripening, the continuous phase serves as a dissolving medium, and there is no need for droplets come into direct contact with each other to coalesce. Stable emulsions do not coalesce or undergo Ostwald
ripening. However, many factors, including interfacial tension and solubility differences between emulsified substances, may affect the ripening rate remarkably, so Ostwald ripening should be considered as an important degradation process for emulsions (Taylor, 1998).

Fig. 7. O/w emulsion, separation processes and forms of droplet loss mechanisms that lead to a broken emulsion (de-stabilization).

**Creaming and sedimentation**

Due to the thermodynamic instability and density differences of the dispersed phase and the continuous phase, emulsions are prone to phase separation processes, most commonly creaming or sedimentation. The creaming phenomenon causes the “cream” layer, which has a lower density and is typically richer in the dispersed phase, to form on the top of the dispersion, while the sedimentation layer appears at the bottom of the system (Robins, 2000). Both of the mentioned mechanisms are enhanced by external gravitational or centrifugal forces and can be often assessed by visual observations or optically (Newling, Glover, Keddie, Lane & McDonald, 1997). The level of creaming over time can also be determined simply by measuring the volume of the emulsion layer in a graduated container. Even though creaming is not a de-emulsification process, it increases the probability of the
coalescence phenomenon due to the tight packing and close contact between adjacent droplets (Robins, 2000).

The separation processes that are based on gravitational forces, such as sedimentation and creaming, can be described by the Stokes Law (Equation 1):

\[ v = \frac{2r^2 \cdot g \cdot \Delta \rho}{9\mu}, \]  

(1)

where \( v \) is the velocity of the phase separated droplet, \( r \) is droplet radius, \( g \) is the acceleration of gravity, \( \Delta \rho \) is the density difference between phases, and \( \mu \) is the viscosity of the continuous phase. The decrease in the dispersed droplet size (e.g., by homogenization) or the diminishing density difference result in slower phase separations. Moreover, increasing the viscosity of the continuous phase (e.g., by adding a thickening agent) similarly affects the velocity of separation (Chanamai & McClements, 2000).

Coalescence

Small-sized oil droplets possess a large interfacial area; thus, they also have high interfacial free energy. Emulsion droplets continuously interfere with one another due to either Brownian motion or gravity. These phenomena cause many collisions to occur, and in some cases, the droplets irreversibly merge and coalesce (Dickinson, 2009). The coalescence phenomenon reduces the free energy, eventually leading to de-emulsification; that is, the dispersed oil phase separates from the water (Anderson & Daniels, 2003). However, the droplet collisions do not always lead to coalescence; the combination of colloidal forces (i.e., van der Waals attractive forces, steric interactions, and electrostatic repulsive forces) will result in a high energy barrier that needs to be overcome for the droplets to coalesce (Mohan & Narsimhan, 1997). The coalescence phenomenon can typically be hindered by using soluble surfactants. Moreover, emulsion stabilization driven by solid particles have gained more interest for the formation of very stable emulsions without coalescence (Yang et al., 2017).
2.4 Emulsion stabilization with different nanoparticles –Pickering emulsion system

Traditionally, emulsions have been stabilized using soluble surfactants (i.e., surface active agents), which are substances that have the ability to adsorb onto the interfaces of both oil and water due to their amphiphilic nature (i.e., they contain both hydrophobic and hydrophilic parts). The aim of surfactants is to form a barrier between droplets to prevent the recoalescence by repulsive interactions. Also, interfacial tension is lowered in systems where surfactants are used (Baret, Kleinschmidt, El Harrak & Griffiths, 2009).

The emulsions stabilized by solid particles are commonly referred to as Pickering emulsions, named after S. U. Pickering, who was among the first scientists to describe this phenomenon at the beginning of the last century (Pickering, 1907). However, Walter Ramsden (1903) already knew about the phenomenon a few years earlier. Both scientists studied fine inorganic particles in emulsion stabilization, and even today, interest in applications for particle-stabilized emulsions continue to attract increased attention. Due to its strong kinetic stability, Pickering stabilization can potentially be used in several fields, including food technology, cosmetics, pharmaceuticals, and environmental technologies such as wastewater treatment or oil recovery (Hunter, Pugh, Franks & Jameson, 2008). In several applications, the long-term stability of Pickering emulsions is the one of the important factors when emulsions are produced. Pickering stabilizers lack the on-off interface kinetics that makes the stabilization phenomenon irreversible and, thus, emulsion very stable. Solid nanomaterials stabilize the emulsions by attaching at the oil-water interface. Nanomaterials are typically considered materials in the form of particles, tubes, fibers, or rods, and have at least one dimension between 1 and 100 nm. The nanoparticles are typically partially wetted in both the oil and water phases and are tightly adsorbed in the oil-water interface, which makes the accumulation irreversible, leading to more efficient stabilization.

Theories of the stabilization mechanisms of Pickering particles include the formation of the nanoparticle layer on the surface of oil droplets, creating a steric barrier between droplets (Monegier du Sorbier, Aimable & Pagnoux, 2015). Moreover, the particles can form bridges or larger aggregates that improve stability (Aveyard, Binks & Clint, 2003; Koroleva, Tokarev & Yurtov, 2017; Yang et al., 2017). The van der Waals forces refer to weak attractive (or repulsive) forces between two atoms or molecules. Van der Waals interactions should not be interfered by more stable chemical bonds such as ionic or covalent bonds. The
interaction decreases as a function of the molecule distance, and therefore, there is attraction with only the nearest molecules. Any disturbance (e.g., salt addition) can interfere with the interaction with the nearest molecule. Recent research shows that the formation of Pickering emulsions is possible with charge induced particles and attractive van der Waals forces. This is a novel approach in Pickering emulsion studies and enables applications where particle detachment is needed (Elbers, van der Hoeven & Matthjis de Winter, 2016; Marina, Cheng, Sedev, Stocco, Binks & Wang, 2018).

**Inorganic nanoparticles in Pickering stabilization**

Inorganic nanoparticles, such as silica (Behzadi & Mohammadi, 2016; Koroleva et al., 2017; Pi, Li, Bao, Mao, Gong & Wang, 2016), clay (Reger, Sekine & Hoffmann, 2012), and magnetic metal nanoparticles (e.g., Fe₃O₄; Zhou et al., 2011; Zhou, Wang, Qiao, Binks & Sun, 2012), have recently attracted the attention of researchers for the formation of Pickering emulsions. From the abovementioned, silica has been widely studied because of its availability and formability. However, in its native state, silica is too hydrophilic to form stable emulsions, and it is highly influenced by pH and salt. Therefore, silica is often modified by attaching suitable molecules, such as fatty acids, at the silica surface (Duan, Chen, Zhou & Wu, 2009). Clay, a popular inorganic Pickering stabilizer (Ashby & Binks, 2000), also may need some pretreatment with organic or amphiphilic molecules or some additional surfactant to improve its performance in o/w stabilization. LAPONITE® XLG for example, is a synthetic, layered clay silicate that had been used to lower the surface tension of water. Although the procedure resulted in a gel-like Pickering emulsion due to the cross-linking of clay particles to oil, the surfactant was needed to promote adsorptivity and the ability of the inherently hydrophilic nature of clay to better stay in the oil-water interface (Reger et al., 2012).

**Organic nanoparticles in Pickering stabilization**

Many types of organic particles are also suitable for emulsion stabilization. Chitin, for example, is abundantly available and can be easily converted into chitosan, which has many functional groups in its structure and, thus, has many possible uses (Wang et al., 2016). Typically, biodegradable and biocompatible chitosan is used in biomedicine and pharmaceutics (Wei, Wang, Liu, Zou & Tong, 2012), but it has also been used as a dispersant in oil spill response (Kalliola et al., 2016). Moreover,
starch, soy protein, and whey protein have been studied as stabilizers in Pickering emulsion systems. Li et al. (2013) studied starch granules and the multiple factors that are supposed to affect emulsion stabilization with native starch. They found that the size and concentration of the particles were mostly determining the emulsion stabilization performance.

Nanocelluloses are suitable in many applications due to their biocompatibility, biodegradability, low cost, and lower density when compared to inorganic particles. Andresen and Stenius (2007) studied hydrophobized microfibrillated cellulose (MFC) in the stabilization of oil in emulsion. They found that stabilized o/w emulsions and w/o emulsions were both stable against coalescence, but the drawback in the system was the aggregation of the elongated MFC. Since the fibril length is uncontrollable, that may also cause problems in the stabilization because larger fibrils are packed more loosely around droplets.

Lately, CNCs have especially been studied for Pickering stabilization (Gong, Wang & Chen, 2017; Kalashnikova, Bizot, Bertoncini, Cathala & Capron, 2013; Liu et al., 2018; Li et al., 2018; Tang, Lee, Zhang, Zhao, Berry & Tam, 2014; Zoppe, Venditti & Rojas, 2012). Dr. Capron and her colleagues were the first to report Pickering emulsion stabilization with bacterial CNCs (Kalashnikova, Bizot, Cathala & Capron, 2011). Later, the continued studies of Capron’s group (Kalashnikova, Bizot, Cathala & Capron, 2012; Kalashnikova et al. 2013) discussed CNCs with different aspect ratios and different origins as Pickering stabilizers. The characteristics of bacterial cellulose and cotton in both native and functionalized form were studied in emulsion stabilization, and the electrostatic interaction was reported to be the most important factor in the stabilization. If the surface charge density was too high, no stable emulsion was formed (Kalashnikova et al., 2012; Kalashnikova et al., 2013). It was also suggested that the size of the stabilizing particles may play a significant role in controlling emulsion characteristics, such as droplet size, viscosity, and reproducibility (Kalashnikova et al., 2013). The dimensions of CNCs are considered to be in the optimal range for providing stable Pickering emulsions by the action of stabilizing capillary forces, although studies have found that their performance still depends on cellulose surface characteristics (Hu, Ballinger, Pelton & Cranston, 2015; Kalashnikova et al., 2012).
3 Materials and methods

3.1 Materials

The chemical pretreatments and modifications of different pulp fibers were conducted before liberation of individual CNCs or CNFs. The bifunctionalized CNCs with amphiphilic features were prepared using a combination of oxidative reactions, followed by amination with n-butylamine (Papers I–II). DES systems were used as pretreatment and reaction media to produce succinylated CNF, non-functionalized CNF, and carboxylated CNC (Papers III–IV). A mechanical microfluidization phase was finally used to liberate the individual CNFs or CNCs from all pretreated cellulose samples. The sample names, as referred to in this thesis, are presented later in Table 3 (and may differ from those mentioned in the original publications).

The chemical compositions of different cellulose pulps are presented in Table 1. The lignin content of all raw materials was analyzed using the TAPPI-T 222 om-02 standard and the extractive content using the SCAN-CM 49:03 standard. The polysaccharide content was determined using high-performance anion exchange chromatography (HPAEC-PAD) (Zuluaga et al., 2009). Bifunctionalized CNCs (Paper I) were synthesized from bleached birch (Betula pendula) chemical kraft wood pulp obtained as dry sheets. The average (length-weighted) length and width of the kraft pulp fibers were 0.90 mm and 19.0 µm, respectively, as determined using a Metso FiberLab image analyzer (Metso Automation, Helsinki, Finland).

The lignin-rich, semichemically produced corrugated board pulp was used to fabricate bifunctionalized CNFs in Paper II. The chemical composition of the pulp was 70.4 wt.-% cellulose, 14.7 wt.-% lignin, 14.1 wt.-% hemicelluloses, and 1.8 wt.-% extractives. The original non-dried pulp was first fractionated with a 150µm sieve to obtain a fraction containing only the fine particles (fragments from the outer layers of fibers and cut fibers). The average length in the fines fraction was roughly half that of the original pulp fibers (0.30 mm vs. 0.65 mm), and the average width was 16.2 µm and 21.2 µm for fines fractions and original pulp, respectively.

The commercial softwood dissolving pulp (Domsjö Fabriker AB, Sweden) was delivered as dry sheets and was disintegrated in deionized water, according to ISO 5263-1:2004, before use as a raw material in the production of non-functionalized CNFs and surface functionalized CNCs and CNFs (Papers III–IV).
The disintegrated pulp was filtered, ethanol-washed for 30 minutes, and dried in an oven at 60 °C for 24 hours.

Table 1. The chemical composition of cellulose raw materials.

<table>
<thead>
<tr>
<th>Fibre source</th>
<th>Wood species</th>
<th>Nanocellulose type</th>
<th>Average composition (wt.-%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cellulose</td>
</tr>
<tr>
<td>Chemical (kraft) pulp</td>
<td>Hardwood (Betula Pendula)</td>
<td>Bifunctionalized CNC (Paper I)</td>
<td>74.8</td>
</tr>
<tr>
<td>Semichemical pulp (Fines fraction)</td>
<td>Hardwood</td>
<td>Bifunctionalized CNC (Paper II)</td>
<td>57.8</td>
</tr>
<tr>
<td>Dissolving pulp</td>
<td>Softwood</td>
<td>NON-F-CNF*, SF-CNF**, SF-CNC*** (Papers III, IV)</td>
<td>96.2</td>
</tr>
<tr>
<td>Acetate grade dissolving pulp (Sixta, 2006)</td>
<td>Softwood (Western Hemlock)</td>
<td>REF-CNC (Papers III, IV)</td>
<td>&gt;97.0</td>
</tr>
</tbody>
</table>

*) Non-functionalized cellulose nanofibers  
**) Surface functionalized cellulose nanofibers  
***) Surface functionalized cellulose nanocrystals

Reference CNCs (BGB Ultra™) that were produced from acetate-grade dissolving pulp (Western Hemlock) using an oxidative process were purchased from Blue Goose Biorefineries Inc. (Canada) and were used as a reference material (Papers III–IV). The acetate-grade dissolving pulp is the highest purity dissolving pulp, containing more than 97% cellulose and < 2% hemicelluloses (Sixta, 2006). According to Blue Goose Biorefineries, crystallinity index of BGB Ultra is 80%, the length is 100–150 nm and width 9–14 nm. The carboxyl content of BGB ultra is 0.15 mmol g⁻¹ (Blue Goose Biorefineries Inc., 2019). The BGB Ultra that is referred as REF-CNC in this thesis is fabricated through naturally oxidative transition-metal-catalyzed method. The produced CNCs are comparable to ‘traditional’ CNCs in their size distribution and carboxyl content; however, there is no sulfate half esters present (Blue Goose Refineries Inc., 2019).
All chemicals used in the fiber modifications and analyses (i.e., conductometric titration and transmission electron imaging) are listed in Table 2. The chemicals were pro analysis grade with high purity and used without additional purification. For all o/w emulsions, lightweight marine diesel oil was used. This diesel oil was a sulfur-free winter grade with a density of 828 kg/m³ at 15 °C and a viscosity of 1.846 mm²/s at 40 °C (Neste Oyj, Finland). Styrene (C₈H₈) and benzoyl peroxide were purchased from TCI Europe (Zwijndrecht, Belgium) and the soybean oil was purchased from Sigma Aldrich (Darmstadt, Germany). The density of styrene was 906–909 kg/m³ at 20 °C and for soybean oil 920 kg/m³ at 25 °C. Deionized water was used in all dilutions and disintegrations throughout the experiments.
Table 2. List of chemicals.

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Chemical formula</th>
<th>Purity</th>
<th>Producer/Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bifunctionalized CNC (Papers I,II)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium metaperiodate</td>
<td>NaIO₄</td>
<td>p.a. grade</td>
<td>Sigma Aldrich (India)</td>
</tr>
<tr>
<td>Sodium chlorite</td>
<td>NaClO₂</td>
<td>p.a. grade</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>CH₃COOH</td>
<td>p.a. grade</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td>2-picoline borane</td>
<td>pic-BH₃</td>
<td>95%</td>
<td>Sigma Aldrich (USA)</td>
</tr>
<tr>
<td>n-butylamine hydrochloride</td>
<td>C₄H₁₂ClN</td>
<td>&gt;98%</td>
<td>TCI (Belgium)</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>HCl</td>
<td>37%</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td><strong>DES-pretreated CNCs and CNFs (Papers III, IV)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline chloride</td>
<td>C₅H₁₄ClNO</td>
<td>&gt;98.0%</td>
<td>TCI Europe (Germany)</td>
</tr>
<tr>
<td>Urea</td>
<td>CO(NH₂)₂</td>
<td>99.0-100.5%</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>LiCl</td>
<td>99%</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td>Oxalic acid dihydrate</td>
<td>C₂H₂O₄·2H₂O</td>
<td>&gt;99%</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td>Succinic anhydride</td>
<td>C₄H₄O₃</td>
<td>&gt;95%</td>
<td>TCI (Japan)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>C₂H₆O</td>
<td>96%</td>
<td>VWR (Finland)</td>
</tr>
<tr>
<td><strong>Aldehyde and carboxyl content analyses of bifunctionalized CNC (Papers I-IV)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxylamine hydrochloride</td>
<td>NH₂OH·HCl</td>
<td>p.a. grade</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>CH₃COONa·2H₂O</td>
<td>p.a. grade</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>NaCl</td>
<td>p.a. grade</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>NaOH</td>
<td>p.a. grade</td>
<td>Sigma Aldrich (Germany)</td>
</tr>
<tr>
<td><strong>TEM imaging (Papers I-IV)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uranyl acetate</td>
<td>UO₂(CH₃COO)₂·2H₂O</td>
<td>98%</td>
<td>Polysciences (USA)</td>
</tr>
</tbody>
</table>
3.2 Chemical modification of pulp fibers

3.2.1 Sequential periodate-chlorite oxidation and butylamination

Synthesis of the partially oxidized dicarboxylic acid celluloses (DCCs) was conducted through a sequential periodate-chlorite oxidation reaction. The reaction was followed by reductive amination to produce bifunctionalized cellulose nanocrystals from kraft pulp (Paper I) and semichemical, lignin rich pulp (Paper II).

The periodate-chlorite oxidation

The oxidation reaction pathway consisted of two distinct stages. First, the pulp was oxidized with sodium metaperiodate (NaIO₄) in a 1 wt.-% consistency in an aqueous environment to attain dialdehyde celluloses (DACs) with different aldehyde contents. Second, the samples were further oxidized with sodium chlorite (NaClO₂) to produce partially oxidized 2,3-dicarboxylic acid celluloses (DCCs). The reaction conditions are summarized in Table 3, and the procedure is described in more detail below.

Table 3. Sodium periodate and chlorite oxidation reaction conditions.

<table>
<thead>
<tr>
<th>Sample names *)</th>
<th>The raw material</th>
<th>NaIO₂ [g]</th>
<th>Metal salt</th>
<th>Temperature [°C]</th>
<th>Reaction time [min]</th>
<th>NaClO₂ [g]</th>
<th>Reaction time [min]</th>
<th>Sample names in original publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNC B1</td>
<td>CP**)</td>
<td>12.3</td>
<td>-</td>
<td>65</td>
<td>180</td>
<td>0.50</td>
<td>8</td>
<td>But-CNC/A</td>
</tr>
<tr>
<td>CNC B2</td>
<td>CP</td>
<td>12.3</td>
<td>-</td>
<td>65</td>
<td>180</td>
<td>0.99</td>
<td>8</td>
<td>But-CNC/B</td>
</tr>
<tr>
<td>CNC B3</td>
<td>CP</td>
<td>12.3</td>
<td>-</td>
<td>65</td>
<td>180</td>
<td>1.24</td>
<td>8</td>
<td>But-CNC/C</td>
</tr>
<tr>
<td>CNC B4</td>
<td>CP</td>
<td>12.3</td>
<td>LiCl</td>
<td>75</td>
<td>180</td>
<td>0.87</td>
<td>8</td>
<td>But-CNC/D</td>
</tr>
<tr>
<td>CNC B5</td>
<td>CP</td>
<td>12.3</td>
<td>LiCl</td>
<td>75</td>
<td>180</td>
<td>2.18</td>
<td>8</td>
<td>But-CNC/E</td>
</tr>
<tr>
<td>Paper II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNC B6</td>
<td>SCP***)</td>
<td>12.3</td>
<td>-</td>
<td>65</td>
<td>180</td>
<td>0.49</td>
<td>8</td>
<td>CNC-LIG-1</td>
</tr>
<tr>
<td>CNC B7</td>
<td>SCP</td>
<td>12.3</td>
<td>LiCl</td>
<td>75</td>
<td>180</td>
<td>0.80</td>
<td>8</td>
<td>CNC-LIG-2</td>
</tr>
<tr>
<td>(CNC B4)</td>
<td>CP</td>
<td>12.3</td>
<td>LiCl</td>
<td>75</td>
<td>180</td>
<td>0.87</td>
<td>8</td>
<td>CNC-CHEM</td>
</tr>
</tbody>
</table>

*) Samples as they are referred in this Thesis
***) CP = Chemical pulp
*** SCP = Semichemical pulp
In Paper I, the chemical pulp (CP) was first wet disintegrated, and the oxidations were conducted in 15 g (oven-dry weight) batches with two different procedures to produce cellulosics with two aldehyde contents. The oxidation with 12.3 g of NaIO$_4$ in 1,500 ml of water at 65 °C for 180 minutes resulted in DAC1 with an aldehyde content of 2.20 mmol g$^{-1}$. The oxidation of cellulose with 12.3 g of sodium periodate and 27 g of lithium chloride (LiCl) in 1,500 ml of water resulted in DAC2 with an aldehyde content of 3.86 mmol g$^{-1}$. Further oxidation for both DACs was performed with sodium chlorite (NaClO$_2$). DAC1 was oxidized with three different NaClO$_2$ amounts (0.497 g, 0.994 g, 1.243 g) by diluting each sodium chlorite amount in deionized water (17.78 g). Then 26.65 g of acetic acid (20 vol.-%) was added together with 2 g of DAC1 in 44.4 ml of water. The oxidation was conducted in a fume hood for 8 minutes. This procedure resulted in three samples (PO-DAC/A, PO-DAC/B and PO-DAC/C) with carboxyl contents of 0.50 mmol g$^{-1}$, 1.13 mmol g$^{-1}$, and 1.58 mmol g$^{-1}$, respectively. The DAC2 was oxidized with two different sodium chlorite amounts (0.87 g and 2.18 g). The procedure was similar to that with DAC1 and resulted in two samples, PO-DAC/D and PO-DAC/E, with carboxyl contents of 1.08 mmol g$^{-1}$ and 1.90 mmol g$^{-1}$, respectively.

In Paper II, semichemical cellulose pulp (SCP; 15 g oven-dry weight) was oxidized using 12.3 g of sodium periodate (NaIO$_4$) in deionized water (1,500 ml) at 65 °C for 180 minutes to attain DAC_A. DAC_B was prepared similarly, except that 27 g of lithium chloride (LiCl) was used as an additive, and a temperature of 75 °C was used. The third sample, DAC_C, was prepared from kraft pulp with a similar chemistry as used for DAC_B. Sodium chlorite (NaClO$_2$) was used to partially oxidize the aldehydes of the DACs to obtain dicarboxylic acid groups (PO-DACs).

The sodium chlorite amounts used were 0.497 g, 0.80 g, and 0.80 g for DAC_A, DAC_B, and DAC_C, respectively. The sodium chlorite was first diluted with deionized water (17.78 g) and then transferred into a beaker with 26.65 ml of 20 vol.-% acetic acid. These solutions were mixed with 2 g of DAC, oxidized, and diluted in 44.4 ml of water. The oxidation reaction was completed under a fume hood with continuous mixing for 8 minutes.

**Bifunctionalization of the cellulose**

After oxidation to DACs, a reductive amination was used to introduce n-butylamino groups to the partially oxidized cellulose. Fig. 8 shows the total chemical reaction route with oxidation and amination reactions.
The amination (Paper I) was conducted by weighing the n-butylamine hydrochloride (C₄H₁₁N·HCl) in 200 ml of deionized water and adjusting the pH to 4.5 with a diluted HCl solution. Next, a 2-picoline borane solution (where twofold excess of 2-picoline borane, based on the assessed amount of the aldehyde groups, had been diluted in 200 ml deionized water) was introduced to the n-butylamine hydrochloride solution with 4 g of each PO-DAC. After 72 hours of continuous mixing at room temperature, the samples were filtered to stop the reaction and were washed with water (V = 200 ml) and ethanol (V = 300 ml). The solid fraction was collected and 200 ml of 0.1 M HCl solution was added. After 5 minutes of mixing in a beaker, the suspension was filtered and washed with 500 ml of deionized water.

The amination (Paper II) for DAC_A was conducted by adding 7.37 g n-butylamine hydrochloride to 200 ml of deionized water. For DAC_B and DAC_C, 12.19 g of n-butylamine was used. The procedure continued by adding 2-picoline borane solution — 1.44 g pic-BH₃/200 ml deionized H₂O (for PO-DAC/A) and 2.38 g/200 ml deionized H₂O (for both PO-DAC/B and PO-DAC/C) — to the n-butylamine hydrochloride solution with 4 g of desired PO-DAC fiber sample. After 72 hours of continuous mixing at room temperature, the samples were filtered to stop the reaction and were washed with water (V = 200 ml) and ethanol (V = 300 ml). The sample was then collected and mixed for 5 minutes in a beaker with 200 ml 0.1 M HCl, after which the suspension was filtered and washed with 500 ml of deionized water. The reaction conditions for the butylamination reaction are presented in Table 4.
Table 4. Butylamination reaction conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pulp amount [g]</th>
<th>n-butylamine hydrochloride [g]</th>
<th>2-Picoline borane [g]</th>
<th>Deionized water [ml]</th>
<th>Temperature [°C]</th>
<th>Reaction time [h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNC B1</td>
<td>4</td>
<td>7.37</td>
<td>1.44</td>
<td>200</td>
<td>rt</td>
<td>72</td>
</tr>
<tr>
<td>CNC B2</td>
<td>4</td>
<td>7.37</td>
<td>1.44</td>
<td>200</td>
<td>rt</td>
<td>72</td>
</tr>
<tr>
<td>CNC B3</td>
<td>4</td>
<td>7.37</td>
<td>1.44</td>
<td>200</td>
<td>rt</td>
<td>72</td>
</tr>
<tr>
<td>CNC B4</td>
<td>4</td>
<td>12.19</td>
<td>2.38</td>
<td>200</td>
<td>rt</td>
<td>72</td>
</tr>
<tr>
<td>CNC B5</td>
<td>4</td>
<td>12.19</td>
<td>2.38</td>
<td>200</td>
<td>rt</td>
<td>72</td>
</tr>
<tr>
<td>Paper II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNC B6</td>
<td>4</td>
<td>7.37</td>
<td>1.44</td>
<td>200</td>
<td>rt</td>
<td>72</td>
</tr>
<tr>
<td>CNC B7</td>
<td>4</td>
<td>12.19</td>
<td>2.38</td>
<td>200</td>
<td>rt</td>
<td>72</td>
</tr>
</tbody>
</table>
3.2.2 Deep eutectic solvents in the production of cellulose nanoparticles

Deep eutectic solvents (DESs) were used as pretreatment and reaction media in order to enhance the liberation of individual cellulose nanoparticles and to introduce functional chemical groups. Three different DES systems were used in the treatment of dissolving cellulose pulp. The amounts of the chemicals used in the production of the different DES systems and reaction conditions in the fabrication of the samples are summarized in Table 5.

Table 5. The reaction conditions and chemical amounts in different DESs.

<table>
<thead>
<tr>
<th>Sample name *)</th>
<th>Pulp (DIS**)</th>
<th>Choline chloride [g]</th>
<th>Urea [g]</th>
<th>Oxalic acid dihydrate [g]</th>
<th>Succinic acid anhydride [g]</th>
<th>Metal salt</th>
<th>Temperature [°C]</th>
<th>Reaction time [min]</th>
<th>Original sample names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper III, IV</td>
<td>Pulp CNF D1</td>
<td>1.20</td>
<td>64.80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>120</td>
<td>NON-F-CNF</td>
</tr>
<tr>
<td></td>
<td>Pulp CNF D2</td>
<td>1.13</td>
<td>-</td>
<td>131.45</td>
<td>9.27</td>
<td>LiCl</td>
<td>70</td>
<td>360</td>
<td>SF-CNF</td>
</tr>
<tr>
<td></td>
<td>Pulp CNC D3</td>
<td>1.50</td>
<td>63.06</td>
<td>-</td>
<td>56.94</td>
<td>-</td>
<td>100</td>
<td>360</td>
<td>SF-CNC (IV) / DES-CNC (III)</td>
</tr>
</tbody>
</table>

*) Samples as they are referred in this Thesis
**) DIS = dissolving pulp

In Paper IV, a non-derivatizing urea-choline chloride pretreatment was used for the preparation of the CNFs without surface functional groups (CNF D1). A urea-choline chloride DES was prepared by heating 64.8 g of choline chloride and 48.9 g of urea in a flask at 100 °C until a clear, colorless liquid was formed. The cellulose sample (1.13 g of dry sheets of pulp) was added to the liquid and was treated at 100 °C for 2 hours. After the DES treatment, 200 ml of water was added while mixing, and the pulp was filtered (Whatman 413) and washed with deionized water (2,000 mL).

The urea-LiCl DES for the production of succinylated CNF D2 (Paper IV) was prepared by mixing 131.45 g of urea and 18.55 g of LiCl (molar ratio 5:1), and heating the mixture at 80 °C in an oil bath until a clear, colorless liquid was formed. The dissolving pulp (1.50 g) and succinic acid anhydride (9.27 g) were mixed into the solution under continuous stirring for 6 hours at 70 °C (Fig. 9). Following the reaction, the mixture was removed from the oil bath, and 150 ml of ethanol was...
added. The resulting mixture was filtered, and the modified pulp was washed with ethanol (100 mL) and deionized water (1,500 mL).

Fig. 9. Succinylation of cellulose in urea-LiCl DES.

The choline chloride-oxalic acid dihydrate DES in Paper III was produced by mixing 63.06 g of choline chloride and 56.94 g of oxalic acid dihydrate at 100 °C for half an hour. After a clear solution was obtained, 1.2 g of dissolving pulp was added, and the suspension was mixed for 6 hours (Fig. 10). The reaction was stopped by adding 100 ml of deionized water before the suspension was filtered and finally washed with 2,000 ml of deionized water to produce carboxylated cellulose nanocrystals. The same procedure was used in Paper IV to produce CNC D3.

Fig. 10. The carboxylation reaction of cellulose in choline chloride-oxalic acid dihydrate DES.

3.2.3 Microfibrillation of the modified fibers

Both oxidized/aminated and DES-pretreated cellulose materials were converted to individual nanocrystals and nanofibers using a homogenization treatment with a M-110EH-30 Microfluidizer® (Microfluidics, USA).

All suspensions (0.1–1 wt.-%) were passed at least once through a 400 µm auxiliary processing module (APM) and a 200 µm interaction chamber (IXC) at a pressure of 1,000–1,300 bar. For further treatment, interaction chamber sizes of 87–100 µm were used at a pressure of 1,500-2,000 bar. The pH was adjusted with
diluted HCl to 10 for all butylaminated samples, to 7 for CNF D2, and to 8 for CNC D3. CNF D1 was used without a pH adjustment. The specifications for microfibrillation are presented for all samples in Table 6.

**Table 6. The conditions during microfluidization of pretreated celluloses.**

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Raw material</th>
<th>pH</th>
<th>Passages and pressures in different chamber sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>400 µm APM*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-200 µm IXC**)</td>
</tr>
<tr>
<td>Paper I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNC B1</td>
<td>CP</td>
<td>10</td>
<td>1/1300 bar</td>
</tr>
<tr>
<td>CBC B2</td>
<td>CP</td>
<td>10</td>
<td>1/1300 bar</td>
</tr>
<tr>
<td>CNC B3</td>
<td>CP</td>
<td>10</td>
<td>1/1300 bar</td>
</tr>
<tr>
<td>CNC B4</td>
<td>CP</td>
<td>10</td>
<td>1/1300 bar</td>
</tr>
<tr>
<td>CNC B5</td>
<td>CP</td>
<td>10</td>
<td>1/1300 bar</td>
</tr>
<tr>
<td>Paper II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNC B6</td>
<td>SCP</td>
<td>10</td>
<td>1/1300 bar</td>
</tr>
<tr>
<td>CNC B7</td>
<td>SCP</td>
<td>10</td>
<td>1/1300 bar</td>
</tr>
<tr>
<td>CNC B4</td>
<td>CP</td>
<td>10</td>
<td>1/1300 bar</td>
</tr>
<tr>
<td>Paper III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DES-CNC</td>
<td>DIS</td>
<td>7</td>
<td>3/1300 bar</td>
</tr>
<tr>
<td>Paper IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNF D1</td>
<td>DIS</td>
<td>*</td>
<td>3/1000 bar</td>
</tr>
<tr>
<td>CNF D2</td>
<td>DIS</td>
<td>8</td>
<td>3/1000 bar</td>
</tr>
<tr>
<td>CNC D3</td>
<td>DIS</td>
<td>7</td>
<td>2/1000 bar</td>
</tr>
</tbody>
</table>

*) APM = Auxiliary processing module
**) IXC = Interaction chamber
3.3 Analyses of the fabricated nanocelluloses

3.3.1 Morphology

*Transmission electron microscopy (TEM).*

A transmission electron microscope (Tecnai G2 Spirit, FEI Europe, The Netherlands) was used to determine the morphological characteristics of the nanocellulose (both CNC and CNF) samples (Papers I–IV). Preparation of the samples was performed by first adding a small droplet of diluted nanocellulose suspension onto the Butvar-coated copper grid. Excess water on the samples was then absorbed using a small piece of filter paper. All samples were negatively stained with uranyl acetate (2 w/v %) and allowed to dry at room temperature before conducting the measurements. Standard conditions with 100 kV were used during TEM analysis. A Quemesa CCD camera and iTEM image analysis software (Olympus Soft Imaging Solutions GMBH, Munster, Germany) were used for imaging and image processing of the nanocellulose samples. Nanocellulose widths and lengths were measured from 50–70 individual cellulose nanoparticles, and the averages as well as standard deviations were calculated for each studied specimen.

*Field emission scanning electron microscopy (FESEM)*

Field-emission scanning electron microscopy (FESEM; Zeiss ULTRA plus, Germany) was used to study the morphology and stabilization mechanisms of nanocellulose stabilized o/w emulsions, using polymerized styrene spheres covered by nanocelluloses as model systems (Paper IV). DES-pretreated surface-functionalized nanofibers (CNF D2) and commercial nanocrystals (BGB Ultra) were used at a concentration of 0.2 wt.-% in an o/w emulsion, which was made with sonication. Styrene was polymerized under magnetic stirring at a constant temperature of 75 °C. From that suspension, droplets were sampled by FESEM. A more precise description of the polymerization procedure is presented in Section 3.4.4.
Scanning electron cryomicroscopy (CryoSEM)

Cryo-scanning electron microscopy (Quanta 200 FEG ESEM, equipped with Quorum’s cryo transfer system and stage) was used to illustrate the nanoparticle adsorption and attachment at the soybean-oil droplet. A ready-made nanoparticle-stabilized emulsion (0.5 ml) was dipped into slushed liquid N₂ and transferred to an SEM chamber (-186 °C) for pre-imaging. After 2 hours of sublimation (-70 °C), the sample was coated (30 seconds) with Pt and transferred back to the SEM chamber for imaging (-171 °C) at 10 kV.

3.3.2 Crystallinity

The crystalline structure of the CNCs (Papers I and III) after mechanical disintegration was measured by wide-angle X-ray diffraction (WAXD), using a Siemens D5000 diffractometer (Germany) equipped with a Cu Kα radiation source (λ = 0.1542 nm; Paper I) and a Rigaku SmartLab 9 kW rotating diffractometer (Japan) using a Co Kα radiation source (λ = 0.1790 nm; Paper III). Due to different radiation sources, the cellulose peaks have slightly different diffraction angles. Scans were taken over a 2θ (Bragg angle) range from 5 to 50° at a scanning speed of 0.10–0.17° s⁻¹ using a step time of 5 s. The crystallinity of the samples was determined based on the Segal method (Segal, Creely, Martin & Conrad, 1959). The peak intensity of the main crystalline plane diffraction (I₀₀₂) was located at 22.5° (Paper I) and 26.1° (Paper III). The peak intensity associated with the disordered fraction of the cellulose (Iₘₐ) was observed at 18.0° (Paper I) and 22.0° (Paper III). The degree of crystallinity was calculated according to Equation 2:

\[ CrI = \frac{I_{002} - I_{m}}{I_{002}} \times 100\% \]  (2)

3.3.3 Chemical characterization

Determination of initial aldehyde content

After sodium periodate oxidation, the initial aldehyde contents of the celluloses were analyzed through an oxime reaction. The procedure is described in more detail.
elsewhere by Sirviö et al. (2011). The reaction is based on the fact that one mole of aldehyde in oxidized cellulose reacts with one mole of \( \text{NH}_2\text{OH}\cdot\text{HCl} \), and, after the reaction, the nitrogen content can be determined from a freeze-dried sample by using an elemental analyzer (PerkinElmer CHNS/O 2400 Series II; PerkinElmer, Waltham, MA, USA). The aldehyde content is calculated from the known nitrogen content.

The carboxyl content after oxidation pretreatment

The carboxyl contents of the periodate-chlorite oxidized samples (Papers I & II) and DES-pretreated samples (Papers III–IV) were analyzed before nanofibrillation by conductometric titration, as described by Katz, Beatson & Scallan (1984) and Rattaz, Mishra, Chabot & Daneault (2011).

The determination of amino groups with elemental analysis

The amino group content of the bifunctionalized CNCs (Papers I & II) were determined with the elemental analyzer CHN/O 2400 Series II (PerkinElmer, USA). The nitrogen contents of the samples were analyzed, from which the amino group content could be calculated. The samples were freeze-dried (Scanvac, CoolSafe, Denmark) at the solids content of 0.5 wt.-% for 54 hours (Paper I) and 0.1 wt.-% for 60 hour (Paper II) before analysis.

Diffuse reflectance infrared fourier transform (DRIFT) spectroscopy

Pure, non-modified cellulose (birch) and dialdehyde cellulose (DAC), partially oxidized dialdehyde cellulose (PO-DAC) and bifunctionalized cellulose (CNC B4; Paper I), and DES-pretreated cellulosics (Paper IV) were analyzed with DRIFT spectroscopy. Spectra were collected with a Bruker Vertex 80v spectrometer (USA) from freeze-dried samples in a 600–4,000 cm\(^{-1}\) range. For each sample, 40 scans were taken at a resolution of 2 cm\(^{-1}\).

3.3.4 Surface tension

The traditional du Noüy ring method was used to measure the changes in the static surface tension as a function of concentration of the aqueous nanocellulose suspensions at room temperature (Papers II & IV; du Noüy, 1925). A platinum wire
ring lying in a plane parallel to the undisturbed liquid surface was submerged into the liquid and then slowly withdrawn, while the net fluid force on the ring was measured (Fig. 11). Measurements were taken three times so that the cellulose nanoparticles had enough time to settle before the last measurement, and the average of these measurements was calculated. All measurements were carried out at ambient temperature (20–22 °C).

![Fig. 11. The surface tensiometer.](image)

The force \( F \) that was required to raise the ring from the liquid is related to the surface tension \( \gamma \) of the liquid, in accordance with Equation 3:

\[
F = 2\pi \cdot (r_i + r_o) \cdot \gamma
\]

where \( r_i \) is the radius of the inner ring of the liquid film pulled and \( r_o \) is the radius of the outer ring of the liquid film.

### 3.3.5 Water contact angles of nanocellulose films

The hydrophilicity (i.e., the wettability of the DES-pretreated cellulose nanoparticles CNF D1, CNF D2, CNC D3, and REF-CNC) was evaluated from nanocellulose films via static sessile-drop contact-angle measurements conducted with Milli-Q water as a probe liquid at room temperature. Self-standing films were
prepared from the nanoparticle suspensions by first mixing with Ultra-Turrax mixer at 10,000 rpm for 3 minutes, and then filtering the suspension using a filter membrane (polyvinylidene fluoride, 0.65 μm, Millipore Durapore, France) in a glass filter funnel (diameter: 7.2 cm). The films were dried at room temperature overnight.

During the water contact angle measurement, a CAM 2000 (KSV Instruments Ltd, Finland) system equipped with a high-speed CCD video camera and analysis software was used. The water contact angle (WCA) was determined as a function of time by dropping a water droplet (volume: 6.5 µl) on the film surface. The WCA was measured for 60 seconds, but due to possible bending of the nanoparticle films when wetted, the water contact angle at 5 seconds was used for comparison. The Laplace/Young method was used for the calculations (Yuan & Lee, 2013). For each sample, four droplets were measured in different locations, and the standard deviations were then calculated from the averaged results.

Fig. 12 presents a schematic illustration of the surface wetting and water contact angle. The wetting degree describes the tendency of the liquid to spread across a solid surface. The degree of wetting, measured using the sessile contact angle technique, determines the ability of nanoparticles to be wetted by water (hydrophilicity < 90°).

Fig. 12. Water contact angle (θ) at a solid-liquid-gas contact line. For example, the surface in the left is the most hydrophobic as the contact angle with water exceeds 90°, while the surface on the right is hydrophilic (water contact angle <90°).
3.4 Emulsification of diesel oil in water

3.4.1 The preparation of nanocellulose-stabilized emulsions

To determine the influence of the different CNCs (Papers I–IV) and CNFs (Paper IV) on the emulsion formation and stability of o/w, cellulose nanoparticles in fixed mass proportions were mixed with water to final concentrations between 0.05–0.45 wt.-%. The diesel oil was mechanically dispersed in the aqueous nanocellulose suspension using the UltraTurrax mixer (IKA T25; IKA-Works, Staufen im Breisgau, Germany) at 7,000 rpm for 15 minutes. The oil to water ratio was held at 1:10 throughout the study in Papers I–III and studied as one variable in Paper IV at concentrations of 5, 10, and 20 wt.-%.

To monitor the stability of the emulsions in cold conditions (Paper II), emulsions were prepared using cooled (approximately 5 ± 2 °C) water, and the emulsions were kept in an ice bath to prevent warming during dispersing. Bifunctionalized nanocellulose samples from semichemical pulp and chemical pulp were studied at two concentrations: 0.05 and 0.1 wt.-% in an oil to water ratio of 1:10.

Different background electrolyte concentrations (Papers I–III) were used to illustrate the effect of the electrolytes on dispersion stability and oil droplet size. In Paper I, sodium chloride (NaCl) was used at concentrations of 0–5 wt.-%, which was equivalent to the salinity of 0–880 mmol l⁻¹. The droplet size in the emulsions and the stability performance of two bifunctionalized nanocelluloses, CNC B1 and CNC B4, were studied at 0.1 wt.-% concentrations. In Paper II, synthetic seawater was used to study the effect of a marine-like environment on o/w emulsion stability. Cellulose nanocrystal samples were studied at a low concentration of 0.05 wt.-%. In Paper III, sodium chloride (NaCl) was used as a background electrolyte at a concentration of 1.0 wt.-%, which was equivalent to a salinity of 171 mmol l⁻¹ (o/w emulsions stabilized with 0.1 wt.-% CNC D3 and with 0.1 wt.-% REF–CNC). The stability of the emulsions against coalescence was studied by measuring the mean droplet size in the emulsions and centrifugation at 2,415 G. The kinetics of the creaming phenomenon and droplet coalescence were also examined by measuring the creaming layer volume and analyzing the droplet size changes over 10 weeks.
3.4.2 The stability of the emulsion against creaming and coalescence

An analytical centrifuge (LUMiFuge; LUM GmbH, Berlin, Germany) was also used to detect the o/w emulsion stability at room temperature and in a colder condition (5 °C; Papers I, II & IV). The LUMiFuge measures the changes in transmitted light at an 800 nm wavelength over the whole sample height as a function of time (Fig. 13). The measurement was conducted at 1,200 rpm for 30 minutes. An increase in transmission indicates phase separation of the emulsion droplets and lower stability against creaming. At least two parallel measurements of each sample were analyzed.

![Fig. 13. The working principle of LUMiFuge analytical centrifuge.](image-url)
3.4.3 Oil droplet size distribution

The size distributions of oil droplets in nanoparticle-stabilized o/w emulsions were analyzed with the laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, USA; Papers I–IV). Each studied specimen was measured immediately after mechanical dispersing, and altogether, three parallel measurements were performed. Changes in the droplet sizes (Paper III) were also monitored over six weeks using the particle size analyzer to detect indications of the long-term stability of the emulsion against coalescence.

3.4.4 The mechanisms of o/w emulsion stabilization with nanocelluloses

The ability of cellulose nanofibrils (CNF D2) and nanocrystals (REF-CNC) to adsorb into the oil-water interfaces was studied using polymerized polystyrene as a model compound in Paper IV.

To enable imaging of the nanocellulose-stabilized emulsions, styrene was polymerized via a reaction with benzoyl peroxide, as previously described by Alduncin, Forcada & Asua (1994). In brief, 4.78 g of styrene, 0.12 g of benzoyl peroxide, cellulose nanoparticles (0.04 g abs) equivalent to a 0.2 wt.-% dosage, and deionized water were added to a total weight of 20 g. The styrene/water emulsifications were done by sonicking the suspensions for 2 minutes, and polymerization was carried out under magnetic stirring at a constant temperature of 75 °C. Dilutions were made using the polymerized solutions and deionized water, and the remaining styrene was allowed to evaporate. From the dilutions, small droplets were sampled for FESEM (Zeiss ULTRA plus, Germany) imaging.
4 Results and discussion

4.1 Fabrication of functionalized nanocelluloses

A chemical oxidative method was used to fabricate functionalized and non-functionalized cellulose nanocrystals for o/w emulsion stabilization. Acid-free periodate-chlorite oxidation combined with butylamination was used to produce amphiphilic cellulose nanoparticles from both bleached kraft pulp and lignin-rich semichemical pulp. Moreover, three different variations of DES-pretreatments were used to produce non-functionalized CNFs and functionalized cellulose nanoparticles (i.e., carboxylated CNCs and succinylated CNFs) from dissolving pulp.

4.1.1 Bifunctionalized nanocelluloses

In Paper I, sequential periodate-chlorite oxidation in an aqueous environment was used in combination with butylamination to produce bifunctionalized cellulose nanocrystals with different anionic charge densities from birch pulp (kraft). In the first phase, through periodate oxidation, cellulose fibers were oxidized to obtain oxidized dialdehyde cellulose (PO-DAC), while in the second phase, chlorite oxidation of DAC was conducted to partially convert the aldehyde groups into carboxyls, followed by butylamination of residual aldehydes to obtain amphiphilic features. The periodate-chlorite–based chemical oxidative pretreatment is known to increase the surface charge density and internal repulsion forces within the fibrils, thereby enhancing the disintegration of fibers (Liimatainen et al., 2012). This chemical treatment differs from the traditional acid hydrolysis used in CNC production, as strong acids in large volumes are not used. Individual CNCs were mechanically liberated from chemically oxidized pulp using microfluidization. Since the surface functionalities were introduced prior to liberation of CNCs, no additional post-treatment was necessary after the homogenization phase. In comparison, when CNCs are produced in traditional acid hydrolysis processes, some purification processes are typically needed to get rid of the excess acid. These can include centrifugation, dialysis, or some extraction processes. Sonication is commonly used as a post-treatment to help to improve the dispersion of individual nanoparticles so that a homogenous suspension is achieved. The use of sequential periodate-chlorite oxidation (or DES system) for the production of CNCs
eliminates the need for laborious post-treatments. Liberation of individual crystals and homogenization of the suspension occurs simultaneously during microfluidization.

In Paper II, a periodate-chlorite oxidation procedure followed by butylamination was used to produce surface functionalized, amphiphilic CNCs from the fines fraction of lignin containing semichemical pulp.

**The initial aldehyde, carboxyl and amino group contents**

The carboxyl content of the oxidized chemical (kraft) pulp samples varied between 0.50 mmol g⁻¹ and 1.95 mmol g⁻¹ (Paper I), the highest being CNC B5, which was produced in the harshest periodate oxidation procedure that had a high initial aldehyde content of 3.86 mmol g⁻¹. After the amination reaction, the amino group content was 0.10–0.73 mmol g⁻¹, determining the hydrophobicity level of the CNCs. These contents corresponded to conversions of 3.8–24.0% and 22.7–71.8% in the amination and carboxylation reactions, respectively, since the initial aldehyde contents were 2.20 mmol g⁻¹ and 3.86 mmol g⁻¹, respectively. The balance of carboxyl groups and butylamino groups determines the nanoparticle amphiphilicity and their ability to adsorb into the oil-water interface i.e. their ability to stabilize the o/w emulsion. The high amino group content increases hydrophobicity of nanoparticles and may lead to phase change in emulsion from o/w to w/o (Yang et al., 2017). The residual aldehydes (23.6–53.4%) were converted back to alcohols by the reductive agent that had been used in the amination reaction. The aldehyde and carboxyl contents of the cellulose samples after periodate and chlorite oxidation and the butylamino group contents of all the bifunctionalized nanocelluloses are presented in Table 7.

The initial aldehyde contents of oxidized nanocelluloses from semichemical pulp (Paper II) were similar for both fabricated samples CNC B6 and CNC B7, being 2.43 mmol g⁻¹ and 2.62 mmol g⁻¹, respectively, despite using different chemical doses and temperatures in the oxidation reaction. Semichemical pulp may contain some metals or other impurities that might have affected the oxidation by catalyzing the reaction. A high lignin content may also negatively affect oxidation by consuming the oxidant for reactions of lignin (Gosselink et al., 2011). This assumption is supported by the high aldehyde content of CNC B4, which was fabricated from low-lignin kraft pulp with similar chemistry in the oxidation reaction. Its initial aldehyde content was 32% higher (i.e., 3.86 mmol g⁻¹ vs. 2.62 mmol g⁻¹). The carboxyl contents varied between 0.70–1.60 mmol g⁻¹, slightly higher than those with nanocelluloses made from kraft pulp with
similar chemical oxidation. The conversion rates for semichemical pulp in the carboxylation reaction varied between 28.8%–61.0% and in the amination reaction between 18.7%–35.0%. The reductive agent that had been used in the amination reaction converted the residual, unreacted aldehydes, 36.2% (CNC B6) and 16.4% (CNC B7), back to alcohols.

Table 7. The aldehyde contents of periodate oxidized cellulose, carboxyl contents after periodate-chlorite oxidation, amino group contents after reductive amination reaction and conversion rates of aldehydes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial aldehyde content [mmol g⁻¹]</th>
<th>Carboxyl content [mmol g⁻¹]</th>
<th>Conversion rate of carboxylation [%]</th>
<th>Aminogroup content [mmol g⁻¹]</th>
<th>Conversion rate of amination [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC B1</td>
<td>2.20</td>
<td>0.50</td>
<td>22.7</td>
<td>0.53</td>
<td>23.9</td>
</tr>
<tr>
<td>CNC B2</td>
<td>2.20</td>
<td>1.13</td>
<td>51.4</td>
<td>0.28</td>
<td>12.6</td>
</tr>
<tr>
<td>CNC B3</td>
<td>2.20</td>
<td>1.58</td>
<td>71.8</td>
<td>0.10</td>
<td>4.5</td>
</tr>
<tr>
<td>CNC B4</td>
<td>3.86</td>
<td>1.08</td>
<td>28.0</td>
<td>0.73</td>
<td>18.8</td>
</tr>
<tr>
<td>CNC B5</td>
<td>3.86</td>
<td>1.95</td>
<td>50.5</td>
<td>0.15</td>
<td>3.8</td>
</tr>
<tr>
<td>CNC B6</td>
<td>2.43</td>
<td>0.70</td>
<td>28.8</td>
<td>0.85</td>
<td>35.0</td>
</tr>
<tr>
<td>CNC B7</td>
<td>2.62</td>
<td>1.60</td>
<td>61.0</td>
<td>0.49</td>
<td>18.7</td>
</tr>
</tbody>
</table>

The crystallinity index

The X-ray diffraction patterns showed the characteristic peaks for cellulose I for all bifunctionalized samples, indicating that no rearrangement had occurred to other allomorphs of the cellulose. The crystallinity indexes were calculated for bifunctionalized CNC B1–B5, being 53.3%, 54.6%, 50.1%, 38.6%, and 45.3%, respectively. The initial aldehyde contents of CNC B1–B3 were 2.20 mmol g⁻¹ and for CNC B4–B5 3.86 mmol g⁻¹, respectively. When the initial aldehyde content is high, and a longer reaction time is used, the crystallinity index is typically lower due to the partially destroyed crystal structure. The harsher reaction conditions used in the production of CNC B4, for example, expose the cellulose structure to chain scission and may produce shorter crystals. Moreover, some disordered parts of cellulose might dissolve during the oxidative chemical reaction, thus causing relative changes in the crystallinity index between samples.
The measurements with the DRIFT spectroscopic technique

The DRIFT spectra (Paper I, Fig. 2) revealed the partial conversion of the aldehyde groups into carboxyls, with a new peak at 1,730 cm\(^{-1}\), representing the C=O stretching of aldehyde, and another peak at 1,614 cm\(^{-1}\), confirming the presence of protonated and dissociated carboxyl groups. However, a small aldehyde band was left, confirming that only partial oxidation was conducted. Due to the overlapping, the appearance of an amine bond could not be directly confirmed by DRIFT analysis. However, the disappearance of the aldehyde band was observed from the bifunctionalized CNC B4 spectrum, indicating that the aldehydes were converted to secondary amines or reduced to hydroxyl groups.

Nanoparticle size and morphology

The size and morphology of the bifunctionalized cellulose nanoparticles were visualized with TEM and imaged with a CCD camera. Image analysis was used to average the lengths (L) and the widths (w) of the individual CNCs from the samples (Table 8). The lengths of cellulose nanocrystals varied, depending on the raw material and the oxidation method. The bifunctionalized CNCs fabricated through an oxidation method from kraft pulp possessed the smallest lengths and narrowest length distribution (CNC B1–B5). The TEM images showed that the samples contained mainly individual rod-shaped CNCs (Fig. 14). For example, CNC B1 and CNC B4 were approximately 89 ± 40 nm and 60 ± 40 nm in length, respectively, and 2–4 nm in width. These findings were also supported by earlier results (Visanko et al., 2015).

Table 8. Examples of average lengths and widths of produced nanocelluloses.

<table>
<thead>
<tr>
<th>Nanocellulose</th>
<th>Functionalization</th>
<th>Length [nm]</th>
<th>Width [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC B1</td>
<td>Bifunctionalized</td>
<td>50–130</td>
<td>2–4</td>
</tr>
<tr>
<td>CNC B4</td>
<td>Bifunctionalized</td>
<td>60–70</td>
<td>2–4</td>
</tr>
<tr>
<td>CNC B6</td>
<td>Bifunctionalized</td>
<td>40–100</td>
<td>2–4</td>
</tr>
<tr>
<td>CNC B7</td>
<td>Bifunctionalized</td>
<td>60–400</td>
<td>2–4</td>
</tr>
<tr>
<td>CNF D1</td>
<td>Non-functionalized</td>
<td>up to micrometre(s)</td>
<td>3–5</td>
</tr>
<tr>
<td>CNF D2</td>
<td>Succinylated</td>
<td>up to micrometre(s)</td>
<td>2–3</td>
</tr>
<tr>
<td>CNC D3</td>
<td>Carboxylated</td>
<td>100–400</td>
<td>3–7</td>
</tr>
<tr>
<td>REF-CNC</td>
<td></td>
<td>50–350</td>
<td>3–8</td>
</tr>
</tbody>
</table>
The cellulose nanoparticles produced from semichemical pulp also appeared mainly as crystal-like structure, but some nanofibril aggregates were present in both the CNC B6 and CNC B7 samples. Nanocrystals formed a major part of the produced nanomaterial, but it is most likely that there were also short CNFs because a purification procedure was not conducted. Averaged from the TEM image analysis, CNC B6 had a length from 40 nm to 100 nm and widths from 2 nm to 4 nm, while CNC B7 possessed a wider length distribution (60–400 nm) but had a similar lateral distribution (2–4 nm). Examples of TEM images of bifunctionalized nanocelluloses form semichemical pulp are presented in Fig. 15.

Fig. 14. Examples of TEM images of bifunctionalized nanocelluloses CNC B1–5. (Reprinted by permission from Paper I © 2016 Elsevier)
4.1.2 Nanocelluloses fabricated using deep eutectic solvents

Three different DES systems were utilized as pretreatments of cellulose materials for nanoparticle production. A choline chloride–urea DES was used in the fabrication of non-functionalized cellulose nanofibrils (CNF D1), a urea–LiCl DES with succinic acid anhydride was used to fabricate functionalized nanofibrils (CNF D2), and an oxalic acid–choline chloride DES was used to fabricate nanocrystals (CNC D3). Fig. 16 presents the fabrication stages of nanoparticles with DES – pretreatment. Yields in the production of cellulose nanoparticles in the DESs were 99%, 97%, and 75% for CNF D1, CNF D2, and CNC D3, respectively. The lower yield for CNC D3 when compared to the other specimens was most probably due to the dissolution of disordered regions of cellulose. However, the yield for CNC D3 was higher than typically reported for mineral acid hydrolysis to produce nanocrystals (Sadeghifar, Filpponen, Clarke, Brougham & Argyropoulos, 2011; Wang, Zhao & Zhu, 2014). The carboxyl content of the DES-pretreated specimens was analyzed with conductometric titration and varied from 0.17–0.67 mmol g\(^{-1}\). The succinylated CNF D2 was the only nanoparticle specimen with a notably higher anionic charge of 0.67 mmol g\(^{-1}\). The REF-CNC (BGB CNC) had a carboxylic acid content of 0.15 mmol g\(^{-1}\) — close to that of CNC D3 (0.17 mmol g\(^{-1}\)) and the non-functionalized CNF D1 (0.28 mmol g\(^{-1}\)). Even though the carboxyl
content was low or moderate, it was enough to prevent the aggregation of cellulose nanoparticles during storage.

**Fig. 16. The schematic figure of the fabrication of nanoparticles using DES-system. (From Paper IV, under CC BY 4.0 license.)**

*The crystallinity index*

The crystalline structures of CNCs fabricated through the oxalic acid-choline chloride DES treatment (Paper III) were analyzed using wide-angle X-ray diffraction (WAXD). Based on the analysis, the samples retained their cellulose I structure. The crystallinity indexes were calculated according to Equation 1 for the DES-pretreated sample (CNC D3) and REF–CNC. The crystallinity index (CrI) for CNC D3 was 68.0%. The REF–CNC had a CrI of 67.0%, as well as the original dissolving pulp without any treatment.

*The measurements from the DRIFT spectroscopic technique*

The DRIFT spectra for the original dissolving pulp raw material and the DES-pretreated samples (CNF D1, CNF D2, and CNC D3) along with the REF-CNC spectra (Paper IV, Fig. 2) showed typical cellulose spectra with OH stretching bands at 4,000–2,995 cm⁻¹ and C=H stretching at 2,900 cm⁻¹. In addition, the band at 1,730 cm⁻¹ confirmed the succinylation of CNF D2 and carboxylation of CNC D3, showing the C=O stretching in the carboxylic acid and ester groups. Moreover,
C=O stretching of carboxylate anions was seen at 1,574 cm⁻¹. The spectrum of non-functionalized CNF D1 followed the spectrum of dissolved cellulose, as expected.

**Nanoparticle size and morphology**

The sizes of the DES-pretreated nanocelluloses were evaluated from TEM images with image analysis software, and the average sizes are presented in Table 8. According to the images, both nanocellulose samples CNC D3 and REF-CNC appeared as rod-like cellulose nanocrystals. The nanocrystal samples and commercial reference were 3–7 nm and 3–8 nm in width, respectively, with the lengths being 50–350 nm. The nanocrystals made with DES from dissolving pulp were of the same size as the REF-CNCs, despite the different wood species and production method.

Nanofibril samples (CNF D1 and CNF D2) appeared as flexible and elongated structures, which could not be accurately measured in length. The average lengths were estimated to vary from hundreds of nanometers to micrometers. The widths were 3–5 nm and 2–3 nm for CNF D1 and CNF D2, respectively. The widths of nanofibrils were smaller after the succinylation reaction (CNF D2) compared to non-functionalized CNF D1 (Fig. 17)
Fig. 17. TEM-images of DES-pretreated nanofibril samples CNF D1 (A), CNF D2 (B) and nanocrystal samples CNC D3 (C) and REF–CNC (D). (From Paper IV, under CC BY 4.0 license.)

4.2 Effect of functionalized nanocelluloses on the surface tension and wettability

4.2.1 Surface tension of aqueous nanocellulose suspensions

Surface tension measurements via the Du Noüy ring method of the aqueous nanocellulose suspensions showed the dependency of surface tension on the nanocellulose concentrations. The bifunctionalized CNC B6 and CNC B7 samples were studied at concentrations of 0–0.1 wt.-%, and bifunctionalized CNC B4, DES pretreated CNF D1, CNF D2, and CNC D3 at various concentrations from 0 to 0.5 wt.-%. The results are presented in Fig. 18. In the Paper II, the reduction of 11%
and 26% with bifunctionalized CNC B6 and CNC B7 was achieved at the concentration of 0.1 wt.-%, respectively. Nanoparticle suspensions made with DESs (Paper IV) showed a reduction in surface tension already at low concentrations, but at higher concentrations, the surface tension tended to approach the surface tension of water ($\gamma_{AW} = 72.4$ mN/m at 24 °C). This phenomenon occurred with all cellulose nanoparticles produced in DESs, except the one with non-functionalized choline-chloride urea DES (CNF D1). With other DES pre-treated samples, the surface tension minimums were reached at quite low nanoparticle concentrations. Total decreases of 22%, 8%, and 12% were observed with samples CNF D2, CNC D3, and REF-CN C at concentrations of 0.10, 0.05, and 0.10 wt.-%, respectively.

It has been suggested that the increase of surface tension in higher concentrations is related to particle aggregation or accumulation of nanoparticles at the air-liquid interface. This phenomenon has been studied more with inorganic nanoparticles, but it is assumed that it also occurs with cellulose nanoparticles (Huminic, G., Huminic, A., Dumitrache, Fleaca & Morjan, 2017; Tanvir & Qiao, 2012; Vafaei, Purkayastha, Jain, Ramanath & Borca-Tasciuc, 2009). In addition, the particle sizes also affect the surface tension as reported by (Binks, 2002) or (Tanvir & Qiao, 2012). They suggested that larger particles have a greater effect on surface tension than smaller particles at the same concentrations. This work did not show such dependency, however, the attachment of particles at interfaces would strongly affect the surface tension thus having significant contribution to emulsion stability.
4.2.2 Wettability of cellulose nanoparticles produced in DES

The surface characteristics of cellulose nanoparticles (i.e., the wettability of nanocellulose films) were studied by analyzing the time-dependent water contact angles (WCAs) from self-standing films made from cellulose nanoparticles. Wettability is the ability of a liquid to maintain contact with a solid surface, and it is depending on both materials through intermolecular interactions. Pickering emulsion stabilization is influenced by wettability of surfaces among particle shape and size from which the main parameter controlling the particle interfacial behaviors and the emulsion stability is the wettability of particles. Fig. 19 presents the results of the WCAs from 60 seconds of measuring time. A small decline in the beginning of the measurement is a result of swelling of the film surface, which is typical with nanocellulose samples since the films are somewhat permeable. The decline seemed to be smaller with CNF films, although the cellulose nanocrystals are not supposed to swell at all. In all likelihood, CNC films adsorb the water between the crystals more effectively than CNF films. This phenomenon was reported earlier by (Dankovich & Gray, 2011). In prolonged measurements, however, some swelling of the nanocellulose films is possible; therefore, the water contact angle values at t = 5s were taken into the comparison. The highest WCA, 71°, was measured with CNF D2, meaning it had the lowest hydrophilicity and,
thus, not yet considered hydrophobic. Surprisingly, CNF D1, the non-modified CNF, had the second highest contact angle (66°), although this same phenomenon could already be seen in the surface tension measurements, where this sample reduced the water surface tension from approximately 74 to 54 mN/m. CNC D3 and REF-CNC had contact angles of 55° and 57°, respectively. The aim was to target lowering the hydrophilicity to better interact with oil, but not reaching too high water contact angle, for samples with WCAs above 90° are hydrophobic and start to form w/o emulsions instead of o/w emulsions. The roughnesses of the films was not measured, and some heterogeneity between them might exist due to the fabrication method of films. CNF D2, that had the lowest hydrophilicity levels, was effective in stabilizing the emulsion against creaming. On the other hand, CNF D1, performed poorly in stabilization of emulsion despite of its low hydrophilicity in water contact angle measurements. In general, the water contact angles of DES – pretreated samples were higher compared to those made with oxidative or hydrolytic methods, where dicarboxylic acid films, for example, had a contact angle of 45° and CNC films, hydrolyzed from microcrystalline cellulose (MCC), had a contact angle of 44° (Li et al., 2018; Visanko et al., 2014).

Fig. 19. Time-dependent water contact angles (WCAs) of the self-standing nanocellulose films made from cellulose nanofibrils (CNF D1 and CNF D2) and cellulose nanocrystals (CNC D3 and REF-CNC). (From Paper IV, under CC BY 4.0 license.)
4.3 Stabilization of o/w emulsions with nanocelluloses

4.3.1 The adsorption of nanocellulose at the oil-water interface

In o/w emulsion, the surface-modified cellulose nanoparticles are distributed on the oil surface evenly in a position where hydrophobic groups are protruding toward oil, and the hydrophilic groups are reaching the continuous water phase. Emulsion stabilization with bifunctionalized nanocelluloses, where the hydrophobicity of the cellulose nanoparticles has been tailored by attaching the amino groups to the cellulose backbone, is presented in the schematic illustration in Fig. 20. The bifunctionalized and, therefore, amphiphilic structure is able to seek the oil-water interface, interacting with the oil phase via n-butylamino moieties, while the hydrophilic carboxylic groups mainly protrude toward the aqueous phase. These functional groups are randomly distributed on nanocrystal surfaces, altering the hydrophobicity/hydrophilicity of CNCs, which will clearly promote their attraction toward the oil-water interface.

Typically, an o/w emulsion with neither a nanoparticle stabilizer nor a surfactant will coalesce quickly because there is no hindrance in the oil-water interface that could prevent the separate phases from coming into contact and coalescing. Amphiphilic cellulose nanoparticles are distributed on the oil droplet surface, thus increasing steric and/or charge hindrance and stabilizing the formed droplets at certain sizes. Nanocrystals tend to cover the interface of an oil droplet more densely when shorter crystals are used; longer crystals have a more steric hindrance, and therefore, more porous and multilayer structures are formed (Kalashnikova et al., 2013). Even though the BGB CNCs that were chosen as reference materials are not typical CNCs (Reid, Villalobos & Cranston, 2017), they possess similar charge density and size distribution to CNCs produced through periodate-chlorite oxidation or DES systems with oxalic acid. Thus, they can be used as bases for comparison in emulsion stability studies.
The distribution of cellulose nanoparticles into the oil-water interface

The distribution of cellulose nanoparticles (crystalline REF-CNC and fibrillar CNF D2) was studied with FESEM imaging to investigate the spatial locations of cellulose nanoparticles on the surface of the diesel oil droplet. Due to its high volatility, diesel oil was replaced with polymerized styrene as a model compound to simulate oil droplet surface coverage in an emulsion. Polymerization of styrene resulted in a broad size distribution of beads, ranging from a few hundred nanometers to approximately 50 µm. Fig. 21 shows the polystyrene beads with 0.2 wt.-% cellulose nanoparticles. The cellulose nanoparticles were clearly visible on the surface of the styrene beads, and their distribution on the interfaces can be observed.

It has been suggested that the stable emulsion obtained by using nanoparticle stabilizers results from the combined effect of particles of different sizes. The large particles are more responsible for preventing coalescence, while the smaller particles define the size of the oil droplets (Matos, Marefati, Bordes, Gutiérrez & Rayner, 2017). Here, the rigid nanocrystals (REF-CNCs) formed a smooth, homogeneous layer where individual nanocrystals can be seen on the surface of beads, whereas the succinylated nanofibrils (CNF D2) formed a heterogeneous web-like structure protruding out from the surface of beads. It was also detected that smaller CNCs were able to stabilize very small styrene beads that were attached at the surface of the larger beads. CNF D2 was connecting beads mechanically via a cellulose nanofibril network. Previously, the coverage of cellulose nanoparticles on the droplet surface was found to be attributed to particle length (or aspect ratio)
as well as the flexibility, which our findings with nanofibrils also support (Kalashnikova et al., 2013).

The cryo-SEM imaging of stabilized o/w emulsion

The adsorption mechanisms and interactions between nanoparticles and oil (soybean oil) were also addressed using the cryo-SEM technique. The cryo-SEM images of soybean oil droplets stabilized with bifunctionalized nanocellulose fibrils are presented in Fig. 22. Nanofibrils attached at the oil droplet surface similarly as the model compound (polystyrene), creating a web-like structure on the surface of the oil and between adjacent oil droplets.

The traditional explanation of emulsion stabilization mechanisms using particles (Pickering stabilization) is the accumulation of particles on the oil-water interface, which is clearly seen in Figs. 21 and 22. A dense layer of particles is formed that prevents coalescence and droplet flocculation by steric interactions. In addition to that, when two droplets stabilized by particles come near each other, a physical hindrance, a bilayer, is formed. This thin film between the droplets greatly increases the stability of the droplet against coalescence (Horozov & Binks, 2006). Generally, smaller particles can be packed more closely on the interface, and therefore, it has a positive effect on stability (Matos et al., 2017).
Cellulose nanoparticles distribute on the oil droplet surface, increasing the steric hindrance between droplets (Tang et al., 2014). The surface coverage of cellulose nanoparticles on the oil droplets depends on the particle size, and therefore, the emulsion stability may be enhanced by tuning the size and shape of stabilizing particles. It has been suggested that the most effective emulsion systems contain both large and small particles as stabilizers. The larger particles will improve the steric barrier while the smaller ones adjust the droplet sizes (Matos et al., 2017).

4.3.2 The oil droplet sizes in nanoparticle stabilized emulsions

The changes in oil droplet size of nanocellulose-stabilized emulsions were analyzed using a laser diffraction particle size analyzer. The emulsion stabilization with bifunctionalized CNCs from kraft pulp (CNC B1–B5; Paper I) resulted in a significant reduction in oil droplet sizes compared to o/w non-stabilized emulsions. Without nanoparticle stabilization, o/w emulsions at room temperature were very prone to coalescence, and the measured particle size ranged from 22.8 µm to 73.6 µm, where more than 50% of the droplets were larger than 45.9 µm (Mean value 48.2 µm). In the Paper II, bifunctionalized CNCs from semichemical pulp (CNC B6–B7) at a nanocellulose concentration of 0.05 wt.-%, had the stabilized oil droplet sizes varied from 8.7 µm to 13.6 µm, thus being yet smaller than with stabilization using cellulose nanoparticles made from kraft pulp. Moreover, the low concentration of 0.05 wt.-% was enough to stabilize the droplets in the very small size. High lignin content likely promotes attachment at the oil interface. The studies of Gould, Garcia-
Garcia & Wolf (2016) with lignin-rich coffee as o/w Pickering emulsion stabilizer also support this conclusion. Table 9 summarizes the average droplet diameters with nanocellulose dosages of 0.05–0.1 wt.-%. The emulsification was done at room temperature.

Table 9. Summary of mean oil-droplet diameters (µm) in o/w emulsion with constant 10 wt.-% oil concentration and 0.05 and 0.1 wt.-% nanoparticle concentration. The analysis was done at room temperature.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nanoparticle dosage [wt.-%]</th>
<th>Droplet diameter [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>O/W Emulsion</td>
<td>No stabilizer</td>
<td>48.2</td>
</tr>
<tr>
<td>CNC B1</td>
<td>0.1</td>
<td>16.4</td>
</tr>
<tr>
<td>CNC B2</td>
<td>0.1</td>
<td>13.3</td>
</tr>
<tr>
<td>CNC B3</td>
<td>0.1</td>
<td>16.9</td>
</tr>
<tr>
<td>CNC B4</td>
<td>0.1</td>
<td>9.8</td>
</tr>
<tr>
<td>CNC B5</td>
<td>0.1</td>
<td>13.2</td>
</tr>
<tr>
<td>CNC B6</td>
<td>0.05</td>
<td>11.0</td>
</tr>
<tr>
<td>CNC B7</td>
<td>0.05</td>
<td>8.7</td>
</tr>
<tr>
<td>CNF D1</td>
<td>0.1</td>
<td>28.2</td>
</tr>
<tr>
<td>CNF D2</td>
<td>0.1</td>
<td>11.0</td>
</tr>
<tr>
<td>CNC D3</td>
<td>0.1</td>
<td>15.8</td>
</tr>
<tr>
<td>REF-CNC</td>
<td>0.1</td>
<td>20.2</td>
</tr>
</tbody>
</table>

Table 10 provides a summary of oil droplet sizes studied in Paper IV for samples CNF D1, CNF D2, CNC D3, and REF-CNC. The oil droplet sizes were studied in three different nanoparticle and oil concentrations as wt.-%. A trend could be seen whereby particle sizes decreased when the nanoparticle concentration increased. However, the CNF D1 sample did not follow that trend, but the sampling issues need to be taken into account since the measurement is very sensitive to the sampling procedure. In addition, the increase in oil volume in the constant nanoparticle concentration showed a trend. The oil droplet sizes seemed to grow along with the higher oil concentrations. This observation is in line with the assumption that more cellulose nanoparticles are needed to provide sufficient coverage and prevent droplet coalescence (i.e., the nanoparticle concentration and surface coverage determine the oil droplet size).
Table 10. Mean values of oil droplet size diameters [µm] in stabilized emulsions with different DES pre-treated cellulose nanoparticles (CNF D1–D2 and CNC D3) and diesel oil concentrations (Modified from Paper IV)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oil [wt.-%]</th>
<th>Nanoparticle concentration [wt.-%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>CNF D1</td>
<td>5</td>
<td>12.1</td>
</tr>
<tr>
<td>CNF D2</td>
<td>5</td>
<td>11.7</td>
</tr>
<tr>
<td>CNC D3</td>
<td>5</td>
<td>15.7</td>
</tr>
<tr>
<td>REF-CNC</td>
<td>5</td>
<td>22.5</td>
</tr>
<tr>
<td>CNF D1</td>
<td>10</td>
<td>15.2</td>
</tr>
<tr>
<td>CNF D2</td>
<td>10</td>
<td>15.3</td>
</tr>
<tr>
<td>CNC D3</td>
<td>10</td>
<td>17.4</td>
</tr>
<tr>
<td>REF-CNC</td>
<td>10</td>
<td>26.4</td>
</tr>
<tr>
<td>CNF D1</td>
<td>20</td>
<td>21.0</td>
</tr>
<tr>
<td>CNF D2</td>
<td>20</td>
<td>15.2</td>
</tr>
<tr>
<td>CNC D3</td>
<td>20</td>
<td>21.9</td>
</tr>
<tr>
<td>REF-CNC</td>
<td>20</td>
<td>26.9</td>
</tr>
</tbody>
</table>

In general, all nanocellulose-stabilized emulsions possessed quite small oil droplet sizes. The range of bifunctionalized CNC stabilized droplets was 8.7–16.9 µm, while the DES-pretreated CNCs and CNFs had a range of 11.0–28.2 µm. It has been stated that oil droplets below 70 µm are small enough for microbial degradation, but fast re-coalescing might hinder effective degradation (Lessard & DeMarco, 2000; Prince, 2015). In this study, we found out that even around 50 µm oil droplet size without any stabilizer present, was very prone to coalescing.

The concentration of cellulose nanoparticles was found to affect the oil droplet size of the stabilized o/w emulsions. The effect was observed, for example, in Fig. 23, where the influence of concentrations of CNC B1 and CNC B4 from 0.05 up to 0.45 wt.-% is presented. The smallest droplet sizes (mean 8.9 µm) and the kinetic stability were already achieved in a concentration of 0.1 wt.-% with CNC B4, and an increase in concentration did not decrease the droplet size further. It is assumed that sufficient coverage of oil droplets by cellulose nanoparticles with close packing is needed. However, an excessive amount of cellulose nanoparticles did not decrease the droplet size and, therefore, most probably would not have an effect on emulsion stability. These findings are in line with earlier reports on the role of stabilizers in emulsification (Chevalier & Bolzinger, 2013).
4.3.3 O/w emulsion stability against creaming and coalescence

Density-driven creaming is known to be the most typical route for phase separation in nanoparticle-stabilized o/w emulsions whereas the coalescence phenomenon is a common de-emulsification process that occurs easily without a stabilizer in the emulsion. The investigation of phase separation for the nanoparticle stabilized o/w emulsion was carried out using an analytical centrifuge.

In Paper II, nanoparticle stabilization was studied at room temperature with cellulose nanoparticles fabricated from chemical kraft pulp (CNC B4) and two nanocrystals from semichemical pulp (CNC B6 and CNC B7). The Fig. 24 shows the phase separation of emulsions as indicated by increased transmittance of the emulsion. Moreover, the kinetics of separation is indicated as a slope of transmittance curve. Here CNC B6 and CNC B7 performed better than CNC B4 with concentration of 0.05%, as their final transmission level was lower and the kinetics revealed a slower separation process. The creaming occurred with CNC B4 soon after analysis started, and the transmission was around 50% already after 10 minutes of centrifugation. At that time, the creaming, i.e. the transmission for
semichemical cellulose nanoparticles CNC B6 and CNC B7 was only approximately 15% and 10%, respectively.

In Paper III, the stability of CNC Pickering emulsions was studied using choline chloride-oxalic acid dihydrate DES-fabricated CNCs (CNC D3) in diesel oil-water. The kinetics of natural creaming were monitored for ten weeks with both CNC D3 and REF-CNC at 0-1 wt.-% nanoparticle concentration with 10 wt.-% oil dosage. The changes in oil droplet size were measured weekly from the creaming layer during storage. In addition, the stability against droplet coalescence was analyzed by centrifugation of samples for 5 min at 2415G. As a result of this experiment, it was found that emulsions with REF-CNC resulted in the smallest oil droplet sizes (Table 1; Paper III). The changes in the emulsion creaming when using different CNC dispersants were presented as a function of time (Fig. 4; Paper III). The creaming layer was changed quickly (i.e., packed more tightly) during the first 2 h after emulsification, but after one week, emulsions reached a constant creaming layer. The changes in oil droplet size were measured with an LS particle size
analyzer weekly for six weeks storing. The mechanical barrier created by nanocellulose particles effectively prevented the coalescence since all the emulsions stabilized with either CNC D3 or commercial reference (REF-CNC) had very minimal droplet size changes (Fig. 7; Paper III), and DES-fabricated CNC D3 as a stabilizer was as resistant to droplet coalescence as were the commercial references (Fig. 8; Paper III).

In the Paper IV, the emulsion stability was studied at room temperature in increasing nanoparticle concentrations 0.05–0.20 wt.-% with a constant oil level (10 wt.-%) during all experiments. The Fig. 25 shows, that all samples were prone to creaming that is typical for nanoparticle stabilized emulsions. The phases started to separate soon after emulsification, and a clear phase below the creaming layer was formed with all samples resulting in final transmission around 70–80%. CNF D2 was resisting the creaming phenomenon for the longest time, as the smallest slope of the curve indicated. CNF D2-stabilized emulsion at the highest nanoparticle concentration of 0.2 wt.-% and 10 wt.-% of oil showed the most efficient performance against creaming. The final transmission decreased from 70 to 30 % when the nanoparticle concentration was increased from 0.05 wt.-% to 0.20 wt.-%. CNF D1, CNC D3, and REF-CNCs ended up to final transmittances of approximately 70–80% at all the dosages i.e. they did not prevent creaming despite of similar droplet sizes in emulsion as with the CNF D2. In addition, there was not any difference between the different nanoparticle concentrations.
Fig. 25. The stability of o/w emulsions against creaming as a function of centrifugation (205 G) time. Nanoparticle samples are analyzed at concentrations of 0.05%, 0.1%, and 0.2% with a constant oil dosage of 10 wt-%. (From Paper IV, under CC BY 4.0 license.)

4.4 The background salt and low temperature in emulsion formation

4.4.1 Effect of water salinity on the stability of the o/w emulsion

Various different factors, such as temperature, mixing energy, and electrolyte concentrations (i.e., the salinity) affect the dispersal efficiency in o/w emulsification (Chandrasekar et al., 2006). Especially, background electrolytes affect the stability of emulsions, since the counter ions screen the electrostatic repulsion between surface-charged oil droplets, which promotes droplet coalescence and leads to emulsion breaking. The extent of this effect is associated with the valence of electrolytes, as stated by the Schulze-Hardy rule (Sano, Kamino & Shinkai, 2000).
The background salt effect on the o/w emulsion stability (Paper I) was studied with CNC B1 and CNC B4, using three different salt (NaCl) concentrations – 1.0, 3.5, and 5.0 wt.-% – of which the closest NaCl concentration to the salinity of the natural sea water was 3.5 wt.-%. The effect of the increased background salt concentration on emulsion stability in terms of droplet size is shown in Fig. 26. Both emulsions (0.1 wt.-% of CNC B1 and 0.05 wt.-% of CNC B4) possessed an oil droplet size increase already with the addition of the lower background salt (1.0 wt.-%) dose. However, at the highest salt concentration (5.0 wt.-%), the droplet sizes for CNC B1 and CNC B4 stabilized emulsions were approximately 18 µm and 23 µm, respectively, which was still over 50% smaller than with the o/w emulsion absence the stabilizing cellulose nanoparticles (droplet size 48.2 µm) and electrolytes. This phenomenon indicates that the stabilization effect of bifunctionalized CNCs can be attributed to a combined electrosterical mechanism, since the electrolytes increased the size of the droplets (electrochemical stabilization), but the stability remained high despite the increased background electrolyte concentration (steric stabilization).

Fig. 26. The effect of NaCl increase on the oil droplet size in the o/w emulsion that was stabilized with 0.1 wt.-% CNC B1 or 0.05 wt.-% CNC B4. (Reprinted by permission from Paper I © 2016 Elsevier)
The influence of electrolytes on phase separation of emulsions was evaluated with 0.1 wt.-% CNC B1 and 0.05 wt.-% CNC B4 stabilized emulsions. Fig. 27 presents the transmission of the samples in the absence of background salt (i.e. no salt addition), and presence of 1.0, 3.5 and 5.0 wt.-% of NaCl.

The stability of the reference o/w emulsion without any stabilizing cellulose nanoparticles (“o/w only” in Fig. 27) was poor, as the de-emulsification had already started in the beginning of the analysis, and the final phase separation occurred after 750 seconds of centrifugation, after which a constant transmittance of approximately 80% was obtained. The oil separation was remarkably less pronounced for the CNC-stabilized emulsions, as indicated by the lower final transmittance of the emulsions; however, there were significant differences in the kinetics of the phase separations as a function of background electrolyte concentration. The creaming phenomenon was quite fast and complete when the CNC B1 at 0.1 wt.-% concentration was used compared to the CNC B4, even though it was used at a lower concentration of 0.05 wt.-%.

In the presence of background electrolytes CNC B1 -stabilized emulsions started to separate more quickly than without any salt addition. The phase separation was slower with CNC B4 –stabilized emulsion. This can probably be attributed to the differences in charge densities and the content of the hydrophobic amino groups and their ratios. For example, the charged carboxyl group content of CNC B1 (0.50 mmol g⁻¹) was approximately half of the carboxyl content of CNC B4 (1.08 mmol g⁻¹). Thus, the smaller effective surface charge of CNC B1, which affected droplet stability, was probably screened and reduced by the electrolytes below the value required to prevent droplet coalescence. The emulsions stabilized by CNC B1 seemed to separate more quickly than those with CNC B4 when there were background electrolytes. It is likely that the electrolytes affect the conformation of functional groups, which, in turn, affect the stabilization effects of CNCs.
In the Paper II, the stability of the emulsions in the presence of background salt was studied by preparing the emulsions in the synthetic seawater. Emulsions stabilized with CNC samples (B4, B6, and B7) at a concentration of 0.05 wt.-% were analyzed with stability analyzer. Initial emulsion formation was good for all samples. However, the creaming effect was visually (Fig. 28) observed after emulsification, which was in agreement with the stability analysis (Fig. 29). Still, no signs of droplet coalescence were observed in any of the studied samples. The results showed that the CNC B4 performed best (among the studied samples) in the saline conditions, as the phase separation was the slowest, and the final transmission was approximately 20%. Interestingly, the bifunctionalized cellulose nanoparticles from semichemical pulp (CNC B6 and CNC B7) were not performing in seawater as good as in de-ionized water. The semichemical nanoparticle CNC B7 performed well in the 0.05 wt.-% concentration when de-ionized cold water was used, but the seawater salinity negatively affected the stability of the emulsion. This is probably due to weaker interactions with oil and lignin-rich CNCs in high salinity. The steric barrier is preventing the oil droplets from separating since no coalescence phenomenon was observed.
Fig. 28. Proper o/w emulsion stabilized with 0.1 wt.-% bifunctionalized cellulose nanoparticles (on the left) and same emulsion after creaming (in the middle). The coalescence phenomenon (on the right) occurs with the absence of stabilizing particles (o/w only). (Reprinted by permission from Paper I © 2016 Elsevier)

Fig. 29. Emulsion stability in synthetic seawater containing at nanoparticle concentration of 0.05 wt.-% and a temperature of +5°C. (Reprinted by permission from Paper II © 2018 Springer Nature)
4.4.2 The emulsification at a low temperature

In Paper II, low temperature conditions in emulsion stability were studied by mixing the nanocellulose suspension in +5 °C deionized water and preparing the emulsion in a container, which was placed in an ice bath to maintain the temperature at the desired level. The emulsion was stabilized with 0.05 wt.-% and 0.1 wt.-% nanoparticle (CNC B4, CNC B6, CNC B7) concentrations, while the oil amount was kept at a constant 10 wt.-% during all experiments. The semichemical, bifunctionalized CNC B7 was effective in preventing creaming already at the concentration of 0.05 wt.-% having the highest resistance the creaming of studied samples and the final transmittance being around 10%. The performance was even better here in colder conditions than at room temperature. Both CNC B6 and CNC B7 stabilized the oil in very small particle size but the differences in stabilization ability was most likely due to different carboxyl and amino group content. The emulsion stabilized with CNC B4 had the lowest resistance to creaming (Fig. 30), but it was observed that the addition of cellulose nanoparticles (i.e., increasing the concentration from 0.05 to 0.1 wt.-%) improved the stability, probably due to changes in droplet sizes. Cold temperatures typically improve stability, or vice versa, emulsions are temperature sensitive, and high temperatures expose emulsions to the coalescence phenomenon e.g. due to viscosity decrease and increased Brownian motion.

Fig. 30. The stability of emulsions at cold temperature (+5°C) in de-ionized water using 0.05 wt.-% (left figure) and 0.1 wt.-% (right figure) of cellulose nanoparticles. (Reprinted by permission from Paper II © 2018 Springer Nature)
4.5 Cellulose nanoparticles as a green biochemical in oil spill response

Cellulose nanoparticles provide novel and easily tunable alternatives for biochemical production. In a chemical oil spill response with oil dispersants, the main aim is to make the oil disperse in the water column and remain in small droplets until the microbes reach the oil and start the degradation process (Lessard & DeMarco, 2000). For biodegradation to occur and the microbes to reach the small oil droplets in time, the stability of the dispersed oil droplets is a prerequisite. Thus, effective Pickering stabilization of oil droplets with nanocellulose provides a novel biochemical platform that can be tailored toward oil dispersion and stabilization.

The fundamental challenge in nanoparticle functionalization beyond production costs is controlling the physical and chemical properties of the final product. To end up with a product with consistent quality is yet a distant dream since production has not been optimized, and many studies still need to be done on a laboratory scale. In addition, pilot-scale testing in actual contaminated areas requires permission from authorities. However, the demand for novel, less toxic, and more socially approved methods to overcome the problems from oil spills puts pressure on material development. In this work, the reaction conditions were not optimized yet, so the chemicals, reaction times, and overall production costs are still to be reduced. Oxidative methods will most likely be replaced by more environmentally friendly chemicals and methods if the recycling of the chemicals turns out to be industrially demanding. The results presented here indicate that deep eutectic solvents (DESs) are a very interesting and new technique to pretreat different cellulosic biomasses, and some of their capabilities have already been shown. However, the pretreatment of lignocellulose using DESs has not yet been sufficiently studied. Thus, thorough investigations on the pretreatment mechanisms with a larger number of different DESs should be conducted. For industrial applications, DES recycling studies need to be carried out, even though DESs are considered biodegradable, biocompatible, and sustainable.

Cellulose nanoparticles are studied extensively to find new and efficient materials for enhanced chemical oil recovery. Kalashnikova et al. (2011, 2012, and 2013) first proposed Pickering emulsions stabilized with cellulose nanocrystals (CNCs), and since then, several modifications and surface functionalization of nanocellulose have been reported for oil emulsification and stabilization. The study of nanocellulose for enhanced oil recovery increased drastically when the capability of modified nanocellulose in that application was found. For example,
grafting was used for CNCs to change surface characteristics and make the extraction of oil possible (Tang, Berry & Tam, 2016; Tang et al., 2014; Zoppe et al., 2012). Nanocellulose functionalization and emulsion destabilization mechanisms still need further study since it has been mainly focused on oil droplet sizes and emulsion stability. Latest research which has gained interest has concentrated on the surface charge induced emulsion stabilization which enables the fast emulsion breaking by e.g. salt addition (Elbers et al., 2016; Marina et al., 2018). Destabilization and nanoparticle de-attachment are essential for applications where nanomaterial has to be collected after use.
5 Summary and concluding remarks

Cleaning oil-polluted water resources can be done mechanically, chemically, burning in-situ, or even by using a combination of processes, depending on the quality and volume of the spilled oil within the environmental restrictions. Oil dispersants are effective and widely used chemicals in oil spill response, however, their use is recently questioned because the dispersant-related health symptoms on humans have been reported. Therefore, there is an urgent need for a novel, “greener” oil-spill response method based on natural resources. Awareness of these negative effects among sustainable thinking have accelerated interest in bio-based and non-toxic alternatives for the treatment of oil spills.

Due to its availability and tunability, cellulose is an impressive raw material for the sustainable production of oil spill dispersants and non-soluble particle stabilizers for o/w emulsion. In this study, both cellulose nanofibrils and nanocrystals were produced from wood cellulose using different chemical treatments. The tailored fabrication of cellulose nanoparticles resulted in different functionalities, with cellulose changing its hydrophobicity level to better adsorb and attach to the surface of oil droplet.

Emulsion stabilization with cellulose nanoparticles is based on different mechanisms that are rather complex systems. One of those mechanisms is based solely on cellulose nanoparticles forming a physical protective layer around the oil droplets, thereby preventing coalescence. Other mechanisms are based on electrostatic forces that keep the droplets far from each other so that the coalescence is unlikely to occur. Thus, the efficiency of the stabilization depends on the morphology and the surface functionalities of the used nanoparticle.

Papers I and II included in this thesis concentrated on fabrication on bifunctionalized cellulose nanocrystals from chemical pulp and semichemical pulp. Functional amino groups were introduced to enhance the amphiphilic nature of produced cellulose nanoparticles. When the dispersion of oil was studied, it was noted that bio-based cellulose nanoparticles at low concentrations formed a Pickering-like stabilized o/w emulsion system, which was highly stable against coalescence. In some conditions, such as in lower temperatures, CNC nanoparticles had the ability to slow down creaming, which is a typical phase separation phenomenon in particle-stabilized emulsions. Semichemical pulp as a raw material for bifunctionalized CNCs performed well in the stabilization studies. The lignin-rich material may provide one good resource for fabrication of amphiphilic cellulose nanoparticles since these results indicated good stabilization capability.
without background electrolytes at room temperature. On the other hand, the stability in the presence of electrolytes seemed to be not as good with CNCs made from semichemical pulp as the CNCs made from chemical pulp.

In Papers III and IV, the DES systems were used as reaction media, and different functionalized and non-functionalized cellulose nanoparticles were fabricated and employed as emulsion stabilizers in different concentrations. Although all DES-pretreated samples prevented coalescence, succinylated CNF D2 was especially effective in that sense because it also prevented the creaming phenomena. This was due to heterogeneous web-like structures protruding out from the surface of beads that increase the steric hindrance between droplets. The mechanism for stabilization was different from those of CNCs that formed a smooth, homogeneous layer on the surface of beads.

These results regarding the possibility to use cellulose nanoparticles in the stabilization of oil in diesel oil-water emulsion are promoting further study around bio-based oil-spill dispersant development. The production of nanocellulose dispersants from wood fibers provides an ecological, bio-based alternative to synthetic dispersant manufacture. Using industrial side streams and waste fibers as raw materials in nanocellulose production offer also solution for fiber value loss and even for some waste management problems.
List of references


French, A.D. (2017). Glucose, not cellobiose, is the repeating unit of cellulose and why that is important. Cellulose 24, 4605–4609. https://doi.org/10.1007/s10570-017-1450-3


Original publications


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FUNCTIONALIZED CELLULOSE NANOPARTICLES IN THE STABILIZATION OF OIL-IN-WATER EMULSIONS

BIO-BASED APPROACH TO CHEMICAL OIL SPILL RESPONSE