ABSTRACT

ZOPPE, JUSTIN ORAZIO. Surface Modification of Nanocellulose Substrates. (Under the direction of Orlando J. Rojas and Richard A. Venditti).

Cellulose fibers constitute an important renewable raw material that is utilized in many commercial applications in non-food, paper, textiles and composite materials. Chemical functionalization is an important approach for improving the properties of cellulose based materials. Different approaches are used to graft polymeric chains onto cellulose substrates, which can be classified by two principal routes, namely ‘grafting onto' or ‘grafting from' methods. Never-dried cellulose nanocrystals (CNCs) or nanowhiskers produced from sulfuric acid hydrolysis of ramie fibers were used as substrates for surface chemical functionalization with various macromolecules. In addition, the use of cellulose nanocrystals to reinforce poly(ε-caprolactone) (PCL) nanofibers was studied. Chemical grafting with low molecular weight polycaprolactone diol onto cellulose nanocrystals was carried out in an attempt to improve the interfacial adhesion with the fiber matrix. Significant improvements in the mechanical properties of the nanofibers after reinforcement with unmodified cellulose nanocrystals were confirmed. Fiber webs from PCL reinforced with 2.5% unmodified CNCs showed ca. 1.5-fold increase in Young’s modulus and ultimate strength compared to PCL webs. The CNCs were also grafted with poly(N-isopropylacrylamide) (poly(NiPAAm)) brushes via surface-initiated single-electron transfer living radical polymerization (SI-SET-LRP) under various conditions at room temperature. The grafting process depended on the initiator and/or monomer concentrations used. No observable damage occurred to the CNCs after grafting, as determined by X-ray diffraction. Size exclusion chromatography analyses of polymer chains cleaved from the cellulose nanocrystals indicated that a higher degree of
polymerization was achieved by increasing initiator or monomer loading, most likely caused by local heterogeneities yielding higher rates of polymerization. In addition, the colloidal stability and thermo-responsive behavior of poly(NiPAAm) brushes grafted from nanoparticles of CNCs of varying graft densities and molecular weights was investigated. Halo areas surrounding grafted CNCs that were adsorbed on silica and imaged with an AFM were indicative of the grafted polymer brushes. Aggregation of nanoparticles carrying grafts of high degree of polymerization was observed. The responsiveness of CNCs in liquid medium and as spin-coated films was determined by using light scattering, viscometry and Colloidal Probe Microscopy (CPM). Light transmittance measurements showed temperature-dependent aggregation originating from the different graft densities and molecular weights and a sharp increase in dispersion viscosity as the temperature approached the LCST. The lower critical solution temperature (LCST) of grafted poly(NiPAAm) brushes was found to decrease with the ionic strength as is the case of neat poly(NiPAAm) in aqueous solution. CPM in aqueous media for asymmetric systems consisting of thin films of CNCs and a colloidal silica probe showed the distinctive effects of the grafted polymer brushes on the interaction (repulsive and adhesive) forces. The origin of such forces was mainly electrostatic and steric in the case of bare and grafted CNCs, respectively. A decrease in the onset of attractive and adhesion forces of grafted CNCs films was observed with the ionic strength of the aqueous solution medium. The decreased mobility of polymer brushes upon partial collapse and decreased availability of hydrogen bonding sites with higher electrolyte concentration were hypothesized as main reasons for the less prominent polymer bridging between interacting surfaces. Finally, poly(NiPAAm)-g-CNCs were utilized as a Pickering emulsions stabilizer. All emulsions formed were oil-in-water confirmed by a drop test.
Various drop sizes were obtained as characterized by laser scattering particle size analysis and optical microscopy. Anisotropic colloidal assemblies of grafted CNCs at the oil-water interface were observed in freeze-fractured samples via Transmission Electron Microscopy. Emulsions were stable for over three months at the time of writing this thesis, however rapidly broke above the LCST as determined by rheometry.
Surface Modification of Nanocellulose Substrates

by
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DEDICATION

This dissertation is dedicated first and foremost to my parents. Without them and the decisions they made for the benefit of my sister and I when we were young I would not be in the place that I am today. This is for my mother especially, who spent countless hours homeschooling me for my first eight years. This is also for Rick and Renee, who became part of the family during my teenage years. This is for my sister Desiree and Colin, who have become my best friends through our weekly family nights. And finally, this is for the love of my life Cristina, whose utmost kindness, understanding and patience have helped me in more ways than she can imagine.
BIOGRAPHY

My first chemistry class was at E. A. Laney High School in Wilmington, NC and taught by Mr. Michael Kelly who deeply inspired me. After high school, I went to the University of North Carolina at Wilmington to receive a B.S. in Chemistry and a minor in mathematics with *cum laude* distinction and Honors in Chemistry in 2005. After working for some time at Linde Electronics & Specialty Gases as an Analytical Chemist, I made the decision to attend NC State for my doctorate in the Fall of 2007 as a USDA National Needs Fellow in the Department of Forest Biomaterials.
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LIST OF PUBLICATIONS

This dissertation is a summary of the following publications and each represents one chapter:


Chapter 8: Zoppe, J. O.; Venditti, R. A.; Rojas, O. J.; Pickering Emulsions Stabilized by Cellulose Nanocrystals Grafted with Thermo-responsive Polymer Brushes. *In preparation*.

Publications not included in this dissertation:

1 CELLULOSE STRUCTURE AND POLYMORPHY

Cellulose is a polysaccharide common in all plant materials. It was first discovered and isolated by Anselme Payen and since has been studied for over 150 years. \(^1\) \(^-\) \(^4\) Cellulose is also present in some fungi, bacteria, algae, and tunicates. It consists of repeating monomer units of \(\beta\)-D-\(\text{anyhydroglucopyranose}\) rings linked by \(\beta\)-1,4-linkages forming a linear chain. Each monomer unit contains three hydroxyl functions which are of interest for chemical modification. Each cellulose chain has a reducing end and nonreducing end, which gives it directionality. The reducing end ring can be opened and reduced to an aldehyde group at the C-1 position (see Figure 1.1). \(^5\)

![Cellulose structure](image)

Figure 1.1 Cellulose structure \(^5\)

There are intramolecular hydrogen bonds within an individual chain between the ring oxygen and C-3 hydroxyl group, and between the C-2 and C-6 hydroxyl groups. This fact also contributes to the chain’s linearity. Individual cellulose chains are usually tightly packed together forming microfibrillar units making up a fiber by this strong intra- and
intermolecular hydrogen bonds making up the crystalline or ordered domains. There can also be more loosely packed domains of microfibrils which form the amorphous or disordered regions. In the case of intermolecular hydrogen bonds between cellulose molecules in crystalline regions, it is of a very complex nature. Depending on the native source and treatment of the cellulose, the hydrogen bonding network and molecular orientation can be completely different. These differences give rise to the polymorphy of cellulose. So far, cellulose has been identified to exist in four different forms depending on native origin and treatment, Cellulose I (α and β), II, III, and IV. Differences in crystal structure of these polymorphs are usually determined by X-ray diffraction and each gives a different diffraction pattern.

Cellulose I (α and β) is the native form and depending on its origin, exist in different ratios. For example, tunicates, wood, cotton and ramie fibers have primarily Iβ form, while some algae and bacterial cellulose have primarily Iα. The differences between these two polymorphs lie within the crystal structure and hydrogen bonding pattern, although both have cellulose chains aligned in parallel. Iα has a triclinic one-chain unit cell, whereas Iβ has a monoclinic two-chain unit cell and is actually more stable between the two forms. Iα can be annealed to produce Iβ, but it cannot be reversed. The difference in these polymorphs was first distinguished by IR Spectroscopy and electron diffraction and has been further studied more recently by solid state CP/MAS C\textsuperscript{13}NMR, where higher plant Cellulose I seemed to be different than bacterial and algal cellulose. In the CP/MAS C\textsuperscript{13}NMR spectrum of Cellulose I (α and β) there are significant differences in the multiplet patterns of the C-4 and
C-6 peaks depending on the origin of the Cellulose I polymorph. The crystal structure and hydrogen bonding pattern of Cellulose I (α and β) was later confirmed by Synchotron X-ray and neutron fiber diffraction, where in the latter, the hydrogen atom positions involved in hydrogen bonding were determined from Fourier-difference analysis with hydrogenated and deuterated samples.9, 10 With these methods it was possible to define all atomic spatial coordinates in this crystal structure for the first time. Cellulose I (α and β) both have sheets stacked in a “parallel-up” fashion, meaning in a defined crystallographic coordinate system, the ring oxygen is greater in the z-axis than C-5. To make matters more complex, Cellulose I (α and β) is not the only native form because it has been shown that Cellulose IV exists in some primary walls.2 Also, interestingly, there has been some controversy about some anomalies within the CP/MAS C\textsuperscript{13}NMR spectra of higher plant celluloses compared to algal, bacteria, and tunicates.11 In fact, Atalla and VanderHart suggested that higher plants contain only Cellulose Iβ, and instead of Iα, they claim there is just a distorted form of Iβ below the surface.6 Although, other researchers usually assume that higher plants synthesize both Cellulose Iβ and Cellulose Iα.

When native Cellulose I (α and β) is treated with alkali or dissolved in a suitable solvent and regenerated, the polymorph is converted to Cellulose II.1-4 Suitable solvents include heavy metal-amine complex solutions, copper with ammonia in water, some inorganic salts above 100°C, thiocyanate/amine, LiCl/DMAC mixtures, and NMMO/water systems, among others. The structure of Cellulose II differs from I in that the chains are in an anti-parallel arrangement where reducing and non-reducing end groups alternate in a...
microfibril, yielding a more stable structural arrangement. Thus, it has been found that Cellulose II cannot be converted back to Cellulose I. It is also important to note that the crystal densities of Cellulose II and Cellulose Iβ are almost identical.² Shown in Figure 1.2 is the major supramolecular distinction between cellulose I and cellulose II.⁵

![Figure 1.2](image)

Figure 1.2 (a) Cellulose I and (b) cellulose II.⁵

If Cellulose I (α and β) or Cellulose II is treated with amines or liquid ammonia and then removed, Cellulose III (either IIII or IIII) is formed.¹⁻⁴ However, if either of these forms is placed in high temperature and humidity environment, it will revert back to its parent structure. Further, if Cellulose I (α and β) or Cellulose II is treated with glycerol at ~260°C, Cellulose IV (IVI or IVII) forms. As in the case of Cellulose III, Cellulose IV can also be reverted back to its parent structure. A summary of possible cellulose polymorph transformations is shown in Figure 1.3.
In order to separate and obtain all of the highly crystalline domains of cellulose from the amorphous regions, workers have employed different types of acid hydrolysis. Some variables in this process are the type of acid used, time, and temperature. For example, if one employs sulfuric acid hydrolysis, a more stable aqueous suspension of crystalline cellulose will be obtained because of anionic sulfate groups present on the surface. There are a few types of products made of mainly crystalline cellulose, namely cellulose nanocrystals (CNCs) or whiskers and microcrystalline cellulose (MCC). CNCs are usually produced by either sulfuric acid or hydrochloric acid hydrolysis which hydrolyzes cellulose chains in amorphous regions, leaving rod or whisker-like crystals that the acid has not penetrated.
MCC are usually derived from pulverized wood pulp hydrolyzed with hydrochloric acid to produce a colloidal cellulose powder. Some current uses for MCC are for fillers in foods and for tableting in pharmaceuticals.\(^2\)

### 1.1 REFERENCES


2 NANOCELLULOSE SUBSTRATES

Cellulose fibers, containing crystalline and amorphous domains, can be broken down into crystallite building blocks which exhibit even more unique surface, optical and mechanical properties.\(^{15, 16}\) These rod-like cellulose nanocrystals (CNCs) or nanowhiskers can be produced after acid hydrolysis of the amorphous regions. Typically, sulfuric acid hydrolysis is employed to yield aqueous suspensions electrostatically-stabilized by the negatively-charged sulfate ester groups installed on the surface of CNCs which promote uniform dispersion in water.\(^{17}\) At a certain solids content, typically between 1 to 10\%, this homogeneous suspension self-organizes spontaneously into spectacular liquid crystalline arrangements with a chiral nematic pitch ranging from 20 to 80 µm.\(^{18}\) However, electrostatic forces affect colloidal stability, making suspensions of CNCs sensitive to the ionic strength of the medium.\(^{19}\) In order to prevent aggregation in aqueous and organic media, steric stabilization could be achieved via polymer brushes grafted from CNCs.\(^{20}\) Importantly, the destabilization effect brought about by the presence of electrolytes can be minimized when nonionic polymer chains are grafted.\(^{21}\)

Nanofibrillated cellulose (NFC) is another example of nanocellulose that offers a sustainable material with extraordinary properties that could be utilized by a number of applications due to its biocompatibility, mechanical properties, reactivity, high surface area and transparency.\(^{22-24}\) NFC is produced by high-pressure and high-shear mechanical disintegration and homogenization yielding fibrils of 3-4 nm thickness and several tens of microns in length. The reinforcing potential and transparency of NFC has been shown in
recent studies. Figure 2.1 summarizes the mechanisms of the production of nanocelluloses.

![Diagram of nanocellulose production]

Figure 2.1 Methods of production of nanocelluloses from macroscopic cellulose fibers.

2.1 REFERENCES


3 METHODS OF SURFACE MODIFICATION

Recently, there have been significant efforts in the development of new lignocellulosic materials because of their abundance, biodegradability, low cost, nonfood agricultural economy, and high thermomechanical properties.\textsuperscript{12-14, 28, 29} Cellulose, being the most abundant biopolymer in the biosphere, is an attractive material source due not only to its biodegradability and renewability, but also because of its low density, high strength properties and potential for chemical modification. There is an interest in incorporating CNCs in nanotechnology in the form of nanocomposites with other polymer matrices. Some of the potential applications lie within packaging, paper, wood building materials, and biomedical. These composites can be made in a variety of ways including electrospinning, solution casting, layer-by-layer deposition, and extrusion. One of the challenges in preparing composites with cellulose and a hydrophobic polymer matrix is the low compatibility. One of the possible routes to overcome this is by cellulose surface modification. One of the issues with this route is that the mechanical strength of composites usually decreases with composites made without chemical modification when compared to unmodified cellulose.

Polymer grafting on cellulose can be accomplished by two main strategies, namely, the “grafting-onto” and “grafting-from”.\textsuperscript{30} The grafting-onto method requires attachment of pre-synthesized and well characterized polymer chains onto cellulose hydroxyl groups. However, steric hindrance can prevent optimal attachment because the polymer chains must diffuse through the grafted “brushes” to reach the reactive sites. Therefore, the grafting-onto
method is limited to low surface density grafts. To increase the grafting density, the “grafting from” approach can be employed. In this method polymer chains are formed by in situ polymerization from substrate-immobilized initiators and molecular growth can be achieved by conventional radical, ionic, and ring opening polymerizations.31

Since the mid 1990s,32 living radical polymerizations (LRP) such as atom transfer radical polymerization (ATRP) has been used extensively as a tool for the development of novel macromolecular structures.33 ATRP attributes include its simplicity, “livingness,” cost and robustness. The use of inexpensive copper catalysts and readily-available initiators also makes it an interesting option for surface modification. Surface-initiated ATRP (SI-ATRP) has been shown to be one of the most efficient ways to ‘graft from’ polymer chains or brushes exhibiting high surface grafting density and low polydispersity.34, 35 Most recently, the discovery of a Cu(0) mediated single-electron transfer living radical polymerization (SET-LRP)36, 37 process has streamled great advances in ultrafast polymerizations with high molecular weights at or below ambient temperature with small amounts of catalyst.38-40 The differences between the latter technique and ATRP are derived from the low activation energy in an outer-sphere electron transfer mechanism and the rapid disproportionation of Cu(I) with N-containing ligands in polar solvents (shown in Figure 3.1).41, 42
Within the last year, it has been shown that Cu(I)-mediated ATRP of NiPAAm actually proceeds via a Cu(0)-mediated SET-LRP, and thus more recent efforts in polymerizations of thermo-responsive copolymers via combinations of SET-LRP, ATRP, and click chemistries have surfaced. In addition, Nguyen et al. reported on the SET-LRP of NiPAAm initiated with 2-methylchloropropionate (MCP) in protic and dipolar aprotic solvents. In consequence to previous efforts, a comparison of surface-initiated ATRP and SET-LRP has been carried out.

### 3.1 REFERENCES


4 RESEARCH OBJECTIVES

- The objective of this research was to generate protocol for the surface functionalization of cellulose nanocrystals with 1) hydrophobic and 2) stimuli-responsive polymers. Methods of surface modification including ‘grafting onto’ and ‘grafting from’ were to be evaluated in terms of efficiency and ease of characterization.

- The interest in hydrophobizing CNCs was for purposes of increasing compatibility and surface adhesion between CNCs surfaces and hydrophobic polymer matrices in the form of nanocomposites produced either by casting or electrospinning.

- Hydrophobized CNCs and unmodified CNCs were to be applied as reinforcing agents in a hydrophobic polymer matrix to evaluate and compare thermo-mechanical properties of non-woven nanofiber mats.

- In addition, the ‘grafting from’ approach was to be used to form stimuli-responsive functionalities and define protocol to fully characterize polymer grafts. The use of stimuli-responsive polymer grafts on CNCs was to be evaluated in terms of their self-assembly behavior and surface interaction forces in aqueous dispersions and as thin films.
Finally, stimuli-responsive CNCs were to be applied to Pickering emulsion formulations and their assembly and stability at the oil-water interface was to be evaluated.
5 REINFORCING POLY(\(\varepsilon\)-CAPROLACTONE) NANOFIBERS WITH CELLULOSE NANOCRYSTALS

5.1 Introduction

Manufacture and use of nanofiber-based scaffolds have recently attracted interest in biomedical applications, especially for tissue engineering\(^{50-55}\). One of the main goals in this area is to create biocompatible and biodegradable scaffolds with mechanical and biological properties similar to those of extracellular matrices (ECM) so as to facilitate surgical implant and promote tissue regeneration\(^{56}\). Natural and synthetic polymers have been examined for this purpose, including collagens, chitosan, hyaluronic acid and biodegradable polyesters such as polylactic acid, polyglycolic acid, and polycaprolactone, among others. Synthetic, biodegradable poly(\(\varepsilon\)-caprolactone), or PCL, has been shown to be particularly useful when used in the production of electrospun fibers to mimic ECM\(^{57-59}\). However, there are a few challenges that need to be overcome in the application of PCL, including effects brought about by its hydrophobicity, which can potentially prevent living cell adhesion, mobility and also limit mechanical strength (required to ensure structural integrity)\(^{60}\). In order to reduce its surface energy, PCL is usually blended with hydrophilic polymers, thereby facilitating cell adhesion\(^{54}\). On the other hand, improvement of the mechanical and thermal properties of PCL-based scaffolds can be accomplished by reinforcing them with suitable fillers\(^{61}\).

Recently, efforts to increase mechanical properties of PCL nanofiber webs have been reported by addition of carbon nanotubes (CNTs)\(^{62, 63}\). Although introduction of CNTs
yielded composite fibers with increased mechanical properties, manufacture protocols required demanding processing conditions \textsuperscript{64}; therefore, it is anticipated that such types of composites can be quite costly. In addition, CNTs possess some degree of toxicity (in \textit{vivo} and \textit{in vitro}), predominately due to the presence of transition metal catalysts and have been shown to cause some cytotoxicity at elevated concentrations \textsuperscript{65}.

Use of biological materials such as proteins from egg shells have been also reported as a reinforcing agent for PCL-based nanofibers \textsuperscript{66}. Though the mechanical and interfacial properties of the composites were improved, the incorporation of such soluble polymers in the PCL matrices required special manufacturing protocols.

Incorporation of cellulose nanocrystals (CNCs) into polymer nanofibers would be advantageous over CNTs because the precursor cellulose in CNCs is obtained from an abundant bioresource. Also, CNCs are less expensive, simple to produce, and, most importantly, are biocompatible and biodegradable. Excellent chemical and thermomechanical properties of CNCs have been reported \textsuperscript{14, 67}, making them suitable candidates as a reinforcing, disperse phase in polymer matrices \textsuperscript{15, 68-70}. CNCs can be produced from a variety of cellulose sources including wood, cotton, sisal, ramie, tunicate, fungi or bacteria \textsuperscript{3}. Bacterial CNCs have been successfully incorporated into hydrophilic poly(ethylene oxide) nanofibers, and an increase in modulus, strength, and strain at break were observed \textsuperscript{66}. Recently, we have successfully used CNCs as a reinforcing phase in composites made from synthetic hydrophobic matrices \textsuperscript{71}. 

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In this work we propose a novel combination of biodegradable CNCs from natural fibers as reinforcing material in PCL nanofibers via electrospinning. The resulting composites were developed to overcome the otherwise low mechanical strength of neat PCL nonwovens. The grafting of low molecular weight polycaprolactone diol chains onto the surfaces of cellulose nanocrystals and their incorporation in electrospun PCL matrices were also investigated. The effect of such functionalization was addressed in an effort to improve their compatibility with the continuous phase. Finally, the main structural and thermomechanical features of the developed composite nanofibers were addressed for various CNCCs loadings.

5.2 Experimental

5.2.1 Materials and Methods

*Materials.* Pure ramie fibers were obtained from Stucken Melchers GmbH & Co. (Germany). Toluene 2,4-diisocyanate (2,4-TDI, 95%), polycaprolactone (PCL, 80,000 g.mol\(^{-1}\)), polycaprolactone diol (PCL, 2000 g.mol\(^{-1}\)), toluene (anhydrous, 99.8%) and dichloromethane (DCM, 99.5%), were all obtained from Sigma-Aldrich. Sulfuric acid (95%), acetic acid (glacial), triethylamine (TEA, 99.5%), acetone (99%), N,N-dimethylformamide (DMF, 99%), sodium hydroxide pellets, and sodium chlorite were all purchased from Fischer Scientific. All solvents were dried over molecular sieves (3 Å, 4-8 mesh beads, Sigma-Aldrich) for 48 h before use.
Cellulose nanocrystals. The procedure for preparing ramie CNCs was adapted from Habibi et al.\textsuperscript{72} In short, ramie fibers were first treated for 2 hours with a 4 wt\% NaOH solution at 80 °C to purify cellulose fibers from residual additives. The purified ramie fibers obtained after repeating the previous procedure were then acid hydrolyzed with 65 wt\% sulfuric acid (55 °C, 45 minutes). The resulting dispersion was filtered into ~500 g ice cubes and washed with distilled water until neutral pH by successive centrifugations at 12,000 rpm at 10 °C for 20 minutes. Finally, dialysis for one week against deionized water with a 3,500 MWCO dialysis membrane was performed to remove trace amounts of residual sulfuric acid.

Surface Grafting. CNCs from the above procedure were grafted with PCL diols and also used to manufacture the composite nanofibers. Grafting PCL onto CNCs was performed according to a slightly modified procedure reported by Habibi et al.\textsuperscript{70} using TDI as coupling agent. 2 g of ramie CNCs were solvent-exchanged from water to acetone (three times) and then to toluene and to anhydrous toluene by using successive centrifugations (12,000 rpm at 10 °C for 20 minutes). The resulting CNCs were placed in 100 mL anhydrous toluene in 250 mL flask equipped with reflux condenser under argon gas and heated to 75 °C while vigorously stirred. 2 mL of triethylamine (TEA) was added followed by drop-wise incorporation of 12.3 mmol of toluene 2,4-diisocyanate (2,4-TDI). The mixture was left to react for 24 hours at 75 °C and then centrifuged three times with anhydrous toluene and once with acetone to remove unreacted TDI. 24.6 g PCL diol and 2 mL TEA were dissolved in 100 mL anhydrous toluene in a 250 mL flask equipped with reflux condenser under argon atmosphere and heated to 75 °C and then, 2,4-TDI modified CN Cs, prepared previously,
were added and the reaction was left to proceed for 5 days while stirring. The reaction mixture was then centrifuged with toluene (three times) and dried overnight under vacuum at 40 °C. The resulting PCL-modified CNCs were Soxhlet extracted with dichloromethane to remove ungrafted PCL diol, and dried in vacuum oven overnight at 40 °C. The grafted CNCs were hydrophobic and dispersible in DCM.

Preparation and characterization of the dispersions.

1. Dispersion of unmodified cellulose nanocrystals in PCL: Aqueous dispersions of unmodified CNCs were solvent exchanged from water to DMF by rotary-evaporation. First, aqueous dispersions were concentrated in a rotary evaporator, then a volume of DMF was added and the remaining water was subsequently evaporated to a desired final volume of DMF. Desired amounts of unmodified CNCs in DMF were sonicated for a few minutes using an OMNI-Ruptor 250 Ultrasonic Homogenizer before adding them to PCL solutions. 15 g of PCL was dissolved in DCM, and then freshly sonicated CNC dispersions were mixed to produce dispersions that lead to CNCs loadings of 2.5, 5.0, and 7.5% in PCL. These dispersions (up to CNCs content of 7.5%) were found to be colloidally stable over long periods of time.

2. Dispersion of grafted cellulose nanocrystals in PCL: Hydrophobic, PCL-grafted CNCs were also dispersed directly in DMF. Desired amounts of PCL-grafted CNCs in DMF were sonicated as previously described before adding them to PCL solutions. 15 g of PCL was dissolved in DCM, and then freshly sonicated CNC dispersions were mixed to produce
dispersions that lead to CNCs loadings of 2.5 and 5.0% in PCL. In this case, the dispersions were only colloidally stable over long periods of time up to CNCs content of 5.0%.

3. Characterization of the CNC dispersions: The viscosity, conductivity, and surface tension of the final dispersions were determined at 25°C by a programmable rheometer (AR2000, TA Instruments), conductivity meter (Corning Inc., model 441), and a Noüy ring (Fisher tensiomat, model 21), respectively (see Table 5.1).

<table>
<thead>
<tr>
<th>Samples</th>
<th>CNC Content (w/w)</th>
<th>Viscosity (Pa.s)</th>
<th>Conductivity (µS/cm)</th>
<th>Surface Tension (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat-PCL</td>
<td></td>
<td>0.59</td>
<td>0.31</td>
<td>32.5</td>
</tr>
<tr>
<td>PCL with unmodified CNC 2.5%</td>
<td></td>
<td>2.41</td>
<td>0.97</td>
<td>33.6</td>
</tr>
<tr>
<td>5.0%</td>
<td></td>
<td>1.21</td>
<td>4.96</td>
<td>33.1</td>
</tr>
<tr>
<td>7.5%</td>
<td></td>
<td>0.96</td>
<td>0.22</td>
<td>32.5</td>
</tr>
<tr>
<td>PCL with PCL-grafted CNC 2.5%</td>
<td></td>
<td>0.42</td>
<td>2.82</td>
<td>32.2</td>
</tr>
<tr>
<td>5.0%</td>
<td></td>
<td>0.62</td>
<td>3.07</td>
<td>32.9</td>
</tr>
<tr>
<td>7.5%</td>
<td></td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Electrospinning. Nonwoven nanofiber webs of neat PCL and PCL reinforced with (unmodified and PCL-grafted) ramie CNCs were produced via electrospinning. The
strength of the electric field used during electrospinning was varied from 0.5 to 1.0 kV/cm between a positive electrode attached to the tip of a syringe needle connected to a pump and a grounded collector plate. Ramie CNCs were dispersed in 15% (w/w) PCL solution (4:1 DCM:DMF solvent) at loads corresponding to 2.5, 5.0, and 7.5% (based on total mixture weight). Freshly prepared dispersions were electrospun with varying flow rates (0.8 to 1.0 mL/hr) and tip-to-collector separations (10-20 cm). The produced nanofiber webs were collected during processing times that were varied between 30 minutes and 4 hours, depending on the desired web film thickness.

*Transmission Electron Microscopy.* Drops of aqueous dispersions of unmodified cellulose nanocrystals (0.01% w/v) were deposited on carbon-coated electron microscope grids (Protochips Inc.), negatively stained with uranyl acetate and allowed to dry. The grids were observed with a Hitachi HF-2000 transmission electron microscope (TEM) operated at an accelerating voltage of 80 kV.

*Scanning Electron Microscopy.* A Hitachi S-3200N variable pressure scanning electron microscope (SEM) was used to image the nanofiber webs formed after electrospinning. The nanofibers, which were collected on aluminum foils placed on the collector, were shadowed with a layer (~150 Å thick) of gold-palladium and observed under a microscope working distance between 3 and 60 mm using an accelerating voltage from 0.3 to 30 kV. Nanofiber diameter distribution was estimated using UTHSCSA Image Tool for Windows freeware and by manually measuring individual diameters across the image. Size distribution
measurements were conducted for each sample considering ca. 40 nanofibers from 10,000X images (shown in Figure 5.5).

**Differential Scanning Calorimetry and Thermogravimetric Analysis.** Differential scanning calorimetry (DSC) was performed using a TA Instruments DSC Q100. In a typical experiment ca. 10 mg of sample was placed in the DSC cell and heated from -100 to 100 °C (heating rate of 10 °C min⁻¹). The melting temperature \( T_m \) was taken as the onset temperature of the melting endotherm. For crystallization studies, the sample was first heated to 100 °C and kept at this temperature for 5 min to ensure complete melting of PCL and then cooled down to 0°C at a cooling rate of 10 °C min⁻¹. The crystallization temperature \( T_c \) was taken as the peak of the crystallization exotherm. Thermogravimetric analyses (TGA) were performed using a TA Instruments TGA Q500. Data was collected after placing ca. 10 mg of sample in a clean platinum pan and heating from ambient temperature to 600 °C (heating rate of 20 °C.min⁻¹).

**Dynamic Mechanical Analysis.** Dynamic mechanical analysis (DMA) was performed in tensile mode (TA Instruments Q800). The measurements were carried out at a constant frequency of 1 Hz, strain amplitude of 0.03 %, for a temperature range of -100 to 100 °C, using a heating rate of 3 °C.min⁻¹ and a gap between jaws of 10 mm. Non-linear deformations (tensile tests) were obtained in controlled force mode at a temperature of 25 °C and ramping at 3 N/min. The DMA samples consisted of 6-mm strips cut from the respective nanofiber webs. Three samples were used to characterize each material.
5.3 Results and Discussion

5.3.1 Characterization of Cellulose nanocrystals

Cellulose nanocrystals were produced by sulfuric acid hydrolysis from ramie fibers. The morphology of these rod-like nanocrystals was characterized by TEM, as shown in Figure 5.1. The CNCs dimensions, which were typically 3-10 nm in diameter and 100-250 nm in length, agreed well with the literature values reported for ramie CNCs produced under the same conditions\textsuperscript{70,72}.

![Figure 5.1 TEM image of ramie cellulose nanocrystals.](image)

One of the challenges in preparing composites with hydrophilic CNCs in hydrophobic polymer matrices is the low interfacial compatibility that may lead to poor composite mechanical strength. To overcome this issue, surface chemical modification of CNCs with
polymer grafts of same chemical nature as that of the matrix was carried out. Polymer grafting can be accomplished either by “grafting-onto” 74 or “grafting from” 75 approaches. In this work, we evaluated the effect of grafting low molecular weight PCL diol onto the ramie CNCs. Surface modification was carried out following the “grafting-onto” method using 2,4-diisocyanate (2,4-TDI) as a coupling agent after a slight modification of the procedure reported by Habibi et al. 70. The first step required the reaction of the surface hydroxyl groups of CNCs with one isocyanate functionality of 2,4-TDI (to produce TDI-g-CN). During the second step, the unreacted second isocyanate of 2,4-TDI was then reacted with the end hydroxyl groups of PCL diol to graft the PCL chain onto the nanocrystals. The isocyanate functionality at the 4 position is reported to react first as this position is seven times more reactive than the one at 2 position 76.

The accomplished surface grafting was confirmed by FTIR (see Figure 5.2). First, the grafting of the coupling agent TDI at the surface of CNCs was confirmed by the presence of new peaks corresponding to the carbonyl stretch, amide stretch, and the amine bend for grafted 2,4-TDI (at wavenumbers of, 1710, 1596, and 1537 cm⁻¹, respectively), in comparison with the spectrum of unmodified ramie CNCs. The peak at 2270 cm⁻¹ was attributed to the unreacted isocyanate functionality. After the reaction of TDI-g-CN with PCL diol, the same distinctive peaks were seen as those for the grafted TDI, however a new and intense peak at 1720 cm⁻¹ was observed and ascribed to the ester carbonyls in PCL chains. It was also noted that there was a significant decrease in the intensity of the isocyanate stretch at 2270 cm⁻¹, confirming attachment of PCL diol.
Successful PCL grafting onto CNCs was also verified by TGA, as shown in the thermograms of freeze-dried unmodified and PCL-grafted CNCs (see Figure 5.3).
thermogram of unmodified CNCs showed the typical degradation profile of CNCs with sulfate groups introduced during fiber hydrolysis with sulfuric acid (see Figure 5.3A). The differential thermogram with a peak around 300°C (see Figure 5.3B) corresponded to cellulose degradation, which typically occurs between 250°C and 325°C due to its depolymerization, dehydration and decomposition of glycosyl units followed by the formation of a charred residue. The small shoulder above 325°C was ascribed to the oxidation and breakdown of the charred residue to lower molecular weight gaseous products. Compared to unmodified CNCs, the thermogram of PCL-grafted CNCs showed distinct degradation behaviors (see Figure 5.3A). The peak around 375°C observed in Figure 5.3B was attributed to the degradation of grafted PCL chains which corresponded to the thermogram peak of neat PCL diol that was used as a reference (Figure 5.3B). Two additional peaks at 275 and 325°C were observed in the thermogram of PCL-grafted CNCs. These peaks were related to the effect of unreacted isocyanate linked to CNCs (see Figure 5.2) and grafted PCL diol onto the CNCs, respectively.
From the weight loss recorded with TGA for PCL-grafted CNCs, the grafting yield was estimated to be 30%, which is in agreement with the weight gain determined after CNC grafting by analytical gravimetry. From this value and with the amount of available hydroxyl groups (carbon 6 of glucopyranose) on the surface of CNCs, the grafting density was calculated to be ca. 4.5%. This low grafting density is typical of “grafting onto” methods which are limited due to high steric hinderance and entropy considerations.

5.3.2 Production and characterization of composite nanofibers

Nonwoven nanofiber webs of neat PCL and PCL reinforced with 2.5, 5.0, and 7.5% (w/w) unmodified and PCL-grafted ramie CNCs were produced via electrospinning. The operating conditions for the production of these nanofibers were thoroughly investigated. The optimum voltage range was found to be between 10 and 15 kV at a distance between the syringe tip and the collector of 15–20 cm (corresponding to an electric field strength of 0.65 to 0.75 kV/cm). The nanofibers were collected on an aluminum foil and the morphology of
the nanofiber webs were characterized by SEM. Figure 5.4 shows typical images of the collected PCL nanofiber webs with and without CNC reinforcement. The corresponding fiber diameter histograms are given in Figure 5.5.
Figure 5.4  SEM images of nanofiber webs produced by electrospinning at a electrical field strength of 0.65 kV/cm and a flow rate 1.0 mL/hr. Shown are neat PCL nanofibers (A) and PCL nanofibers reinforced with unmodified CNCs at weight loads of 2.5 (B), 5.0 (C) and 7.5% (D). PCL nanofibers reinforced with grafted-CNCs at 2.5 (E) and 5% (F) are also included.
Figure 5.5 Diameter frequency distribution of nanofibers of neat PCL obtained at a electrospinning flow rate of 0.8 mL/hr and electrical field strength of 0.65 kV/cm (A) and 0.75 kV/cm (B). The histograms corresponding to PCL reinforced with 2.5% (C), 5.0% (D) and 7.5% (E) ramie CNCs (at 0.65 kV/cm and 0.8 mL/hr) are also included.
Figure 5.4A shows a typical SEM image of neat PCL nanofibers produced under the stated conditions. The fibers were homogenous and their diameters were in the nanoscale range. Some anomalies such as beads and/or diameter engrossment within single fibers were observed, which are typical of these systems 73. By varying the electric field strength from 0.65 to 0.75 kV/cm a slight shift in distribution towards smaller fiber diameters was observed (see Figures 5.5A and 5.5B, respectively). A reduction in average fiber diameter from 210 nm (0.65 kV/cm) to 180 nm (0.75 kV/cm) was observed. This reduction in fiber diameter can be explained by the electrically-induced stretching of fibers during the electrospinning process, as reported in other systems 78.

Figures 5.4B-D show PCL nanofibers reinforced with 2.5, 5.0, and 7.5% (w/w), never-dried, unmodified CNCs. The corresponding diameter frequency distribution is reported in Figures 5.5C-E. All CNC-loaded PCL fibers were nanometric in size and their surfaces appeared to be smooth, except those filled with 7.5% CNCs. By adding 2.5% of CNCs, thinner fibers were produced, as compared to unfilled PCL nanofibers. In fact, the diameter of the nanofibers produced under the same conditions decreased from 210 to 120 nm for neat and 2.5% PCL-reinforced fibers, respectively. This observed reduction in diameter with the (low level) addition of reinforcing CNCs can be attributed to the effect of increased viscosity of the latter dispersion during electrospinning, as reported in Table 5.1. Although the viscosities of all dispersions were greater than that of neat PCL solutions, a decrease in viscosity was observed relative to unmodified CNC loadings. This effect may be attributed to the formation of CNCs aggregates in the case of high loads, which were not
apparent by visual inspection. In addition, the ionic strength of the electrospinning solution brought about by the negatively-charged CNCs, from the sulfate groups grafted during the hydrolysis process, could also contribute with the reduction in diameter of the electrospun nanofibers. This is because the increase in electrostatic charge density of the electrospinning solution induces more extensive filament stretching during jet whipping. However, as the CNC concentration increased the effect of the charge density on the produced nanofiber diameter was offset by a lower dispersion viscosity (see Figures 5.5D and 5E where larger fiber diameters are observed). According to the aspect ratio of the used CNCs, the percolation threshold was calculated to be around 3 %. Above this concentration the likelihood of CNC aggregation is expected to increase and therefore the fiber diameter may increase. Accordingly, the contribution of CNCs overlapping adds to the effect described earlier to produce larger fiber diameters (see Figures 5.5C and 5.5E).

Figures 5.4E and 5.4F show SEM images of electrospun nanofiber webs of PCL reinforced with 2.5 and 5.0% PCL-grafted (ramie) CNCs produced at electrospinning conditions corresponding to those found as optimum in the case of neat PCL. While the general aspect and morphologies of the unmodified CNC-loaded PCL nanofibers were preserved compared to neat PCL nanofibers, the addition of PCL-grafted CNCs to the PCL matrix produced different morphological features. Despite the fact that a precursor random nanofibrillar structure seemed to take place (see Figures 5.4E and 5.4F), the nanofibers were annealed and formed a more continuous film with many, broad nodes. Therefore, the diameter size distribution of nanofibers could not be evaluated and compared to other
This phenomenon can be explained by three main reasons. First, suspension instabilities caused aggregation by the reduction in the polarity of the dispersion. Second, the electrospinning conditions were optimized for the high molecular weight PCL matrix (which also were suitable for processing PCL with unmodified CNCs). In the case of CNC grafted with short PCL chains the required conditions to preserve nanofiber integrity is expected to be different due to the different respective rheological behavior. Finally, the different morphologies observed for composite fibers with grafted-CNC can be the consequence of different crystallization rates for the different PCL chains (those of the PCL matrix and the grafted chains on CNCs). Such features in crystallization rate are expected to occur due to the different molecular masses (80,000 from PCL matrix and 2000 for the PCL grafts). As a consequence, the solution jet from the high molecular weight PCL matrix was elongated and solidified rapidly after spinning from the syringe tip while the grafted chains on CNCs may have taken more time to crystallize and probably did not elongate during the electrospinning process.

5.3.3 Thermomechanical properties of PCL-based fiber nanocomposites

In order to determine the reinforcing effect of CNCs and PCL-grafted CNCs on the thermal and mechanical properties of PCL nanofibers, DSC and DMA tests were conducted. Table 5.2 shows melting temperature ($T_m$), degree of crystallinity ($\chi_c$), and crystallization temperature ($T_c$) for all composite fibers studied. An increase in the degree of crystallinity ($\chi_c$) was observed when comparing bulk, as-received PCL and electrospun webs from the same polymer (0.51 and 0.55, respectively). The corresponding melting temperatures ($T_m$)
slightly decreased (from 53.4 to 52.2 °C), as expected. Based on these results, it is hypothesized that the electrospinning process restrained the mobility of the polymer chains in such a way that more but smaller PCL crystallites were formed within the electrospun nanofibers.

Table 5.2 Summary of DSC results for commercial PCL, PCL nanofiber webs after electrospinning (ES) with and without reinforcing ramie CNCs (unmodified and PCL-grafted).

<table>
<thead>
<tr>
<th>Samples</th>
<th>CNCs Content</th>
<th>Tm</th>
<th>ΔH</th>
<th>Xc(*)</th>
<th>Tc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial PCL</td>
<td></td>
<td>53.40</td>
<td>80.30</td>
<td>0.51</td>
<td>26.02</td>
</tr>
<tr>
<td>ES-PCL</td>
<td></td>
<td>52.20</td>
<td>86.00</td>
<td>0.55</td>
<td>28.07</td>
</tr>
<tr>
<td>ES-PCL with unmodified CNC</td>
<td>2.5%</td>
<td>53.23</td>
<td>79.81</td>
<td>0.52</td>
<td>38.85</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
<td>53.71</td>
<td>79.56</td>
<td>0.53</td>
<td>37.34</td>
</tr>
<tr>
<td></td>
<td>7.5%</td>
<td>53.13</td>
<td>77.97</td>
<td>0.54</td>
<td>35.47</td>
</tr>
<tr>
<td>ES-PCL with PCL-grafted CNC</td>
<td>2.5%</td>
<td>52.20</td>
<td>70.19</td>
<td>0.46</td>
<td>32.79</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
<td>51.80</td>
<td>70.53</td>
<td>0.47</td>
<td>33.32</td>
</tr>
<tr>
<td></td>
<td>7.5%</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

(*) $X_c = \Delta H/\Delta H_{100%}*(1-w)$, where $\Delta H_{100\%\ PCL} = 157 \text{ J/g}$

Addition of CNCs into PCL produced minimal changes in the thermal properties of the electrospun fibers. Only small differences in the melting temperature ($T_m$) as well as the degree of crystallinity were observed when unmodified CNCs were used as reinforcement. A larger $T_m$ and degree of crystallinity was expected since in the case of composite cast films CNCs acted as nucleating agents in the crystallization of PCL $^{81,82}$. In associated, very slow
processes, the lattice structure and the differences in chemical composition of CNCs and PCL were beneficial for the matrix to nucleate on the surface of CNCs. It was observed that in cast films the matrix crystals nucleated epitaxially on the surface of the added CNCs and crystal growth occurred radially, relative to the axis of CNCs. Therefore, a transcrystalline layer was formed. In contrast, during electrospinning the very high shear stress and the very fast solvent evaporation induced rapid crystallization before the polymer nanofibers reach the collector. This fast process, as well as the volume restriction within the nanofibers, was a possible cause for restricted crystallite growth.

In contrast to the case of unmodified CNCs, a reduction in the degree of crystallinity and melting temperature of PCL reinforced with PCL-grafted CNCs was observed. This fact can be attributed to the effect of grafted, low molecular mass chains on CNCs that impairs the formation of PCL crystallites during electrospinning. This is consistent with observations made for cast films with similar composition; it was observed that grafting interrupted the nucleation effect of CNCs. Also, it can be argued that during the electrospinning the crystallization of the grafted short chains was delayed. This last phenomenon was confirmed by the observed crystallization temperature of the nanofiber webs: significant differences in crystallization temperatures were evident, as seen in Table 5.2 and in agreement with the results reported in the case of cast films 81, 82. Similar Tc values measured for cast films and electrospun webs are noteworthy because in the latter case the nanofiber webs melted and crystallized more like a cast film. In contrast, and as a confirmation of the previous hypothesis, an important increase in Tc was observed for electrospun PCL filled with
unmodified CNCs. In fact, the $T_c$ of nanofiber webs shifted significantly (by 11 °C), after loading PCL with unmodified CNCs.

The mechanical properties of nanofibers have important roles in many applications, including mechanical support in cell growth and migration in scaffolds. However, the mechanical properties of neat PCL electrospun fiber webs are known to be deficient. Several studies have explored various blends and/or reinforcing systems, and various processing parameters and post-processing treatments have been proposed to improve associated mechanical properties\textsuperscript{57, 58, 60, 66}. Electrospun fibers form nanostructured networks that are expected to influence mechanical properties such as the tensile strength, elongation, etc. Elastomer-based electrospun fibers have shown a 40% reduction in the peak tensile strength and 60% reduction in elongation at maximum applied stress compared to cast films. According to Wang et al., the mechanical properties of electrospun nanofiber webs are closely related to the fiber orientation, bonding between fibers and fiber slippage, rather than the mechanical properties of individual fibers within the web\textsuperscript{83}.

In order to evaluate the mechanical properties of nanofiber webs from PCL and CNC-filled PCL, longer electrospinning collection times were used to produce suitable, thicker samples. In these measurements nanofiber samples were limited to those from neat PCL and PCL filled with unmodified CNCs (so as to avoid complicating effects from the different morphological features observed in nanocomposites with PCL-grafted CNCs). The morphologies of such thicker (0.15 mm) webs were observed under SEM and the
preservation of the nanofibrillar structure was confirmed. DMA measurements were performed on these nanofiber webs and the change of storage modulus with temperature is reported in Figure 5.6. The storage modulus curves displayed the typical behavior of semicrystalline polymers with four distinctive zones. At low temperature (below -60 °C), the modulus slightly decreased with temperature. This observed limited temperature effect is explained by the fact that the polymer was in the glassy state and molecular motions were largely restricted to vibration and short-range rotation. Around -60 °C, a transition shown as a sharp drop in the storage modulus was observed. At this temperature the main relaxation process corresponded to amorphous PCL domains associated to the glass transition of the polymer. At higher temperatures range, -60 to 40 °C, the storage tensile modulus decreased due to the progressive melting of the PCL matrix. Here amorphous, rubbery and crystalline domains coexisted. At higher temperatures (above 50-60 °C) the modulus dropped sharply and unrecoverable deformations occurred due to the complete melting of the crystalline zones of the PCL matrix.
A significant increase in the storage modulus was observed in the case of webs of PCL filled with unmodified CNCs (2.5 and 7.5%) in comparison with neat PCL. In the case of PCL webs with 2.5% unmodified CNCs, the reinforcing effect resulted from the filler loading but it also can be partially attributed to the smaller fiber diameters observed at this load during electrospinning (relative to other CNCs loads tested). As noted earlier, the fiber diameters tended to increase with the reinforcement load. Smaller fiber diameters yielded higher overall relative bonded area between fibers because of the increased surface area, bonding density and better distribution of bonds. In the case of 5% CNC load in PCL, the variations in the storage modulus compared well with the corresponding profile obtained for the neat PCL matrix. According to the morphology results presented previously, at this loading the fiber diameter was similar to that of neat electrospun PCL. At 7.5% CNCs
loading, the storage modulus increased compared to that of neat PCL. This was despite the fact that the nanofiber diameter was larger at this CNCs load. Therefore, the improved mechanical behavior in this case can be related to the reinforcing contribution of CNCs within the fiber (intrinsic strength). From these results it becomes clear that the effect of CNCs and the fiber diameter on the mechanical properties of the nanocomposites are intimately related. It is expected that different optimum CNCs loading apply to different fiber diameters.

PCL nanofiber webs reinforced with PCL-grafted CNCs presented poor mechanical properties (results not presented). In this case, the storage modulus dropped drastically above the glassy transition and did not follow the trend observed in other PCL-based nanofiber webs. The poor mechanical properties after loading modified CNCs are related to the heterogeneous morphology of the webs which contains both nanofibrillar and annealed structures. The defective morphology in this case makes the adhesion within the web and stress transfer deficient.

The behavior of electrospun webs under non-linear deformations was also investigated. Table 5.3 summarizes the results (Young’s modulus, strength, and strain at rupture) obtained from these tensile tests. The neat PCL nanofiber webs showed an ultimate strength of 1.10 MPa and a maximum strain of 43.5%. The Young’s modulus was calculated to be 3.89 MPa. With the incorporation of unmodified CNs, a distinctive increase of these mechanical properties was realized (for loadings of 2.5 and 7.5%). The system with 5%
CNCs loading gave similar mechanical properties to the neat PCL webs. Nanofiber webs reinforced with 2.5% unmodified CNCs showed the most notable increase (ca. 1.5-fold increase in Young’s modulus and ultimate strength compared to neat PCL webs). If it is assumed that the slippage between fibers and fiber bonding was uniform over all nanofiber webs, it is clear that the mechanical properties of the PCL-CNC composites (nanofiber webs) were effectively improved by incorporating unmodified CNs.

Table 5.3 Results from tensile tests of electrospun PCL nanofiber webs without reinforcing CNC (ES-PCL) and with reinforcing (unmodified) CNCs (ES-PCL-CNC) added at various concentrations.

<table>
<thead>
<tr>
<th>Samples</th>
<th>CNC Content</th>
<th>Young’s Modulus (MPa)</th>
<th>Strength (MPa)</th>
<th>Strain at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES-PCL</td>
<td></td>
<td>3.89 ± 0.10</td>
<td>1.10 ± 0.08</td>
<td>43.5 ± 10.4</td>
</tr>
<tr>
<td>ES-PCL-CNC</td>
<td>2.5%</td>
<td>6.54 ± 0.26</td>
<td>1.51 ± 0.06</td>
<td>64.8 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
<td>3.94 ± 0.58</td>
<td>0.86 ± 0.08</td>
<td>82.6 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>7.5%</td>
<td>6.26 ± 0.73</td>
<td>1.32 ± 0.21</td>
<td>85.8 ± 14.4</td>
</tr>
</tbody>
</table>

5.4 Conclusions

Nanofiber web composites based on biodegradable PCL and cellulose nanocrystals were successfully produced via the electrospinning technique. Chemical grafting of cellulose nanocrystals with short PCL chains was used in an attempt to obtain better compatibility
between the hydrophobic PCL matrix and the hydrophilic CNC dispersed phases. It was demonstrated that cellulose nanocrystals can be used effectively as reinforcing material in electrospun PCL and to produce nanofibers with unique structural properties. Homogeneous nanofibers with diameters in the nanoscale and improved thermomechanical properties were obtained when unmodified CNCs were incorporated in the PCL matrix. When short PCL chains were grafted on the surface of CNCs, annealed fibrillar morphologies were obtained. The interfering effect of short PCL chains during the electrospinning process was elucidated. The differences in the crystallization rates of the high and low molecular mass PCL in the matrix and in the CNC grafts impaired properties of PCL filled with PCL-grafted CNCs.

Finally, a significant increase in the storage modulus at all temperatures tested and non-linear deformation strength properties were observed when CNCs were incorporated in the PCL nanofibers. The reinforcement effect of CNCs was demonstrated and explained in terms of differences in fiber diameter, CNCs loading and crystallization processes.

5.5 REFERENCES


6 POLY(N-ISOPROPYLACRYLAMIDE) BRUSHES GRAFTED FROM CELLULOSE NANOCRYSTALS VIA SURFACE-INITIATED SINGLE-ELECTRON TRANSFER LIVING RADICAL POLYMERIZATION

6.1 Introduction

The use of responsive polymer grafts has been of interest in recent years in the design of “smart” materials that can respond to changes in their environment, such as light, heat, ionic strength, and pH. Related materials are especially interesting for drug-delivery and sensing applications. Thermo-responsive polymers may have an upper or a lower critical solution temperature (UCST and LCST), depending on entropic changes and phase behaviors associated with their structural features. Poly(N-isopropylacrylamide) (poly(NiPAAm)), one of the most studied thermo-responsive polymers, has an LCST in aqueous solution ranging between 30 to 35 °C, depending on its detailed molecular architecture. Above the LCST, poly(NiPAAm) phase-separates due to thermal-driven chain dehydration. Since the LCST of poly(NiPAAm) is near the physiological temperature of ca. 37 °C, it has been extensively used in applications involving controlled drug release. The utility of nanocellulosic substrates as nanocarriers of responsive functionalities for such applications has an obvious advantage related to their biocompatibility and cost.

Lindqvist et al. have reported the development of novel dual-responsive surfaces via SI-ATRP, now known to have proceeded via SI-SET-LRP, from cellulose fibers in filter paper. In their work, NiPAAm as well as 4-vinylpyridine were utilized to produce thermo- and pH-responsive materials after grafting the respective block copolymer brushes on the
fiber surface. A large increase in the water contact angle (~120 °) of the functionalized fibers was observed when increasing the temperature above the LCST of poly(NiPAAm). However, few reports are available on the use of surface-initiated LRP to modify CNCs. Yi et al. investigated the liquid crystalline phase behavior of CNCs resulting from grafting poly(styrene) (PS), poly(6-[4-(4-methoxyphenylazo)phenoxy]hexyl methacrylate (PMMAZO), and poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA) from the surface of CNCs via SI-ATRP, however the effects of reaction conditions were not undertaken. On the other hand, Morandi et al. used various grafting conditions to control grafting density of polystyrene brushes on CNCs using excess concentrations of initiator during modifications. In these three reports, freeze-dried CNCs were utilized as the substrate for surface modification. Since drying of the CNCs can lead to aggregation, it can be argued that surface modification was limited due to the reduced available surface area. It has been shown that solvent exchange with a number of solvents promotes the self-assembly of a CNC gel and is more suitable for redispersion. To our knowledge, grafting from never-dried CNCs via LRP has not been reported. Therefore, this study addresses the important issue of grafting poly(NiPAAm) from CNCs to develop brushes with various surface densities and degrees of polymerization. Such systems are expected to not only provide steric stabilization of colloidal suspensions of CNCs, but also provide a bio-based platform for nanotechnological systems that may benefit from thermo-responsive functionalities.
6.2 Experimental

Materials. Pure ramie fibers were obtained from Stucken Melchers GmbH & Co. (Germany). 2-bromoisobutyryl bromide (BriB), 2-dimethylaminopyridine (DMAP), tetrahydrofuran (THF, 99%), N-isopropylacrylamide (NiPAAm), copper (I) bromide and \(N,N,N',N''\)-pentamethyldiethylenetriamine (PMDETA), were all obtained from Sigma-Aldrich. Sulfuric acid (95%), acetic acid (glacial), triethylamine (TEA, 99.5%), acetone (99%), ethanol (95%), methanol (99%), sodium hydroxide pellets, and sodium chlorite were all purchased from Fisher Scientific. All solvents were dried over molecular sieves (3 Å, 4-8 mesh beads, Sigma-Aldrich) for 48 h before use.

Preparation of Cellulose nanocrystals (CNCs). CNCs were obtained from ramie fibers, which were first treated for 2 hours with a 4 wt% NaOH solution at 80 °C to remove any residual or unwanted components or additives. The purified ramie fibers obtained after repeating the previous procedure were then acid hydrolyzed with 65 wt% aqueous sulfuric acid solution (55 °C, 45 minutes). The resulting dispersion was filtered into ~500 g ice cubes and washed with distilled water until neutral pH by successive centrifugations at 12,000 rpm at 10 °C for 20 minutes. Finally, dialysis for one week against deionized water with a 3,500 MWCO dialysis membrane was performed to remove trace amounts of residual sulfuric acid. Further details about this procedure can be found elsewhere.72

Preparation of Initiator-modified Cellulose Nanocrystals. Never-dried CNCs were reacted with BriB initiator at different ratios with respect to anhydroglucose units in CNCs ([Br]:[AGU]), 5:3, 5:4, 5:6, and 5:12. For simplicity, these were referred to as very high
density (VHD), high density (HD), medium (MD), and low density (LD), respectively. In a typical experiment, 1 g of CNCs (6.17 mmol of anhydroglucose units, equivalent to 18.5 mmol OH groups) were solvent-exchanged from water to acetone (three times) and then to dry THF by using successive centrifugations (12,000 rpm at 10 °C for 20 minutes). The resulting CNCs were placed in a 250 mL flask under argon gas containing 100 mL of dry THF at 25 °C while keeping the dispersion continuously stirred. In the case of VHD condition, 2.3 mL of triethylamine (TEA) was added followed by 18.5 mmol of DMAP. Finally, 10 mmol of BriB was mixed dropwise and the dispersion was left to react for 24 hours at 25 °C and then centrifuged twice with dry THF, twice with 1:1 THF:EtOH, once with acetone, then redispersed in deionized water by sonication. These rigorous centrifugal rinsing and separation steps assured that free initiator has been removed from CNC suspensions. Component makeup for other [Br]:[AGU] ratios was adjusted according to the respective molar inventory.

**SET-LRP of Poly(NiPAAm) from Initiator-modified Cellulose Nanocrystals.** Initiator-modified CNCs were grafted with poly(NiPAAm) by surface-initiated single-electron transfer living radical polymerization at three ratios of monomer to anhydroglucose unit [NiPAAm]:[AGU], 50:3, 75:3, and 100:3. For simplicity, these were referred to as low degree of polymerization (LDP), medium (MDP), and high (HDP), respectively. For these various monomer concentrations during polymerization, medium density (MD) initiator-modified CNCs were used. 300 mg of initiator-modified CNCs dispersed in water were placed in a 250 mL Schlenk flask. A desired amount of methanol was then added. In the
case of LDP, this co-solvent containing initiator-modified CNCs was first degassed under argon (3 cycles) before adding 11.3 g NiPAAm and 0.143 g Cu(I)Br. The reaction mixture was then degassed again three times before injecting 0.209 mL PMDETA. For all polymerizations, the ratio of monomer to catalyst was held constant. The mixture was left to react for 24 hours at ambient temperature while stirring vigorously. The resulting products, poly(NiPAAm)-g-CNCs, were then centrifuged and dialyzed (MWCO 3,500) against deionized water for one week followed by lyophilization. The absence of free sacrificial initiator and centrifugal separation assured the absence of free poly(NiPAAm) in the lyophilized products. A summary of reaction conditions can be found in Table 6.1. Scheme 1 shows a summary of the key steps involved in the production of poly(NiPAAm)-g-CNCs.

Table 6.1  Summary of reaction conditions for initiator-g-CNCs and poly(NiPAAm)-g-CNCs.

<table>
<thead>
<tr>
<th>Molar ratios</th>
<th>amount of initiator, mmol [mM]</th>
<th>amount of monomer, mmol [mM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Br]:[AGU]</td>
<td>5:12 (LD) 2.5 [25 mM] 100 [667 mM]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5:6 (MD) 5.0 [50 mM] 100 [667 mM]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5:4 (HD) 7.5 [75 mM] 100 [667 mM]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5:3 (VHD) 10 [100 mM] 100 [667 mM]</td>
<td></td>
</tr>
<tr>
<td>[NiPAAm]:[AGU]</td>
<td>50:3 (LDP) 5.0 [50 mM] 100 [667 mM]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75:3 (MDP) 5.0 [50 mM] 150 [1 M]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100:3 (HDP) 5.0 [50 mM] 200 [1.33 M]</td>
<td></td>
</tr>
</tbody>
</table>

§ The [NiPAAm]:[AGU] monomer molar ratio used in this set of experiments corresponded to 50:3 or LDP

† The [Br]:[AGU] initiator molar ratio used in this set of experiments corresponded to 5:6 or MD
Scheme 1 The synthesis route for the grafting of poly(NiPAAm) from the surface of cellulose nanocrystals.

Fourier Transform Infrared Spectroscopy (FT-IR). FTIR was used in order to confirm the presence of the initiator and thus, poly(NiPAAm) brushes grafted from CNCs. Pellets of freeze-dried samples were made with KBr and then directly analyzed in a Nicolet FTIR spectrometer. All spectra were collected with a 2 cm\(^{-1}\) resolution after 32 continuous scans.

Wide-angle X-ray diffraction (WAXS). WAXS experiments were carried out at ambient temperature on dried pellets of unmodified or modified CNCs. A Philips (XLF, ATPS XRD 1000) diffractometer operated with a CuK\(_\alpha\) anode (\(\lambda = 0.15406\) nm) was used with a 2\(\theta\) range from 5\(^{\circ}\) to 40\(^{\circ}\) with steps of 0.1\(^{\circ}\) and a counting time of 60 s. The crystallinity index (\(CrI\)) was calculated by the method of Segal et al.\(^\text{94}\) according to:

\[\text{CrI} = \frac{I_c}{I_t} \times 100\]%
\[
CrI = 1 - \frac{I_{am}}{I_{002}},
\]

where \(I_{002}\) is the maximum intensity (arbitrary units) of the 002 lattice diffraction and \(I_{am}\) is the intensity of diffraction (in the same units) at \(2\theta = 18^\circ\).

The dimension of the crystal \(D_{hkl}\) was evaluated by using Scherrer’s formula:

\[
D_{hkl} = \frac{0.9 \times \lambda}{\beta_{1/2} \times \cos \theta},
\]

where \(\theta\) is the diffraction angle, \(\lambda\) the X-ray wavelength and \(\beta_{1/2}\) the peak width at half of maximum intensity.

**Atomic Force Microscopy (AFM).** CNC suspensions were diluted to the desired concentration and spin-coated directly onto UV-treated silicon wafers at 4000 rpm. The deposition of the suspension was performed on a substrate which was then spun for 30 seconds. The surfaces were then dried at 80 °C for 1 hour. In the case of unmodified CNCs, poly(ethylene imine) was used as an anchoring layer to the substrate. The spin-coated CNC films were imaged using a scanning probe microscope (model Multimode Nanoscope IIIa, Digital Instruments) in tapping mode, which was equipped with an E or J scanner and with non-coated silicon-etched probes (model LTESP, Veeco).

**Differential Scanning Calorimetry and Thermogravimetric Analysis.** Differential scanning calorimetry (DSC) was performed using a TA Instruments DSC Q100. In a typical experiment ca. 10 mg of dry sample was placed in the DSC cell and heated from 25 to 265 °C (heating rate of 10 °C min\(^{-1}\)). The glass transition temperature \(T_g\) and heat capacity \(C_p\)
were calculated using TA Universal Analysis software. Thermogravimetric analyses (TGA) were performed using a TA Instruments TGA Q500. Data was collected after placing ca. 10 mg of sample in a clean platinum pan and heating from ambient temperature to 600 °C (heating rate of 20 °C.min\(^{-1}\)).

**X-Ray Photoelectron Spectroscopy.** XPS measurements were performed on freeze-dried samples of CNCs before and after surface modifications first with a Riber electron spectrometer equipped with dual Mg/Al irradiation. More detailed XPS investigations were later performed with an AXIS 165 electron spectrometer and monochromatic Al irradiation. In the latter case, samples were placed in the spectrometer and evacuated overnight in order to stabilize vacuum conditions. All samples were measured at three locations on the surface (analysis area of ca. 1 mm\(^2\)) and the average values were reported. Elemental surface compositions were determined from low-resolution scans recorded with an 80 eV analyzer pass energy and a 1 eV step. Nominal cellulose contents were determined from carbon high-resolution spectra recorded at binding energies in the C 1s region using 20 eV analyzer pass energy and 0.1 eV step. In the curve fitting procedure for C 1s, symmetric Gaussian components with Shirley background were used.\(^{96, 97}\) The binding energy scale was shifted using the C-C binding energy at 285.0 eV.\(^{98}\) Cellulose content was calculated by the following equation:

\[
\% \text{ cellulose} = \frac{\% \text{ C1s}(\% \text{ CO})(1.1)(0.01)}{5}
\]
where C 1s is total atomic percentage of carbon from low resolution spectra and CO is percentage of carbon with one oxygen bond (also referred as C2), from high resolution spectra. Whatman filter paper was used as reference for 100 % cellulose.

*Saponification of poly(NiPAAm)-g-CNCs.* Saponification of poly(NiPAAm)-g-CNCs was performed in order to cleave the polymer grafts and analyze their structures and determine their molecular masses. For this purpose 300 mg of each poly(NiPAAm)-g-CNCs sample was placed in 50 mL 2 % NaOH solution for 48 hours while stirring. Following the saponification, each dispersion was brought to neutral pH with 6 N HCl and then centrifuged (12,000 rpm at 10 °C for 20 minutes) to separate CNCs from the cleaved poly(NiPAAm) grafts in the supernatant. The obtained CNCs were washed successively by redispersion and centrifugation (3 times) in DI water to remove residual salts. The supernatant from each sample was then dialyzed against DI water until a minimum in conductivity was reached. CNCs and free, cleaved poly(NiPAAm) samples were then freeze-dried for further analyses.

*NMR Spectroscopy.* Cleaved poly(NiPAAm) chains from the CNCs surface were analyzed by NMR. $^{13}$C NMR experiments were carried out with an AC 300 Bruker spectrometer operated at a frequency of 75.468 MHz. Spectra, from solutions in D$_2$O at 25°C in 5 mm o.d. tube (internal acetone $^{13}$C (CH$_3$) at 31.5 ppm relative to Me$_4$Si), were recorded in $^{13}$C NMR conditions using relaxation delay of 2 s, 8 000 data points, 0.54 s acquisition time, and 10 000 transients.

*Size Exclusion Chromatography.* Cleaved poly(NiPAAm) chains were characterized by a size exclusion chromatograph (Labora, Czech Republic) equipped with RI and UV detectors.
and using a set of two PSS-SDV columns (5 µm; 10³ and 10⁵ Å, respectively) (PSS, Germany). The eluent used was THF at room temperature at a flow rate of 0.3 mL/min. Poly(N-isopropylacrylamide) standards and the Mark-Houwink constants for the PNiPAAm/THF system (K = 5.754 x 10⁻³ mL g⁻¹, α = 0.78) were used to establish the number-average molecular weight (Mn) and polydispersity index (PDI).⁹⁹

6.3 Results and Discussion

Attachment of initiator on Cellulose Nanocrystals. The SET-LRP-mediated grafting and polymerization of polymer brushes from the surface of CNCs required immobilization of an initiator on the surface of the CNCs. The hydroxyl groups of the CNCs were treated with 2-bromoisobutyryl bromide and converted to the corresponding ester; the resultant product was then used as the SET-LRP agent for graft polymerization of the vinyl monomer, i.e., NiPAAm. This two step synthetic route is illustrated in Scheme 1. The 2-bromoisobutyryl-CNCs ester, or initiator-modified CNCs, was synthesized by surface esterification of CNCs with 2-bromoisobutyryl bromide in THF in the presence of TEA and a catalytic amount of DMAP (Step 1). The success of this reaction was confirmed by Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Photoelectron Spectroscopy (XPS).

When compared to the spectrum of unmodified CNCs, a typical FTIR spectrum of initiator-modified CNCs, as that shown in Figure 6.1, indicated the appearance of a carbonyl ester stretch at 1735 cm⁻¹, which confirmed covalent attachment of the initiator. Typically, the OH stretch did not show visible changes because of the low percentage of available surface hydroxyl groups compared to those in the interior of the crystallite.⁹²
XPS analysis was conducted in order to quantify the concentration of the initiator on the surface of the CNCs. Freeze-dried samples were analyzed to give relative intensities of carbon, oxygen, and bromine. Whatman filter paper was used as a reference of pure cellulose. Figure 6.2 shows typical XPS spectra of unmodified CNCs and initiator-modified CNCs. Unmodified CNCs showed distinct peaks for carbon and oxygen with trace amounts of sulfur due to the presence of grafted sulfate groups. In the case of initiator-modified CNCs, the same peaks were observed; at higher XPS resolution a peak corresponding to the binding energy of bromine (Br 3d) was observed. Even though this
small peak was not suitable for reliable quantification of Br surface concentration, it did confirm the presence of the initiator.

Figure 6.2  XPS spectra showing C1s, N1s and O1s peaks for (A) unmodified CNCs, (B) initiator-g -CNCs and (C) poly(NiPAAm)-g-CNCs. The inset displays higher XPS resolution indicating the presence of Br (Br 3d signal). Trace amounts of sulfur were detected, but they are not distinguishable in these wide scan spectra.

To determine the effect of the initiator esterification reaction on the crystalline structure of CNCs, freeze-dried samples were exposed to X-ray diffraction (XRD) analysis. The WAXD analyses of both unmodified and initiator-modified CNCs showed typical patterns (see Appendix A) of the native crystalline form of cellulose (cellulose I). They displayed 2θ diffraction peaks at 14.8° (0.60 nm), 16.5 (0.54 nm), 22.6 (0.39) nm and 34.5
(0.258 nm). This fact confirmed that the crystalline core of the CNCs was not affected by the immobilization of the initiator, which is therefore limited only to the surfaces of the nanocrystals.

From the wide angle X-ray diffraction spectra, the crystallinity index was calculated along with the average crystallite width using Scherre’s formula and a shape constant of 0.9. These parameters are summarized in Table 6.2. Unmodified CNCs from ramie fibers showed a crystallinity index of 87.8 and average width of 5.8 nm. After attachment of the initiator and considering the experimental deviations of the measurements, the crystallinity index remained about the same as that of unmodified CNCs. This confirmed that no or minimal damage occurred after installation of the initiator.

Table 6.2 Width and crystallinity index of unmodified CNCs and initiator-g-CNCs for different [Br]:[AGU] ratios.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average crystallite width*, nm</th>
<th>Crystallinity Index, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified ramie CNCs</td>
<td>6.2</td>
<td>87.8</td>
</tr>
<tr>
<td>Initiator-g-CNCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VHD</td>
<td>6.1</td>
<td>86.0</td>
</tr>
<tr>
<td>MD</td>
<td>5.7</td>
<td>85.4</td>
</tr>
<tr>
<td>LD</td>
<td>5.9</td>
<td>87.4</td>
</tr>
</tbody>
</table>

*calculated by Scherre’s formula
**SI-SET-LRP of Poly(NiPAAm) from Cellulose Nanocrystals.** The obtained initiator-modified CNCs were used to polymerize NiPAAm via SET-LRP using a grafting from approach (step 2, scheme 1). A series of poly(NiPAAm)-g-CNCs were prepared by varying the ratio between the immobilized initiator and the monomer as shown in Table 6.1. After purification to remove unreacted chemicals by centrifugation and dialysis, samples were further analyzed by AFM, FTIR and XPS.

First, the effect of the SET-LRP chemical process on the morphology of CNCs was studied by AFM imaging. The structural and morphological integrity of the CNCs did not appear to be affected by the immobilization of initiator nor by further polymerization of poly(NiPAAm), as shown in Figure 6.3. The size of the obtained rod-like CNCs was determined to be in the range of 3-15 nm in diameter and 50-250 nm in length. The height AFM image of the poly(NiPAAm)-g-CNCs showed a halo that could be taken as indication of the presence of the polymer brushes. AFM phase images showed this effect more markedly due to the apparent contrast produced by the large difference in the hardness between the soft poly(NiPAAm) grafts and the CNCs.
Figure 6.3  Tapping mode AFM 2x2 µm height images of unmodified CNCs (left) and poly(NiPAAm)-g-CNCs (right) on silicon wafers.

After SET-LRP of NiPAAm, new FTIR peaks corresponding to the amide stretch and amine bend of poly(NiPAAm), 1650 and 1546 cm⁻¹ were observed, indicating successful ‘grafting from’ polymerization. FTIR spectra of all grafting conditions displayed similar FTIR spectra as those shown in Figure 6.4.

XPS analyses were conducted in order to quantify surface concentration of the poly(NiPAAm) brushes grafted from the CNCs surfaces. Freeze-dried samples were analyzed to give relative intensities of carbon, oxygen, and nitrogen. Figure 6.5 shows typical XPS spectra of poly(NiPAAm)-g-CNCs.

Poly(NiPAAm)-g-CNCs showed the appearance of nitrogen from NiPAAm monomers. In some samples, trace amounts of sulfur were detected from residual sulfate
groups on CNCs surfaces resulting from the respective sulfuric acid hydrolysis used during their preparation. The overall concentration of sulfur which was determined by elementary analysis to be 0.76% on dry matter corresponded to 0.019 mol per anhydroglucose unit (or 0.0066 mol per mol cellulose hydroxyl groups) in agreement with other reports. Therefore it was not surprising that the sulfur signal was difficult to detect since only ca. 2.1% of surface hydroxyl groups were converted to sulfate groups. This estimation was derived from morphological considerations, in that only ca. 33% of the total hydroxyl groups were available on the surface of the crystallites. Given this estimated small percentage of sulfate groups on the surface, it is expected that the sulfate groups had a minimal effect on surface reactions. Finally, in agreement with recent investigations on the advantages of SET-LRP-derived polymers, the XPS spectra for grafted CNCs did not show Cu signals.

From the peak areas of carbon, C1s, and more specifically those for C–O (also known as C2) signals in high resolution spectra, the relative molar quantities of poly(NiPAAm) and cellulose in each sample were determined (see Table 6.3). In reference to the molar quantities of initiator present on CNCs after each reaction condition, it was observed that in the LD case, the lowest poly(NiPAAm) content was quantified, as expected. As the relative amount of initiator was increased, no effect on the subsequent grafted poly(NiPAAm) content was observed. Such observation could be explained by the concurrent effects of initiator surface density favoring more but shorter poly(NiPAAm) brushes. When increased molar quantities of monomer were introduced for polymerization, from LDP to HDP, a
distinctive trend was observed in that the poly(NiPAAm) content of the final product increased with the amount of monomer added in the SET-LRP reaction.

Table 6.3 Atomic percentages (XPS) and molar % content of Poly(NiPAAm) and cellulose for unmodified CNCs and poly(NiPAAm)-g-CNCs.

<table>
<thead>
<tr>
<th>Condition</th>
<th>%O 1s</th>
<th>%C 1s</th>
<th>%N 1s</th>
<th>%S 2p</th>
<th>O/C</th>
<th>% Poly(NiPAAm)</th>
<th>% Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>unmodified CNCs</td>
<td>42.8</td>
<td>57.1</td>
<td>0.0</td>
<td>0.1</td>
<td>0.75</td>
<td>0</td>
<td>94.2</td>
</tr>
<tr>
<td>LD</td>
<td>17.1</td>
<td>72.1</td>
<td>10.7</td>
<td>0.1</td>
<td>0.24</td>
<td>76.0</td>
<td>18.2</td>
</tr>
<tr>
<td>MD</td>
<td>15.8</td>
<td>73.2</td>
<td>10.9</td>
<td>0.2</td>
<td>0.22</td>
<td>75.5</td>
<td>18.7</td>
</tr>
<tr>
<td>HD</td>
<td>14.5</td>
<td>74.5</td>
<td>11.0</td>
<td>0.1</td>
<td>0.19</td>
<td>78.0</td>
<td>16.2</td>
</tr>
<tr>
<td>VHD</td>
<td>11.8</td>
<td>75.7</td>
<td>12.5</td>
<td>0.0</td>
<td>0.16</td>
<td>81.6</td>
<td>12.6</td>
</tr>
<tr>
<td>LDP</td>
<td>15.8</td>
<td>73.2</td>
<td>10.9</td>
<td>0.2</td>
<td>0.22</td>
<td>75.5</td>
<td>18.7</td>
</tr>
<tr>
<td>MDP</td>
<td>11.9</td>
<td>76.4</td>
<td>11.7</td>
<td>0.0</td>
<td>0.16</td>
<td>83.0</td>
<td>11.2</td>
</tr>
<tr>
<td>HDP</td>
<td>11.7</td>
<td>76.0</td>
<td>12.3</td>
<td>0.0</td>
<td>0.15</td>
<td>85.1</td>
<td>9.09</td>
</tr>
<tr>
<td>Filter paper</td>
<td>43.6</td>
<td>56.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.77</td>
<td>0</td>
<td>95.4</td>
</tr>
</tbody>
</table>

The ratio of oxygen to carbon atomic percentages shown in Table 6.3 was also an indicator of the presence of poly(NiPAAm) grafts. The XPS O/C ratio for unmodified nanocrystals was found to be 0.75, close to 0.77 obtained for a cellulose reference such as Whatman filter paper (note a theoretical O/C ratio of 0.8 was expected for pure cellulose). The small difference in these values was explained by the presence of adsorbed organic contaminants, as typically found on high energy substrates. As poly(NiPAAm) grafts were grown from the CNC surface, the respective O/C ratios were expected to decrease.
significantly due to the relatively larger contribution of C signal from the polymer grafts. Accordingly, Table 6.3 indicates a distinctive reduction in O/C ratios with monomer addition (to values as low as 0.15 in the HDP case).

To better determine the surface chemistry of grafted CNCs, high resolution XPS was utilized to deconvolute the C1s signal into C–C (C–H), C–O, C=O, O–C=O, and C-N peaks (see Table 6.4).

Table 6.4  Carbon deconvolution of unmodified CNCs and poly(NiPAAm)-g-CNCs determined by high resolution XPS of C 1s.

<table>
<thead>
<tr>
<th>Condition</th>
<th>%C–C (C–H)</th>
<th>%C–N</th>
<th>%C–O</th>
<th>%C=O</th>
<th>%O–C=O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified CNCs</td>
<td>6.7</td>
<td>0.0</td>
<td>75.0</td>
<td>17.5</td>
<td>0.9</td>
</tr>
<tr>
<td>LD</td>
<td>50.1</td>
<td>22.6</td>
<td>11.5</td>
<td>13.9</td>
<td>1.9</td>
</tr>
<tr>
<td>MD</td>
<td>53.7</td>
<td>19.7</td>
<td>11.6</td>
<td>14.3</td>
<td>0.7</td>
</tr>
<tr>
<td>HD</td>
<td>55.4</td>
<td>19.3</td>
<td>9.9</td>
<td>14.2</td>
<td>1.3</td>
</tr>
<tr>
<td>VHD</td>
<td>57.5</td>
<td>19.3</td>
<td>7.6</td>
<td>13.9</td>
<td>1.8</td>
</tr>
<tr>
<td>LDP</td>
<td>53.7</td>
<td>19.7</td>
<td>11.6</td>
<td>14.3</td>
<td>0.7</td>
</tr>
<tr>
<td>MDP</td>
<td>59.8</td>
<td>18.2</td>
<td>6.7</td>
<td>14.2</td>
<td>1.2</td>
</tr>
<tr>
<td>HDP</td>
<td>62.1</td>
<td>17.5</td>
<td>5.4</td>
<td>14.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Filter Paper</td>
<td>4.2</td>
<td>0.0</td>
<td>76.8</td>
<td>18.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Unmodified CNCs again showed similar results to that of cellulose from Whatman filter paper used as a reference; however, there was slightly higher signal intensity for non-oxygen bound carbon which was most likely related the presence of contaminants. When
compared to unmodified CNCs a marked increase in intensity of this peak was observed for poly(NiPAAm)-g-CNCs. This was ascribed to the isopropyl groups in grafted poly(NiPAAm) chains. In addition, a significant decrease in C–O peak intensity was realized after grafting, which indicated the presence of poly(NiPAAm).

Since the XPS O–C=O peak is expected to increase after the introduction of ester functionalities from the attachment of the initiator on the CNCs, the degree of polymerization (D.P.) and surface substitution (DSS) could have been determined from the ratio of C–N to O–C=O and O–C=O to C 1s peaks, respectively. However, it was found that the O–C=O peak did not increase as markedly and reproducibly as otherwise would have been expected. This was likely to be the result of interference from adsorbed contaminants, as has been shown to occur in XPS analyses of lignocellulosic materials. For this reason the D.P. and DSS were not determined from high resolution XPS data but instead they were derived from SEC measurements, which were more appropriate to this end (see later sections).

Thermogravimetric analyses were conducted to investigate the thermal degradation behavior of the obtained grafted CNCs. Figure 6.4 shows representative thermograms for unmodified CNCs, bulk poly(NiPAAm) and poly(NiPAAm)-g-CNCs. Unmodified CNCs showed typical degradation behavior of CNCs obtained from sulfuric acid hydrolysis with an onset temperature of ca. 270 °C. This value was comparatively lower than that for neutral, sulfate-free cellulose; the difference was explained by the in-situ formation of acid in CNCs which induces thermal degradation at lower temperatures. On the other hand, bulk poly(NiPAAm) showed an onset point of degradation at ~400 °C. These two distinct onset
degradation temperatures of CNCs and poly(NiPAAm) can be observed in the weight loss profile of poly(NiPAAm)-g-CNCs. From the derivative weight loss profiles (not shown here), trends in the relative molar concentrations of initiator and monomers could be estimated after normalization of the thermograms; for example, by using a derivative maximum weight loss of poly(NiPAAm) as unity. As the molar quantity of initiator was increased, the quantity of polymer present also increased, most likely due to the presence of a larger number of initiator sites on the surface of the CNCs. The thermograms of increased molar amounts of monomer indicated larger quantities of NiPAAm or longer poly(NiPAAm) grafts.

Figure 6.4 Thermograms of unmodified CNCs, bulk poly(NiPAAm) and poly(NiPAAm)-g-CNCs (VHD).
Differential scanning calorimetry curves of unmodified CNCs, bulk poly(NiPAAm) and poly(NiPAAm)-g-CNCs are shown in Figure 6.5. Unmodified CNCs showed only the onset of thermal degradation in differential scans due to their highly crystalline nature. Bulk poly(NiPAAm) showed the glass transition of the amorphous polymer at ca. 130 °C. A glass transition was also observed in all grafted CNCs samples, at ca. 143 °C, with only minimal variations between samples obtained from various initiator and monomer loadings. Restricting the mobility of grafted poly(NiPAAm) chains to the surface of CNCs caused this increase in glass transition temperature. This was in agreement with Yin et al.\textsuperscript{102} who showed that single-chain poly(NiPAAm) has different glass transition temperatures depending on the microstructure of the polymer; for example, if it formed loose coils or complex globules. By increasing the entanglement density of poly(NiPAAm), the mobility of chain segments could be hindered, thus resulting in an increased glass transition temperature.
From the DSC profiles, $\Delta C_p$ was also determined for each glass transition; and when compared to bulk polymer, the $\Delta C_p$ of grafted chains was used to estimate the relative quantities of poly(NiPAAm) grafts present in each sample (see Table 6.5). A similar trend from the XPS results (Table 6.3) was observed: with increased initiator and/or monomer loading larger amounts of poly(NiPAAm) were determined. The slight difference in absolute values when the results from these two techniques were compared was most likely due to the inherent difference in their application since one is used for bulk analyses (DSC) while the other is surface sensitive (XPS).
Table 6.5 DSC results of poly(NiPAAm)-g-CNCs and bulk poly(NiPAAm).

<table>
<thead>
<tr>
<th>Molar ratio</th>
<th>T$_g$, °C</th>
<th>$\Delta$C$_p$, J/g °C</th>
<th>% poly(NiPAAm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>142.7</td>
<td>0.31</td>
<td>62</td>
</tr>
<tr>
<td>MD</td>
<td>142.3</td>
<td>0.31</td>
<td>62</td>
</tr>
<tr>
<td>HD</td>
<td>143.0</td>
<td>0.37</td>
<td>74</td>
</tr>
<tr>
<td>VHD</td>
<td>141.7</td>
<td>0.40</td>
<td>80</td>
</tr>
<tr>
<td>LDP</td>
<td>142.3</td>
<td>0.31</td>
<td>62</td>
</tr>
<tr>
<td>MDP</td>
<td>142.1</td>
<td>0.39</td>
<td>78</td>
</tr>
<tr>
<td>HDP</td>
<td>142.2</td>
<td>0.43</td>
<td>86</td>
</tr>
<tr>
<td>Bulk poly(NiPAAm)</td>
<td>130.1</td>
<td>0.50</td>
<td>100</td>
</tr>
</tbody>
</table>

*Cleavage of poly(NiPAAm) chains from poly(NiPAAm)-g-CNCs.* Generally, characterization of polymer brushes after grafting from a surface by surface-initiated living radical polymerizations are carried out by using free sacrificial initiator.$^{34}$ In these cases it has been assumed that the polydispersity and molecular weight of the obtained polymer brushes are comparable to those measured for the free polymer. However, it could be argued that the length of polymer brushes depends on the loading of sacrificial initiator. Also the polymerization could be affected by i) the conditions of the reaction (homogenous for the free chains and heterogeneous for the grafted ones) and ii) the steric hindrances at different initiator surface densities. Therefore, in the present study direct quantification of the degree of polymerization (D.P.) and polydispersity indices (PDI) of the grafts was performed by SEC of cleaved polymer brushes. Such cleavage was performed by saponification (2 % aq. 

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NaOH solution, 48 hours) of ester linkages on the surface. Structural characterization of the
grafted polymer was also carried out by using NMR and to confirm that no side reactions
occurred.

After saponification of poly(NiPAAm)-g-CNCs that resulted from different
polymerization conditions, samples were dialyzed and freeze-dried. Cleaved poly(NiPAAm)
corresponding to LD and HDP were analyzed by $^{13}$C-NMR to verify its main structural
features and CNCs were analyzed by FT-IR to verify the absence of poly(NiPAAm) grafts.
As such, Figure 6.6 (left) depicts the $^{13}$C-NMR spectra of the NiPAAm monomer and of free
poly(NiPAAm) chains. The characteristic peaks for vinyl carbons present in the spectrum of
NiPAAm monomer, at 129.7 and 127 ppm, were not detectable in the spectrum of free
poly(NiPAAm) which indicated a full success of the polymerization. In addition, the
spectrum corresponding to poly(NiPAAm) chains was identical to reported spectra recorded
for pure poly(NiPAAm) in water,$^{103}$ which confirmed that no side reactions occurred during
the polymerization from the surface of CNCs. The peaks at 22 and 42.5 ppm were attributed
to the CH$_3$ and CH, respectively, of the isopropyl group and those at 42.9 and 43.5 were
assigned to the CH groups of the polymer backbone. The signals at 39 ppm belonged to the
CH$_2$ groups of the polymer backbone that relaxed much more slowly because of their
restricted mobility. Figure 6.6 (right) shows FT-IR spectra of CNCs after saponification of
grafted poly(NiPAAm) chains compared to the unmodified starting material. Very little, if
any, grafted poly(NiPAAm) was detected in all cases verifying a sufficient saponification
reaction, and thus representative cleaved polymer chains.
Figure 6.6 $^{13}$C-NMR spectra of NiPAAm monomer (left, top) and cleaved poly(NiPAAm) from the surface of CNCs after saponification (left, bottom). FT-IR spectra are also shown for CNCs after saponification of grafted poly(NiPAAm) chains of medium degree of polymerization (MDP) (right, A), low density (LD) (right, B), and unmodified CNCs (right, C).

Size Exclusion Chromatography (SEC) was employed in order to determine the relative molecular mass and polydispersity indices of cleaved poly(NiPAAm) grafts. Examples of the obtained chromatograms are depicted in Figure 6.7 and the corresponding data are reported in Table 6.6.

An increase in molecular mass of polymer brushes was observed with the increase of initiator loading, with monomer conversions varying between 11% (LD) and 17% (VHD) estimated from XPS. In a study performed by Behling et al.\textsuperscript{104} they reported that the rate of polymerization of poly(styrene) grafted from montmorillonite clay particles via SI-ATRP increased almost seven-fold with a higher grafting density. The explanation proposed was
that local concentration heterogeneities shifted the ATRP equilibrium to the active state. To the best of our knowledge, a corresponding study has not yet been undertaken for the case of SET-LRP, however a similar argument may apply. Near the surface of suspended CNCs during polymerization, heterogeneities in the local concentrations of activator and deactivator ligand complexes may have caused an increase in the activation rate and thus an increased monomer conversion (up to 17% for VHD).

In all cases, moderate polydispersity indices (PDIs) were obtained for poly(NiPAAm) brushes grafted from CNCs. The PDI of bulk poly(NiPAAm) synthesized in the same fashion as grafted CNCs with a target molecular weight of 11,300 Da was much higher than that of detached polymers from CNCs. This also supported the previous hypothesis of local heterogeneities yielding higher rates of polymerization, and thus more control on the PDI. In this case, it was inferred that the polymerization from the surface of the dispersed phase gave better control without the addition of deactivator when compared to the bulk polymerization from free initiator.

In the case of a fixed initiator content and increased monomer concentrations, the molecular masses of the grafts also showed a distinct increase, as expected. However, monomer conversion was only between 12-13% for these cases, thus monomer concentration did not have a significant effect on the rate of polymerization. Addition of small amounts of deactivator, CuBr$_2$, during polymerization is expected to yield better control according to Rosen et al.$^{38}$ In future work, these methodologies will be attempted in order to achieve lower polydispersity indices for the polymer brushes grafted from CNCs.
Figure 6.7  Size Exclusion Chromatograms of cleaved poly(NiPAAm) chains obtained according to the grafting conditions of MD (A), VHD (B), and HDP (C).
Table 6.6  Relative molecular masses (M<sub>n</sub> and M<sub>w</sub>) and polydispersity indices of cleaved poly(NiPAAm) chains from CNCs surface.

<table>
<thead>
<tr>
<th>Molar ratio</th>
<th>M&lt;sub&gt;n&lt;/sub&gt;</th>
<th>M&lt;sub&gt;w&lt;/sub&gt;</th>
<th>M&lt;sub&gt;w&lt;/sub&gt;/M&lt;sub&gt;n&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>12,170</td>
<td>25,770</td>
<td>2.117</td>
</tr>
<tr>
<td>MD</td>
<td>10,630</td>
<td>19,540</td>
<td>1.838</td>
</tr>
<tr>
<td>HD</td>
<td>15,630</td>
<td>34,060</td>
<td>2.179</td>
</tr>
<tr>
<td>VHD</td>
<td>23,820</td>
<td>41,090</td>
<td>1.725</td>
</tr>
<tr>
<td>LDP</td>
<td>10,630</td>
<td>19,540</td>
<td>1.838</td>
</tr>
<tr>
<td>MDP</td>
<td>40,330</td>
<td>85,450</td>
<td>2.119</td>
</tr>
<tr>
<td>HDP</td>
<td>42,860</td>
<td>90,840</td>
<td>2.12</td>
</tr>
<tr>
<td>Bulk poly(NiPAAm)</td>
<td>5,385</td>
<td>14,200</td>
<td>2.637</td>
</tr>
</tbody>
</table>

*target molecular mass = 11,300

In the present case where CNCs were utilized as a substrate for chemical functionalization, the reaction conditions, amounts and molecular masses of grafts obtained were significantly different from previously reported studies involving dried CNCs. There was no available report that could be used for direct comparison of the present poly(NiPAAm) brushes grafted from CNCs via SI-SET-LRP. However, ATRP from CNCs using a different monomer was reported by Morandi et al. In this case, up to ca. 50 mL of 2-bromoisobutyryl bromide was used to modify one gram of dried CNCs for grafting poly(styrene) brushes. In contrast, the present case showed an excess of initiator to surface OH group was already achieved with 6.2 mmol, or 0.77 mL per gram of CNCs, based on
previously stated morphological considerations. In addition, a large excess of monomer was used (up to 400 mmol) in the previously cited study, and the resulting poly(styrene) molecular masses were up to ca. 18,000 Da, as measured by the use of a sacrificial initiator during polymerization. In the study of Yi et al., ATRP was conducted without the use of sacrificial initiator; the resulting PS grafts were cleaved and analyzed by SEC and a molecular mass of ca. 75,000 Da (PDI = 1.2) was achieved with 192 mmol styrene per gram of dried CNCs. This PDI was relatively lower than the values obtained in this study (see Table 6.6); however, it was expected that a lower PDI could have been obtained if deactivator was added, as noted before. All in all, in the present study the effect of various reaction conditions was considered and main effects discussed so as to unveil the main chemical changes that occurred during the grafting of CNCs with hydrophilic polymers. Such efforts were aimed at obtaining new thermo-responsive materials based on nanocellulosics.

Evidence for the effect of temperature on the colloidal behavior of CNC dispersions can be found in Supporting Information in the form of viscosity curves at different temperatures. A detailed account of associated properties is under extensive investigation and will be reported in an upcoming publication.

6.4 Conclusions

Thermo-responsive poly(N-isopropylacrylamide) brushes were grafted from never-dried cellulose nanocrystals via surface-initiated single-electron transfer living radical polymerization (SI-SET-LRP) under various conditions at room temperature. Cu(I) was rapidly disproportionated to Cu(0) and Cu(II) yielding simple catalyst removal. XPS
revealed that with increased initiator and/or monomer loadings, increased amounts of poly(NIPAAm) were grafted from the surface of cellulose nanocrystals. Polymer brushes were detached from cellulose nanocrystals via saponification of ester linkages and analyzed by SEC. It was determined that with increased initiator loading, increased molecular masses of polymer brushes were obtained. This effect could be explained by local heterogeneities shifting the SET-LRP equilibrium to the active state. Likewise, with increased monomer loading, increased molecular masses of resulting polymer brushes were obtained. Finally, the results presented are expected to provide the basis for the development of temperature-responsive materials based on cellulose nanocrystals.

6.5 REFERENCES


7 SURFACE INTERACTION FORCES OF CELLULOSE NANOCRYSTALS GRAFTED WITH THERMO-RESPONSIVE POLYMER BRUSHES

7.1 Introduction

Understanding the interfacial forces between particles with adsorbed polymer layers has great relevance, not only in the area of colloid and surface chemistry, but also in the design of new materials in an array of emerging technologies. Coating, papermaking, water treatment, emulsification, oil and mineral extraction, are among the many applications that dependent on such interfacial interactions. The control of the adhesion, lubrication and rheology behaviors as well as colloidal stability implicit in these systems can be gained by physical adsorption and covalent grafting of polymers on surfaces. Within the development of biomedical and smart devices lies more recent interests in the manipulation of bio-interfaces for drug-delivery, biosensing, and cell adhesion, etc.

Stimuli-responsive polymers have been extensively studied, mostly driven by new applications in biotechnology. Since the first reported synthesis of N-isopropylacrylamide (NiPAAm) in 1956, poly(N-isopropylacrylamide) (poly(NiPAAm)) has been one of the most widely studied thermo-responsive polymers due to its coil-to-globule transition at 32 °C in aqueous solution, within the proximity of physiological temperature, also called Lower Critical Solution Temperature (LCST). This transition comes about from thermal dehydrations of the polymer chain that yields an entropic gain in free energy of the system. In fact, the LCST of poly(NiPAAm) has been known for decades, most recently explained by Matsuyama et al. as the mixing entropy produced by solvation. Importantly, factors
such as ionic strength and pH of the respective aqueous dispersion or solution can affect the LCST.  

Great interest in poly(NiPAAm) grafts has developed due to the ability of such layers to reversibly attach and detach cultured cells above and below the LCST, respectively. Among the approaches to generate polymer brushes based on poly(NiPAAm), “grafting onto” and “grafting from” methods are the more prevalent ones. The first report on the thermo-responsive behavior of poly(NiPAAm) grafts was performed by Okahata et al., in which the permeability of NaCl and dyes through a nylon capsule membrane grafted with poly(NiPAAm) could be reversibly controlled by solution temperature. Despite its importance, the characterization of the surface phase transition of thermo-responsive polymer coatings has proven to be challenging; however, a number of efforts have been undertaken by using contact angle, AFM, colloidal probe microscopy (CPM), quartz crystal microgravimetry, etc.

Important contributions relevant to surface responsiveness of grafted polymer brushes are the type and magnitude of interaction forces involved when grafted surfaces are brought to close proximity. In addition to DLVO van der Waals and electrostatic interactions, non-specific interactions, specifically steric repulsion and polymer bridging, are expected to play an important role. Some of the theories developed to describe steric forces of tethered or adsorbed polymers at interfaces include those from Alexander-de Gennes and Milner-Witten-Cates (MWC), which differ in the description of the segment density profile of the adsorbed or grafted polymer layer.
In addition to well established systems in which polymer grafting has made important inroads, applications involving lignocellulosics have emerged in the recent years. Examples are provided by reported efforts to develop “smart” materials based on cellulosic materials\textsuperscript{13, 14} including filter paper,\textsuperscript{89} cellulose nanocrystals (CNCs) or whiskers,\textsuperscript{91, 137, 138} and nanofibrillated cellulose.\textsuperscript{139} CNCs in particular, are attractive due to their unique surface, optical and mechanical properties.\textsuperscript{15, 16}

Interfacial forces between cellulosic surfaces have been previously studied using the colloidal probe technique\textsuperscript{140-156} and the Surface Force Apparatus.\textsuperscript{157-159} Lefebvre et al.\textsuperscript{160} first studied deflection versus distance curves of standard silicon nitride tips on spin-coated cellulose nanocrystal films and adsorbed layers of poly(diallyldimethylammonium chloride) via AFM. Later, Notley et al.\textsuperscript{143} studied the surface forces between a mostly amorphous colloidal cellulose sphere and cellulose surfaces of varying crystallinity, one being a film of sulfate-stabilized CNCs. The observed forces corresponded well to DLVO behaviors due to dominant electrostatic forces of the sulfate-charged nanoparticles. In addition, amorphous cellulose films resulted in surface forces of predominantly steric origin. Cranston et al.\textsuperscript{156} have reported on direct surface force measurements of polyelectrolyte multilayer films containing cellulose crystals and poly(allylamine hydrochloride) (PAH) with varying ionic strength and pH. In recent work the surface interactions of polyelectrolyte-cellulose systems were investigated utilizing colloidal probe AFM with cellulosic spheres in which the measured surface forces correlated well with practical observations related to flocculation.\textsuperscript{145}
In our previous publication,\textsuperscript{138} we reported on the synthesis of poly(NiPAAm) brushes grafted from CNCs via surface-initiated single-electron transfer living radical polymerization (SI-SET-LRP). The resulting materials were proposed to not only provide steric stabilization of colloidal dispersions of bio-based CNCs, but also to enable nanotechnological systems that may benefit from thermo-responsive functionalities. The aim of the current study was therefore to probe the thermo-responsive behavior of poly(NiPAAm) brushes grafted from CNCs (poly(NiPAAm)-g-CNCs) in aqueous dispersion and as spin-coated films. As such, unmodified and grafted CNCs were probed and compared using rheometry, light transmittance, and colloidal probe microscopy (CPM).

7.2 Experimental

Materials. Pure ramie fibers were obtained from Stucken Melchers GmbH & Co. (Germany). 2-bromoisobutyryl bromide (BriB), 2-dimethylaminopyridine (DMAP), tetrahydrofuran (THF, 99\%), N-isopropylacrylamide (NiPAAm), copper (I) bromide and \(N,N,N',N''\)-pentamethyldiethylenetriamine (PMDETA), were all obtained from Sigma-Aldrich. Sulfuric acid (95\%), acetic acid (glacial), triethylamine (TEA, 99.5\%), acetone (99\%), ethanol (95\%), methanol (99\%), sodium hydroxide pellets, and sodium chlorite were all purchased from Fisher Scientific. All solvents were dried over molecular sieves (3 Å, 4-8 mesh beads, Sigma-Aldrich) for 48 h before use.

Preparation of Cellulose nanocrystals (CNCs) and poly(NiPAAm)-g-CNCs. Details about this procedure can be found elsewhere.\textsuperscript{138}
Preparation of Cellulose nanocrystals (CNCs) Films by Spin-Coating. CNC dispersions were diluted to the desired concentration and spin coated directly onto UV-treated silicon wafers at 4000 rpm. The deposition of the dispersion was performed on a substrate which was then spun for ca. 30 seconds. The surfaces were then dried at 80 °C for 1 hour. In the case of unmodified CNCs, poly(ethylene imine) was used as an anchoring layer to the substrate.

Atomic Force Microscopy (AFM). The spin-coated CNC films were imaged using a scanning probe microscope (model Multimode Nanoscope IIIa, Digital Instruments) in tapping mode, which was equipped with an E or J scanner and with noncoated silicon-etched probes (model LTESP, Veeco).

Light scattering. Aqueous dispersions were first subjected to sonication using an ultrasonic microtip (Branson Sonifier S-450 D, Danbury, USA) and then constantly pumped through a multiple light scattering apparatus (Turbiscan Online by Formulaction, France) at a flow rate of 300 mL/min. The transmission (detection angle 0°) light flux was measured through the aqueous dispersions. The temperature of each dispersion was first equilibrated at 20 °C, followed by the placement of the flask into a water bath with a temperature setting of 50 °C and temperature and light transmittance was monitored simultaneously with time.

Rheology. Viscosity measurements of aqueous dispersions of CNCs and poly(NiPAAm)-g-CNCs were undertaken using a TA Instruments AR 2000 Rheometer. A Peltier plate and 25 mm 1.59° cone geometry was used at a constant shear rate of 150 s⁻¹. Viscosity was monitored at a temperature ramp range of 25 to 40 °C.
Surface Force Measurements with the Colloidal Probe Technique. Force measurements of spin-coated CNC films were performed in liquid using a scanning probe microscope (model Multimode Nanoscope IIIa, Digital Instruments) in contact mode, which was equipped with a J scanner and with triangular tipless silicon nitride probes with spring constants between 0.1-0.2 N/m (model NP-O10, Veeco) calculated by the thermal noise method, followed by the attachment of a 25-45 µm silica sphere according to the method of Ducker et al. Each probe was UV-treated and cleaned thoroughly with water:ethanol solution immediately before use. The forces were normalized with the radius of the used silica sphere. Scanning Probe Image Processor software (SPIP, Image Metrology, Denmark) was used to process force curves and calculate pull-off forces. The separation distances obtained were taken as relative to a hard wall contact.

7.3 Results and Discussion

Cellulose nanocrystals from ramie fibers were produced and characterized according to a procedure reported previously. The size of the obtained rod-like CNCs was determined to be 3-15 nm in diameter and 50-250 nm in length. Poly(N-isopropylacrylamide) was grafted from CNCs via surface-initiated single-electron transfer living radical polymerization (SI-SET-LRP). The grafting density was varied by using different amounts of the 2-bromoiodobutryl bromide initiator (BrI) with respect to the total hydroxyl groups in cellulose and denoted here as the molar ratio between initiator [Br] and anhydroglucose units [AGU] in CNCs ([Br]:[AGU]): 5:3, 5:4, 5:6, and 5:12. For simplicity, these are respectively referred to as very high (VHD), high (HD), medium (MD), and low (LD) density.
poly(NiPAAm)-grafted CNCs (poly(NiPAAm)-g-CNCs). To vary the chain length of the grafted polymer chains a series of poly(NiPAAm)-g-CNCs were prepared by varying the ratio between the immobilized initiator and the NiPAAm monomer at three ratios of monomer to total anhydroglucose units [NiPAAm]:[AGU]: 50:3, 75:3, and 100:3, in which the 5:4 [Br]:[AGU] (MD) graft density condition was used. These are thereafter referred to as low (LDP), medium (MDP), and high (HDP) degree of polymerization, respectively. A summary of the molecular weight and polydispersity index of each sample is shown in Table 7.1.

**Table 7.1** Molecular weights and polydispersity indices (Mw/Mn) of poly(NiPAAm) chains grafted from CNC surfaces via SI-SET-LRP.

<table>
<thead>
<tr>
<th>Molar ratio</th>
<th>(M_n)</th>
<th>(M_w)</th>
<th>(M_w/M_n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>12,170</td>
<td>25,770</td>
<td>2.117</td>
</tr>
<tr>
<td>MD</td>
<td>10,630</td>
<td>19,540</td>
<td>1.838</td>
</tr>
<tr>
<td>HD</td>
<td>15,630</td>
<td>34,060</td>
<td>2.179</td>
</tr>
<tr>
<td>VHD</td>
<td>23,820</td>
<td>41,090</td>
<td>1.725</td>
</tr>
<tr>
<td>LDP</td>
<td>10,630</td>
<td>19,540</td>
<td>1.838</td>
</tr>
<tr>
<td>MDP</td>
<td>40,330</td>
<td>85,450</td>
<td>2.119</td>
</tr>
<tr>
<td>HDP</td>
<td>42,860</td>
<td>90,840</td>
<td>2.12</td>
</tr>
<tr>
<td>Bulk poly(NiPAAm)</td>
<td>5,385</td>
<td>14,200</td>
<td>2.637</td>
</tr>
</tbody>
</table>

Thin films of cellulose nanocrystals were produced via spin-coating onto oxidized silicon wafers. In the case of unmodified CNCs anchoring polyethyleneimine was pre-
adsorbed on the wafers to increase adhesion of the sulfate-charged CNCs. Figure 7.1 shows the AFM height images obtained after air-drying the respective spin-coated films. In general, images obtained of unmodified CNCs showed uniform coverage over a number of locations. Poly(NiPAAm)-g-CNCs were spin-coated directly without the need of anchoring polymer due to the presence of secondary amine moieties. As expected, the uniformity of the films obtained varied greatly with various graft densities and degree of polymerization. In general, larger amounts of polymer grafted from the surfaces of CNCs produced larger aggregates during spin-coating process. The films were dried at room temperature overnight but similar aggregation behavior was observed when drying at 80 °C. It was hypothesized that with larger density of polymer grafts and higher degrees of polymerization, aggregation occurred in the precursor aqueous dispersion, and it was not necessarily related to the effect of drying after spin-coating. This hypothesis was later supported by the results from light transmittance discussed in the following paragraphs. The images of LD poly(NiPAAm)-g-CNCs showed similar uniformity to unmodified CNCs, however the grafted crystals were slightly broader and fuzzier in the edges, as seen in Figure 7.1. This fuzziness increased consistently with increasing grafting density and Figure 7.1(f) of HDP shows poly(NiPAAm)-g-CNCs without uncertainty a polymer “halo” surrounding the nanoparticles that is even more pronounced in the respective phase image, Figure 7.1(g). This may be taken as indication of the presence of a soft polymer layer around the CNCs. MD grafted CNCs showed a similar trend in uniformity, although small aggregates were more prominent. In the case of HD grafted CNCs, spin-coating was unsuccessful most likely due to very large
aggregates formed in dispersion which could not adhere to the oxidized silicon wafer surface. VHD grafted CNCs gave films with a distribution of small aggregates as shown in Figure 7.1(e). Overall, it was observed that the degree of polymerization had a pronounced effect on the aggregate sizes of spin-coated films. MDP and HDP grafted CNCs yielded aggregates a few microns in size, most likely due to the screening of electrostatic forces of sulfate groups by longer poly(NiPAAm) chains.

![AFM height images](image)

Figure 7.1 AFM height images (5 x 5 µm) of spin-coated films of unmodified CNCs (a) and poly(NiPAAm)-g-CNCs: LD (b), MD/LDP (c), VHD (d), MDP (e), and HDP (f). The inset in Figure 7.1f corresponds to an AFM phase image (1 x 1 µm) of the area indicated.

Aqueous dispersions of poly(NiPAAm)-g-CNCs were prepared and thermo-responsiveness analyzed by light transmittance. Aqueous dispersions contained in a 250 mL Erlenmeyer flask were pumped constantly at a rate of 300 mL/min through a temperature-
controlled light scattering chamber. The temperature of each dispersion was first equilibrated at 20 °C, followed by the placement of the flask into a water bath set at 50 °C and light transmittance was recorded every second over a 20 minute time period, while the system was heated. Although the same concentration was used for each dispersion, differences in the baseline % transmittance were noted due to differences in aggregation behavior of grafted CNCs at 20 °C. Figure 7.2(a) shows normalized % light transmittance versus temperature for unmodified CNCs and for six poly(NiPAAm)-g-CNCs samples. Unmodified CNCs showed no change in light transmittance with varying temperature, as expected (no thermal responsiveness). With the presence of poly(NiPAAm) brushes grafted from CNCs, dispersions in each case (varying graft density and degree of polymerization) showed a large shift in light transmittance; the onset of such change was close to the LCST of poly(NiPAAm) of 32 °C. This was taken as an indication of the thermo-responsiveness of the grafted CNCs which correlated well with the graft molecular weights shown in Table 7.1. In fact, in earlier work by Okahata et al. it was shown that poly(NiPAAm) maintains its thermo-responsive behavior when tethered to a substrate. The flocculation behavior of CNCs in aqueous dispersions depends highly on the surface interactions between adjacent CNCs. Such interactions are expressed in the observed results. For example, LD poly(NiPAAm)-g-CNCs containing the smallest amount of grafted polymer showed thermal-response and the smallest aggregates above the LCST. Larger flocs were observed above the LCST as the graft density and/or degree of polymerization were increased, which indicated significant changes in the respective surface forces. With increased grafted amounts of
polymer on CNCs, the electrostatic stabilization of the CNC dispersion was hindered. Light scattering did not resolve the forces that induced the more pronounced aggregations above the LCST of poly(NiPAAm) brushes; this will be discussed later in the section on surface interaction forces.

As previously shown, the LCST of free poly(NiPAAm) depends highly on the ionic strength of aqueous solutions due to the Hofmeister effect on dehydration of polymer chains. This effect can be translated to poly(NiPAAm) chains tethered to a surface which have been shown to be without exception of the Hofmeister effect in our earlier reports. 

Figure 7.2(b) shows the LCST versus salt (NaCl) concentration of MD poly(NiPAAm)-g-CNCs, which decreased similarly as in the case of bulk polymer solutions. However, the decreasing trend in the LCST was more marked when compared to previous reports of the Hofmeister effect on poly(NiPAAm) chains. An LCST as low as ca. 23 °C was achieved with the addition of 1 M NaCl, which was significantly lower than previous reports of free and tethered poly(NiPAAm) chains at ca. 28 °C. All the remaining grafted CNCs samples showed a similar trend.
Figure 7.2  Normalized light transmittance versus temperature of aqueous dispersions of unmodified CNCs and poly(NiPAAm)-g-CNCs (LD, MD/LDP, HD, VHD, MDP, and HDP) (a). Figure (b) corresponds to the Lower Critical Solution Temperature of aqueous dispersions of MD poly(NiPAAm)-g-CNCs at various NaCl concentrations. The lines are added to guide the eye.
Viscosity measurements were performed to provide further evidence as to the results of light scattering experiments. After a desired concentration of aqueous dispersion was equilibrated on a Peltier plate at 25 °C, the relative viscosity was monitored over a temperature ramp to 40 °C at a constant shear rate. The results of the rheological measurements are summarized in Figure 7.3. Typically, the relative viscosity of colloidal dispersions in a Newtonian fluid decreases as the temperature is raised due to increased fluidity most simply explained by Arrhenius behavior of molecular kinetics. Thus, a dispersion of unmodified CNCs showed this behavior as seen in Figure 7.3. The viscosity in aqueous solution of free poly(NiPAAm) that was synthesized previously was measured to confirm the LCST of ca. 32 °C. In stark contrast to the behavior of neat CNCs, poly(NiPAAm)-g-CNCs showed significant increases in viscosity as the temperature approached the LCST of the grafted polymer brushes. Figure 7.3(a) indicated a response to temperature at ca. 30-32°C for various graft densities of poly(NiPAAm) brushes on CNCs. Below the LCST of grafted poly(NiPAAm) brushes, aqueous dispersions of poly(NiPAAm)-g-CNCs displayed viscosity values similar to that of the solvent (water), however as the LCST was reached, the dehydration of polymer brushes on the surfaces of CNCs created more hydrophobic dispersed nanoparticles, and thus the onset of colloidal instability produced a ca. two-fold increase in apparent viscosity. Similarly in Figure 7.3(b), as the degree of polymerization of the grafts was varied, increased viscosity of dispersions was noted at ca. 30-32 °C. Although it was unclear whether differences in LCST from that of free poly(NiPAAm) derived from differences in molecular weight or graft density, it was
noted that with increased amounts of poly(NiPAAm) present on CNCs surfaces as determined previously by XPS,\textsuperscript{138} the thermo-responsive behavior was more similar to that of free poly(NiPAAm).
Figure 7.3  Viscosity of CNC dispersions at different temperatures normalized by the viscosity of the respective dispersions at 25 °C of poly(NiPAAm)-g-CNCs with various graft densities (a) and degrees of polymerization (b). The viscosity curves for dispersions of neat CNC and free poly(NiPAAm) solution are included as reference. The lines were added as guide to the eye.
The responsiveness of poly(NiPAAm)-g-CNCs was not only affected by temperature, but by ionic strength as well, as shown previously by light transmittance (Figure 7.2b). Although the results of light transmittance clearly showed the effect of ionic strength on the LCST of grafted poly(NiPAAm) brushes, colloidal probe microscopy in aqueous media was further employed to shed light on the nature of the interfacial forces close to poly(NiPAAm) grafted from CNCs. Increasing the ionic strength of poly(NiPAAm)-g-CNCs dispersions not only decreased the Debye length of the electrostatic double-layer derived from sulfate groups on CNCs surfaces, but also induced a partial collapse of poly(NiPAAm) brushes by means of the Hofmeister effect, i.e., electrolytes disrupted the solvent’s ability to fully dissolve polymer chains.

To further unveil the nature of the interactions, DLVO (electrostatic and van der Waals) and non-DLVO (steric), the forces between a silica sphere and a surface coated with unmodified CNCs and poly(NiPAAm)-g-CNCs were probed by CPM at different ionic strengths. CPM could also be utilized to study the LCST behavior of poly(NiPAAm) brushes at various temperatures in aqueous media, however precise temperature control of the liquid cell chamber proved to be challenging considering the heat produced by the laser. Films of unmodified CNCs, LD, and MD poly(NiPAAm)-g-CNCs were uniform enough for force measurements (see Figure 7.1a-c), however, during attempts to measure the surface forces of LD poly(NiPAAm)-g-CNCs, it was evident that these spin-coated films were unstable during the course of the measurement, most likely due to a low graft density of poly(NiPAAm) brushes on CNCs that could not remain adhered to the silicon wafer substrate after
immersion in water. Since a stable and even coating is essential for successful force measurements, unmodified CNCs and the MD systems were chosen (Figure 7.1b). Other surfaces were not tested due to high roughness and lack of uniformity. Shown in Figure 7.4 are typical semi-log profiles of force versus separation of spin-coated films of MD poly(NiPAAm)-g-CNCs and unmodified CNCs in 10 mM NaCl. The onset of repulsive forces was observed at a relative surface separation of about 20-30 nm. The semi-log plot of normalized force versus separation between the surface coated with unmodified CNC and silica gave a linear relationship. This can be ascribed to osmotic repulsion between overlapping counter-ion clouds close to the negatively charged CNC and oxidized silica. The poly(NiPAAm)-g-CNCs on the other hand showed a non-linear trend. The repulsion was hypothesized in this case to be due to loss of conformational degree of freedom for the grafted polymer chains when the silica sphere approached.
Figure 7.4  CPM interaction forces versus separation between a silica sphere against a flat layer spin-coated on silica wafers consisting of unmodified CNCs (○) and MD poly(NiPAAm)-g-CNCs (●). The best fit to a model of steric interactions, Alexander–de Gennes, is shown in the case of MD poly(NiPAAm)-g-CNCs (see grey dash line) while a linear fit to indicate the expected slope for DLVO forces is included in the case of unmodified CNCs (see black dash line). The forces were measured in liquid medium, across aqueous 10 mM NaCl solution and normalized with the radius of the probing silica sphere.

Shown in Figure 7.5 are the normalized forces versus separation distance of an unmodified CNC film at various NaCl concentrations (10, 100, and 250 mM with corresponding DeBye lengths\textsuperscript{107} of 3.0, 0.96, and 0.61 nm at 25 °C, respectively). As salt concentration was increased, the decay length of the repulsion decreased, supporting the assumption that the forces are in this case of electrostatic origin. The grafting of poly(NiPAAm) brushes from CNCs changed the interfacial interactions of the silica sphere.
with the poly(NiPAAM)-g-CNC coated surface. As shown in Figure 7.6, a semi-log plot showed clearly repulsion derived from steric forces and the effect of an increased ionic strength. At a low salt concentration (10 mM), poly(NiPAAm) brushes were in a highly extended state in solution, which gave steric repulsion starting at ca. 25 nm relative separation. According to the average molecular weight of the MD grafted poly(NiPAAm) chains (Table 7.1), the length of a fully-stretched polymer chain was calculated to be ~14.5 nm, however poly(NiPAAm) brushes were expected to be in a coiled confirmation, thus one could suspect a smaller brush layer thickness. Additionally, poly(NiPAAm) brushes were expected to be coated on the entire surface of CNCs, thus a partially swollen polymer layer may have existed adsorbed to the silica substrate. The observed onset of steric repulsion at ~25 nm may have been an indication that a partially swollen polymer layer existed underneath CNCs while adhered to the silica surface in addition to an extended polymer brushes free for interaction with the silica probe. As the concentration of salt was increased ten-fold, steric forces were not apparent until ca. 18 nm separation, which indicated a partial collapse of grafted poly(NiPAAm) brushes and the adsorbed polymer layer (see Figure 7.6). At 250 mM, steric repulsion was not evident until separations of ca. 10 nm, revealing increased collapse of the grafted polymer chains. Although, Alexander–de Gennes best fit curves were applied to each salt concentration to verify forces of steric origin the model failed at large separation. In the past, Alexander–de Gennes theory was developed strictly for tethered polymers at high graft densities,\textsuperscript{134,135} but it has been shown to be applicable also to adsorbed polymer layers.\textsuperscript{166} The graft densities in poly(NiPAAm)-g-CNCs were expected
to be relatively low, given that the polymers were tethered to CNCs. However, in a study performed by Notley\textsuperscript{133} on the conformation of adsorbed poly(NiPAAm) layers in a binary solvent mixture, steric forces were apparent, and it was determined that the Alexander de Gennes model could be used as a reasonable fit at intermediate to large surface separations, even with low graft densities. In addition, steric forces were evident at ca. 100 nm, however in the current study, steric forces were not evident until ca. 30 nm, which can be explained by the fact that an asymmetric system was dealt with (brushes only present on one of the surfaces) and also because grafts of much smaller molecular weights (10 kDa) were used.

![Graph showing CPM interaction forces normalized with the radius of the silica sphere probe as a function of separation distance against a flat film of unmodified CNCs. The forces were measured in aqueous NaCl solutions of various concentrations, 10 mM (\textbullet), 100 mM (\textcircled{v}) and 250 mM (\textbullet).](image)

Figure 7.5 CPM interaction forces normalized with the radius of the silica sphere probe as a function of separation distance against a flat film of unmodified CNCs. The forces were measured in aqueous NaCl solutions of various concentrations, 10 mM (\textbullet), 100 mM (\textcircled{v}) and 250 mM (\textbullet).
Figure 7.6 CPM interaction forces between a silica sphere probe and flat films of MD poly(NiPAAm)-g-CNCs as a function of the separation distance. The forces were measured in aqueous NaCl solutions of various concentrations (10 mM (●), 100 mM (○) and 250 mM (●)) and were normalized with the radius of the silica sphere. The corresponding best fits to the Alexander–de Gennes equation are added as a reference (see respective dash lines).

Shown in Figure 7.7(a-b) are linear force versus separation distance profiles of a silica colloidal sphere approaching (IN) to and retracting from (OUT) a spin-coated film of MD poly(NiPAAm)-g-CNCs in 10 mM (a) and 100 mM NaCl aqueous solution (b). Some initial attraction was observed upon approaching extended poly(NiPAAm) brushes, most likely due to bridging attraction between the poly(NiPAAM) brushes containing secondary amine moieties and the anionic silica sphere in aqueous medium. As mentioned earlier, poly(NiPAAm)-g-CNCs readily adsorbed to oxidized silicon wafers without the use of
anchoring polymer, thus an attraction between poly(NiPAAm) brushes and the silica colloidal probe was expected. Hydrogen bonding between the amide functionalities of poly(NiPAAm) has been the proposed mechanism of adsorption to silica surfaces below the LCST.\textsuperscript{133, 167} Attraction was detected at separations of ca. 60 nm at low electrolyte concentration. A long range attractive force was also noted for the case of adsorbed poly(NiPAAm) layers in a partially collapsed state in a previous study by Notley.\textsuperscript{133} At ca. 30 nm separation, repulsion between the silica sphere and the grafted CNCs was observed. Significant adhesion between the silica sphere and grafted CNCs was evident at 10 mM NaCl, seen in Figure 7.7(a), most likely due to strong interactions between poly(NiPAAm) brushes and the silica surface. Increasing the electrolyte concentration to 100 mM decreased the adhesion between the silica surface and grafted CNCs and no attraction on approach was noted (Figure 7.7 b). This was explained by the partial collapse of poly(NiPAAm) brushes. From the retraction force curves, pull-off forces were calculated using the SPIP code (see Table 7.2). Overall, as the salt concentration was increased, a partial collapse of poly(NiPAAm) brushes took place and a decrease in adhesion was observed. Partial collapse of poly(NiPAAm) brushes was believed to have decreased non-electrostatic polymer bridges formed between the silica sphere and spin-coated surface. Further, this finding could have been due to ion adsorption at hydrogen bonding sites on NiPAAm monomers leading to decreased adsorption of poly(NiPAAm) brushes to the silica probe as has been described in similar systems.\textsuperscript{168, 169} In addition, Meagher et al.\textsuperscript{170} noted unexpected attractive interactions between silica and low graft density poly(ethylene oxide) (PEO) brushes which was
hypothesized to originate from the adsorption of PEO chains onto the silica probe during approach and that increased adsorption occurred with decreased separation distance. Also, a small fraction of longer PEO chains due to a degree of polydispersity in the coating could have given rise to force interactions at farther than expected separations. In another study, Goodman et al.\textsuperscript{171} found that low graft density polymer brushes gave rise to attractive forces that became repulsive upon increased graft density, which was explained by a decreased mobility of the polymer brushes within the layer. In the present case, poly(NiPAAm) brushes also readily adsorbed to silica surfaces and were found to have 1.8 polydispersity index (see Table 7.1) that could have caused some long distance interactions. As the ionic strength was increased, poly(NiPAAm) brush collapse was evident which also decreased polymer brush mobility. This effect could explain the decreased attractive forces upon approach of the silica probe and thus, the decreased pull-off force observed with increased ionic strength. In the cases of unmodified CNCs films, a minimal adhesion was observed with pull-off forces typically less than 1 nN (see Table 7.2).
Figure 7.7 CPM normalized interaction force versus separation distance for a silica sphere probe against a flat film of MD poly(NiPAAm)-g-CNCs measured in NaCl aqueous solutions of 10 mM (a), and 100 mM (b) concentrations. The force profiles recorded upon approach (In, ●), and on separation (Out, ○) are included in each case.
Table 7.2  Pull-off (adhesion) forces of unmodified CNCs and MD poly(NiPAAm)-g-CNCs against silica in aqueous solution of various salt concentrations.

<table>
<thead>
<tr>
<th>[NaCl]</th>
<th>Adhesion (nN), unmodified CNCs</th>
<th>Adhesion (nN), MD poly(NiPAAm)-g-CNCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM</td>
<td>-0.4 ± 0.3</td>
<td>-9.9 ± 1.0</td>
</tr>
<tr>
<td>100 mM</td>
<td>-0.7 ± 0.3</td>
<td>-5.8 ± 1.0</td>
</tr>
<tr>
<td>250 mM</td>
<td>-0.6 ± 0.4</td>
<td>-5.5 ± 0.6</td>
</tr>
</tbody>
</table>

Cellulose nanocrystals are expected to be interesting substrates for functional surface modifications, especially due to their low cost and abundance as compared to other varieties of nanoparticles. By means of stimuli-responsive polymer grafting, their self-assembly behavior can be controlled, as shown in the preceding discussion. In general, higher grafting densities and longer polymer brushes yielded larger aggregations or flocs after the LCST of poly(NiPAAm) was reached. We have demonstrated that ultrathin films of grafted CNCs give rise to steric repulsion that responded similarly to changes in ionic strength as observed in experiments in solution. Colloidal probe microscopy allowed better understanding of the origins of repulsion forces in these systems. The onset of steric repulsion was correlated with the theoretical length of the free polymer brushes grafted from cellulose nanocrystals and a partially swollen polymer layer adsorbed to the silica substrate. Thus, by varying the grafting density and/or molecular weight of stimuli-responsive polymer brushes grafted from CNCs, their surface interaction forces in dispersion and as surface coating could be controlled by
temperature and/or ionic strength. In this way, phenomena such as rheological behavior, adhesion, lubrication, and drug-release properties could also be tuned making these types of functional biomaterials especially interesting in a broad range of applications.

7.4 Conclusions

We have demonstrated the thermo-responsive behavior of cellulose nanocrystals grafted with poly(NiPAAm) brushes. AFM topographical images showed initially differences in the aggregation behavior of poly(NiPAAm)-g-CNCs films produced by spin-coating. In addition, a polymer ‘halo’ was observed, especially for brushes with the highest degree of polymerization, ca. 40 kDa. The poly(NiPAAm)-g-CNCs dispersion behavior was further elucidated by light transmittance which showed a precise control over grafted CNCs aggregation following the LCST of poly(NiPAAm) brushes. The LCST of grafted poly(NiPAAm) brushes was decreased upon increasing ionic strength, as expected. In all cases, rheological measurements showed increased viscosities of grafted CNCs as temperature was ramped above the LCST as well. In the aforementioned experiments, unmodified CNCs showed no thermo-responsive behavior. Spin-coated films of MD poly(NiPAAm)-g-CNCs were subjected to colloidal probe microscopy in liquid and showed steric surface forces and response to ionic strength which was correlated with the theoretical poly(NiPAAm) chain length grafted from CNCs and a partially swollen polymer layer adsorbed on the silica surface. Films of unmodified CNCs showed forces of primarily electrostatic origin, as expected due to the presence of negatively-charged sulfate ester
groups on CNCs surfaces. Due to changes in ionic strength, differences in adhesion between
the silica colloidal probe and MD poly(NiPAAm)-g-CNCs was observed, and thus pull-off
forces were calculated which showed decreased adhesion as ionic strength was increased.
Pull-off forces calculated for films of unmodified CNCs showed very little differences upon
increased ionic strength. It is expected that this study on the aggregation behavior and
interaction forces of cellulose nanocrystals grafted with stimuli-responsive polymer brushes
will provide the basis for the development of novel nanomaterials derived from abundant
natural resources and bring forth new and advanced applications of cellulosic nanoparticles.

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8 PICKERING EMULSIONS STABILIZED BY CELLULOSE NANOCRYSTALS GRAFTED WITH THERMO-RESPONSIVE POLYMER BRUSHES

8.1 Introduction

The irreversible adsorption of solid particles at the oil-water interface has been known for over a century.\textsuperscript{172} Such phenomenon is critical in the stabilization of surfactant-free emulsions, in the so-called Pickering emulsions,\textsuperscript{173} which have recently found use in applications spanning cosmetics, biomedical, and food products.\textsuperscript{174} The high stability of emulsions stabilized by colloidal particles is derived from the energy barrier required to remove the particles from the interface in order to facilitate droplet coalescence. Pickering emulsions have a number of advantages over conventional surfactant-stabilized emulsions in that they can reduce tissue irritation and their viscosity can be easily adjusted by solid content and/or solid type.\textsuperscript{175} Nanoparticles modified with surface-active polymers have been shown to be very efficient emulsifiers with nanoparticle concentrations as low as 0.04 wt \\textsuperscript{\%}.\textsuperscript{176} In addition, the colloidal assembly of solid particles within Pickering emulsions can be used as templates for advanced materials such as Janus colloids,\textsuperscript{177} composite microcapsules,\textsuperscript{178-180} or microspheres.\textsuperscript{181} In particular, recent efforts focused on the development of environmentally friendly alternatives have led to bio-based materials such as renewable nanocomposite foams\textsuperscript{182} and bio-inorganic microcapsules\textsuperscript{183} from Pickering emulsion templates.

The development of renewable biomaterials for advanced applications has been recently gained momentum in the research community.\textsuperscript{13, 14} Cellulose, in particular, is an
attractive material source due to its availability, biodegradability, renewability, and amenability for surface modification. Cellulose fibers, containing crystalline and amorphous regions, can be subjected to strong acid hydrolysis yielding rod-like crystals having even more unique surface, optical and mechanical properties.\textsuperscript{15, 16} Cellulose nanocrystals (CNCs) or nanocrystalline cellulose (NCC) produced by sulfuric acid hydrolysis yields aqueous suspensions electrostatically-stabilized by sulfate ester groups installed on the surface which promotes uniform aqueous dispersions,\textsuperscript{17} and the ability to self-assemble into chiral nematic liquid crystals.\textsuperscript{18} The utility of cellulosic nanoparticles as the stabilizer of Pickering emulsion is expected to be advantageous over some inorganic nanoparticles when biocompatibility, degradability, density and cost issues are considered.

Due to the hydrophilicity of cellulose surfaces, surface chemical modification is needed in order to impart an appropriate balance of surface energies or amphiphilicity, especially when stabilizing water-in-oil (w/o) emulsions.\textsuperscript{184} In the past, microcrystalline cellulose (MCC) has been shown to stabilize oil-in-water (o/w) emulsions through network formation around oil droplets without surface modification.\textsuperscript{185, 186} The long-term stabilization of Pickering emulsions and foams with microparticles from hydrophobic cellulose has been shown by Wege, et al.\textsuperscript{187} Ougiya et al. first studied the use of unmodified bacterial cellulose to stabilize o/w emulsions in 1997.\textsuperscript{188} In addition, a number of patents have been filed involving the use of fibrillated cellulose as emulsion stabilizers.\textsuperscript{189, 190} Furthermore, recent efforts have focused on the preparation of w/o emulsions by using silylated micro- or nanofibrils from wood pulp and bacterial cellulose.\textsuperscript{182, 191-194}
As previously noted, Pickering emulsifiers irreversibly adsorb at the oil-water interface and require a much higher energy for desorption as compared to conventional surfactants. The use of responsive polymer grafts that react to changes in their environment, such as light, heat, ionic strength, and pH\textsuperscript{84-86} can be used as a means to control Pickering emulsion stability.\textsuperscript{195} Thermo-responsive polymers may have an upper or a lower critical solution temperature (UCST and LCST), depending on entropic changes and phase behaviors associated with their molecular structure. A number of researchers have demonstrated thermo-responsive Pickering emulsions mostly by the use of (2-(dimethylamino)ethyl methacrylate)-carrying silica and polystyrene latex nanoparticles.\textsuperscript{195, 196} Poly(N-isopropylacrylamide) (poly(NiPAAm)), one of the most studied thermo-responsive polymers, has an LCST in aqueous solution ranging between 30 to 35 °C.\textsuperscript{88} Above the LCST poly(NiPAAm) phase-separates due to thermal-driven chain dehydration. Since the LCST of poly(NiPAAm) is near the physiological temperature of ca. 37 °C, it has been extensively used in applications involving controlled drug release.\textsuperscript{87} In addition, poly(NiPAAm) has been shown to be surface-active due to the presence of amide and isopropyl functional groups.\textsuperscript{197-199} The surface activity of particles containing poly(NiPAAm) functionalities have been confirmed at the air-water interface.\textsuperscript{200, 201} Pickering emulsions stabilized by particles carrying copolymers of NiPAAm, methacrylic acid,\textsuperscript{202, 203} and acrylic acid\textsuperscript{204} have been demonstrated. Most recently, silica\textsuperscript{205} and polystyrene\textsuperscript{206} particles modified by poly(NiPAAm) have been developed as thermo-responsive Pickering emulsion stabilizers.
In previous publications, we have reported on the synthesis of poly(NiPAAm) brushes grafted from CNCs via surface-initiated single-electron transfer living radical polymerization (SI-SET-LRP) and their surface interaction forces. The thermo-responsive behavior of poly(NiPAAm)-g-CNCs and response to ionic strength was demonstrated. The aim of this study was to demonstrate the use of cellulose nanocrystals grafted with thermo-responsive functionalities as stabilizers of o/w Pickering emulsions. The effect of solids content, temperature and ionic strength on emulsion stability and droplet size was investigated. The self-assembly of poly(NiPAAm)-g-CNCs at the oil-water interface was visualized via Freeze-Fracture Electron Microscopy (FFEM) and Scanning Electron Microscopy (SEM).

8.2 Experimental

Materials. Pure ramie fibers were obtained from Stucken Melchers GmbH & Co. (Germany). 2-bromoisobutyryl bromide (BriB), 2-dimethylaminopyridine (DMAP), tetrahydrofuran (THF, 99%), N-isopropylacrylamide (NiPAAm), copper (I) bromide and \( N,N,N',N'',N''' \)-pentamethyldiethylenetriamine (PMDETA), were all obtained from Sigma-Aldrich. Sulfuric acid (95%), acetic acid (glacial), triethylamine (TEA, 99.5%), acetone (99%), ethanol (95%), methanol (99%), heptane (99%), sodium hydroxide pellets, and sodium chlorite were all purchased from Fisher Scientific. All solvents were dried over molecular sieves (3 Å, 4-8 mesh beads, Sigma-Aldrich) for 48 h before use.

Cellulose nanocrystals and surface functionalization. Poly(NiPAAm) chains were grafted from cellulose nanocrystals (CNCs) by Surface-Initiated Single-Electron Transfer Living
Radical Polymerization (SI-SET-LRP). Details about this procedure and main properties of the obtained poly(NiPAAm)-g-CNCs can be found in our earlier reports.\textsuperscript{138,207}

\textit{Water Contact Angle.} The initial water contact angle (WCA) of spin-coated films of both unmodified and poly(NiPAAm) grafted CNCs (poly(NiPAAm)-g-CNCs)\textsuperscript{207} were determined using a Phoenix 300 contact angle analyzer (SEO Co. Ltd, Lathes, South Korea). Contact angles were measured at room temperature and immediately after being placed in an oven at 80 °C for 1 hour.

\textit{Preparation of Pickering Emulsions.} All emulsions (total volume 10 mL) were prepared with a water-to-oil ratio (WOR) of one containing given concentrations of poly(NiPAAm)-g-CNCs in the aqueous phase and emulsified with an Ultraturrax T18 basic homogenizer (IKA, Wilmington, NC USA) at 6,000 rpm for 60 seconds at 25 °C. Volume fractions of the organic and aqueous phases were noted 30 minutes and 4 days after emulsification at 25 °C. The stability index of Pickering emulsions was determined by the change in volume fraction of emulsified phase from 30 minutes after preparation to 4 days. The type of emulsion was determined by the drop test. A drop of emulsion was added to neat water and neat to heptane to observe its dispersion ability. An oil-in-water emulsion disperses readily in water while a water-in-oil emulsion disperses readily in oil.

\textit{Optical Microscopy.} Emulsion droplets were visualized using an Olympus BH2-UMA optical microscope (Olympus, Center Valley, Pennsylvania, USA). Emulsion droplets were placed directly onto a glass microscope slide and viewed under 5-50x magnification.
**Particle Size Analysis.** Particle size distribution of emulsion droplets were obtained using a HORIBA LA-910 Laser Scattering Particle Size Distribution Analyzer (HORIBA Instruments, Inc., Irvine, CA USA) using the refractive index of heptane (1.39). The particle size distribution was calculated on a number average basis. The reservoir was filled with water, and 1 mL of emulsion phase was added and circulated during the course of the measurement.

**Rheology.** Viscosity measurements of unmodified CNCS and Pickering emulsions with poly(NiPAAm)-g-CNCs were undertaken using a TA Instruments AR 2000 Rheometer. A Peltier plate and 25 mm 1.59° cone geometry was used to obtain viscosity at a temperature ramp range of 25 to 45 °C at constant shear rate of 100 s⁻¹.

**Freeze-fracture Electron Microscopy.** Emulsion samples were placed in a gold freeze-fracture carrier (Technotrade International, Manchester, NH USA) and frozen by placing into re-solidifying propane cooled with liquid nitrogen. After knife fracture in the freeze-fracture instrument (CFE-50; Cressington, Watford, UK), emulsion samples were subjected to etching to remove ice with a liquid-nitrogen-cooled knife and raising the stage temperature. Shadows were deposited with an electron gun (2-3 nm Pt/C; 45°) and replicated (6-8 nm carbon; 90°). Freeze-fractured samples were deposited on Formvar-coated copper mesh grids and observed with a Hitachi HF-2000 transmission electron microscope (TEM) operated at an accelerating voltage of 80 kV.
Scanning Electron Microscopy. A Hitachi S-3200N variable pressure scanning electron microscope (SEM) was used to image freeze-fractured emulsion samples. The samples deposited on Formvar-coated copper mesh grids were directly observed under a microscope working distance between 3 and 60 mm using an accelerating voltage from 0.3 to 30 kV.

8.3 Results and Discussion

Cellulose nanocrystals from ramie fibers were produced and characterized according to a procedure reported previously. The size of the obtained rod-like CNCs was determined to be 3-15 nm in diameter and 50-250 nm in length. Poly(N-isopropylacrylamide) was grafted from CNCs via surface-initiated single-electron transfer living radical polymerization (SI-SET-LRP) with various reaction conditions producing a variety of graft densities and molecular weights. Of the various molecular weights of the polymer grafts obtained in the previous study, one case was examined as Pickering emulsion stabilizer on the basis of work by Saigal et al. in which silica nanoparticles with the lowest graft densities of (2-(dimethylamino)ethyl methacrylate) (PDMAEMA) were shown to be the most robust and efficient emulsifiers. From the high emulsifying efficiency of low graft density nanoparticles, it was implied that they had a high-affinity adsorption isotherm at the oil-water interface, which required low particle concentrations. The CNCs utilized in this study were carrying poly(NiPAAm) grafts polymerized from CNCs containing a molar ratio between initiator [Br] and anhydroglucose units [AGU] in CNCs ([Br]:[AGU]) of 5:3 and a number average molecular weight ($M_n$) of 12,170 g/mol.
Table 8.1 Initial water contact angle (WCA) and heptane contact angle (HCA) of spin-coated films of unmodified and grafted CNCs with temperature.

<table>
<thead>
<tr>
<th>Sample</th>
<th>WCA (T&lt;LCST °C)</th>
<th>WCA (T&gt;LCST °C)</th>
<th>HCA (T=25 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified CNCs</td>
<td>22</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Poly(NiPAAm)-g-CNCs</td>
<td>38</td>
<td>44</td>
<td>14</td>
</tr>
</tbody>
</table>

Pickering emulsions were prepared by first sonicating in aqueous solution a given amount of poly(NiPAAm)-g-CNCs, followed by mixing with an equivalent amount of heptane. In the lowest concentration case (0.05 %), the effect of ionic strength of the aqueous media was also investigated. Emulsions made with unmodified CNCs were used as control. After homogenization for one minute at 6,000 rpm, volume fractions of emulsions were measured for stability after 30 minutes and 4 days.

To verify the wettability of unmodified CNCs and poly(NiPAAm)-g-CNCs with water and heptane, contact angle measurements were performed on spin-coated films. As shown in Table 8.1, the initial water contact angle (WCA) of unmodified CNCs was 22° at ambient conditions. After being placed in an oven at 80 °C for one hour, the contact angle decreased slightly as expected due to the decreased surface tension of water at higher temperatures. In the case of poly(NiPAAm)-g-CNCs, the opposite effect was noted with temperature. Initially, below the LCST of poly(NiPAAm), the WCA was determined to be 38°, however, after being heated above the LCST, the WCA increased to 44°, which was expected due to the insolubility of poly(NiPAAm) in water above the LCST. This led to a
decreased wettability of the spin coated film, thus a slightly less hydrophilic surface. Additionally, the heptane contact angle was measured to determine nanoparticle wettability with the oil phase of subsequent Pickering emulsions. Due to the low surface tension of heptane, spin coated films of unmodified and grafted CNCs showed low heptane contact angles ca. 14-16°. The slightly lower HCA of poly(NiPAAm)-g-CNCs was explained by the increased wettability of poly(NiPAAm) grafts due to the presence of isopropyl groups within polymer chains.

It is well known that Pickering emulsion stability can depend on a number of parameters, such as particle concentration, surface activity and ionic strength. The concentration of nanoparticles utilized will determine the droplet size formed and thus stability of emulsion droplets to coalescence. Ionic strength plays an important role stabilizing electrostatic interactions that may occur between adjacent nanoparticles at the oil-water interface. In the case of thermo-responsive polymer grafted nanoparticles, such as poly(NiPAAm), ionic strength plays an additional role in the solvation of polymer brushes in aqueous media. These two key factors were chosen as variables to study the stability of Pickering emulsions stabilized by poly(NiPAAm)-g-CNCs. In addition, attempts were made to use unmodified CNCs as Pickering emulsion stabilizers as a control. Figure 8.1 shows examples of the appearance of Pickering emulsions without stabilizer, with unmodified CNCs, and with poly(NiPAAm)-g-CNCs.

Nanoparticle concentration. Shown in Table 8.2 are the compositions and stability indices calculated for heptane-in-water emulsions containing poly(NiPAAm)-g-CNCs (0.05
to 0.5 wt % in the aqueous phase) and unmodified CNCs. Interestingly, equivalent concentrations of unmodified CNCs were not able to stabilize the emulsions with heptane and oil (extreme, very high concentrations of CNCs were required in order to produce a small fraction of an emulsified phase, 2.2% or higher, as reported in Table 8.2). This is explained by the hydrophilicity of the CNCs which enabled a good dispersion of the nanoparticles in water but hampered their ability to adsorb and stabilize the oil-water interface. On the basis of favorable interaction between water and poly(NiPAAm) a preferential affinity of the grafted CNCs is expected with the aqueous phase. Also, according to the Bancroft rule, for conventional emulsions and the water contact angle of the grafted CNCs, it was expected that o/w emulsions were formed. This is in fact what was observed, i.e., o/w emulsions were obtained in all cases as confirmed by the drop tests.

![Figure 8.1](image)

Figure 8.1 Appearance of heptane and water without stabilizer (left), Pickering emulsions with 2.2 % unmodified CNCs (center) and with 0.05 % poly(NiPAAm)-g-CNCs (right) after >3 months at ambient conditions.
After 30 minutes, emulsions homogenized with the largest concentration of grafted CNCs (0.5 %) produced the largest volume fraction of emulsified phase as indicated by Table 8.2. At lower CNC concentrations, the volume fraction of emulsified phase decreased, as expected due to coalescence of larger oil droplets formed (see Figure 8.2A). The lower concentrations of grafted CNCs (0.05 and 0.1 %) emulsified the most oil at 94 and 95 %, respectively. The amount of oil emulsified was assumed to be the volume fraction of oil not present in the oil phase after emulsification. This was expected due to lower concentrations of nanoparticles being available to stabilize oil-water interfaces leading to larger droplet sizes (discussed later). Higher concentrations yielded smaller droplets, thus a decreased total volume of oil emulsified. After 4 days at ambient conditions, Pickering emulsions prepared with 0.25 % grafted CNCs showed the highest stability index. After 4 days, emulsions stored at ambient conditions showed very little, if any, phase separation and have been stable for up to three months at the time of writing this manuscript.

Emulsion samples were subjected to drop sizing by laser light scattering (Figure 8.2). In these experiments only the emulsions stabilized with 0.25 and 0.5% of grafted CNCs were studied since these conditions ensured better stability of the droplets when they were diluted and dispersed in the aqueous medium flowing in the stirred cell used to measure light scattering intensity (the large emulsion droplets stabilized with low concentrations of grafted CNCs broke under shear). Emulsions (WOR=1) prepared with 0.25 and 0.5% of grafted CNCs showed similar mean drop diameters ca. 38 µm, however the larger concentration of grafted CNCs produced a more narrow particle size distribution.
Table 8.2  Phase composition and emulsion stability after emulsification of heptane (organic or oil phase, O) in water (W) at a water-to-oil ration of 1 (WOR=1). The systems were equilibrated at room temperature. The volume fraction of emulsified, middle phase (E) is reported along with the volume fractions separated on the top (oil phase) and in the bottom (aqueous phase).

<table>
<thead>
<tr>
<th>Grafted CNC, % of aqueous phase</th>
<th>Fraction of phase separated after 30 min equilibration, %</th>
<th>Fraction of phase separated after 4 days equilibration, %</th>
<th>O fraction in emulsion (a), v/v%</th>
<th>Stability index (4 days) (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O  E  W</td>
<td>O  E  W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>0  3  59  38</td>
<td>3  53  44</td>
<td>94</td>
<td>90</td>
</tr>
<tr>
<td>0.05</td>
<td>1  3  56  41</td>
<td>6  49  46</td>
<td>94</td>
<td>86</td>
</tr>
<tr>
<td>0.05</td>
<td>10  8  50  42</td>
<td>6  48  46</td>
<td>83</td>
<td>96</td>
</tr>
<tr>
<td>0.05</td>
<td>100 10  48  42</td>
<td>6  48  46</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>N/A  2  64  33</td>
<td>3  59  39</td>
<td>95</td>
<td>91</td>
</tr>
<tr>
<td>0.25</td>
<td>N/A  5  70  24</td>
<td>3  66  31</td>
<td>89</td>
<td>94</td>
</tr>
<tr>
<td>0.5</td>
<td>N/A  11  71  18</td>
<td>9  59  32</td>
<td>79</td>
<td>83</td>
</tr>
<tr>
<td>2.2 unmodified CNCs</td>
<td>N/A  47  6  47</td>
<td>47  3  50</td>
<td>6</td>
<td>51</td>
</tr>
</tbody>
</table>

\(^a\) (Heptane volume fraction in the emulsion)/(total heptane volume)*100.

\(^b\) (Volume fraction of emulsion after 4 days equilibration)/(Volume fraction emulsion after 30 min equilibration time)*100. Note that 4 days equilibration time was arbitrarily chosen to measure the stability of the emulsions.
Ionic strength of the aqueous phase. Emulsion systems with a nanoparticle concentration of 0.05 w/v were used to study the effect of the ionic strength of the aqueous phase, especially with regard to emulsion stability. Aqueous solutions with 1, 10, and 100 mM NaCl concentrations were added to aqueous dispersions containing the respective amount of grafted CNCs. After addition of an equivalent amount of heptane (for a WOR=1) and emulsification, it was observed that the ionic strength had a negative effect on the volume fraction of the emulsified phase, according to Table 8.2. It is hypothesized that the partial collapse of poly(NiPAAm) brushes grafted from CNCs as found in a previous study made the nanoparticle surfaces less hydrophilic and produced less stable oil droplets. Also, increased ionic strength yielded less electrostatic repulsion between dispersed oil droplets, thus were more likely to coalesce. In addition, the amount of oil emulsified also decreased with ionic strength. However, after a period of 4 days, the emulsions prepared with 100 mM NaCl showed the most stable emulsified phase. Emulsions prepared with 0.05 wt % poly(NiPAAm)-g-CNCs yielded very large droplets (>100 µm), as shown in Figure 8.2A, compared to those made with higher concentrations, as 0.5 wt % in Figure 8.2B. Larger emulsion droplets were much less stable under low shear by mixing compared to the smaller droplets formed at higher nanoparticle concentrations. Shown in Figure 8.3(A-B) are results of laser scattering particle size analysis of 0.25 wt % (A) and 0.5 wt % (B) grafted nanoparticle concentration. In both cases, polydisperse uninodal distributions were obtained with mean particle diameters of about 38 µm, on number-average basis. The highest wt % (0.5) of grafted nanoparticles gave a slightly lower polydispersity than 0.25 wt %. For all
cases of emulsion prepared with concentrations lower than 0.25 wt %, the large oil droplets obtained (>100 µm) were unstable to low shear by mixing, and accurate particle size distributions were not obtained.

Figure 8.2 Optical Microscopy Images of emulsions prepared with 0.05 (A) and 0.5 % (B) poly(NiPAAm)-g-CNCs using a WOR=1.

Figure 8.3 Laser Scattering Particle Size Analysis of emulsions prepared with 0.25 (A) and 0.5 % (B) poly(NiPAAm)-g-CNCs using a WOR=1.
In order to determine the nanoparticle wt % needed to stabilize Pickering emulsions, samples were extracted from the aqueous phase of formulations without salt added and nanoparticle concentration was calculated gravimetrically (seen in Table 8.3). In the case of 0.05 wt % formulations, there was not a detectable amount of grafted CNCs in the lower aqueous phase. However, in cases of higher concentration, significant amounts of grafted CNCs were in the aqueous phase, indicating an excess. Thus, it was assumed that the optimum concentration of nanoparticles for these formulations was about 0.25 wt %.

Table 8.3 Pickering emulsion nanoparticle composition of selected samples (calculated gravimetrically).

<table>
<thead>
<tr>
<th>Sample Phase</th>
<th>Wt %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original content in aqueous phase</td>
<td>0.05</td>
</tr>
<tr>
<td>Aqueous phase content after emulsification</td>
<td>0.0</td>
</tr>
<tr>
<td>Emulsion phase content after emulsificationa</td>
<td>0.05</td>
</tr>
</tbody>
</table>

aNanoparticle wt % relative to aqueous phase

Samples of the Pickering emulsion formulated with 0.25 wt % poly(NiPAAm)-g-CNCs were subjected to Freeze-fracture Electron Microscopy (FFEM), in which the emulsion was frozen in propane (-189 °C) and fractured in a cryo-chamber, followed by shadowing with Pt/C to create a mask so as to be further examined in a Transmission
Electron Microscopy (TEM). Interestingly, TEM grids containing freeze-fractured samples, individualized poly(NiPAAm)-g-CNCs which were broken from Pickering emulsions were visualized (Figure 8.4). CNCs dimensions correlated well with those determined previously.\textsuperscript{138}

![TEM images of poly(NiPAAm)-g-CNCs broken away after freeze-fracture of 0.25 wt % containing Pickering emulsions.](image)

**Figure 8.4** TEM images of poly(NiPAAm)-g-CNCs broken away after freeze-fracture of 0.25 wt % containing Pickering emulsions.

Seen in Figure 8.5 are TEM images of a freeze-fractured Pickering emulsion droplet stabilized by poly(NiPAAm)-g-CNCs at the oil-water interface. The appearance of anisotropically aligned rectangular shaped inclusions suggested that grafted CNCs are aligned at the oil-water interface of Pickering emulsions. It is well known that above a critical solids content (>1\%), CNCs can form anisotropic assemblies.\textsuperscript{18} It was interesting to note that the initial concentrations of grafted nanoparticles in all emulsions prepared was less
than 1%, however, due to the formation of three phase systems, the local concentration of grafted nanoparticles in the emulsified phase was expected to be much higher. Interestingly, the size of inclusions visualized (~500 nm x ~2 µm) when compared to the size of grafted CNCs in Figure 8.4 suggested large aggregations of grafted CNCs to form microparticles. This finding was in agreement with a study performed by Lin et al.\textsuperscript{211} in which larger particles were more strongly confined to the oil-water interface than smaller particles, indicating more effective emulsification by micron-sized particles.

![TEM image](image.png)

Figure 8.5 TEM images of emulsion droplet (insert) and oil-water interface after freeze-fracture.

Shown in Figure 8.6 is another emulsion droplet present on the same freeze-fractured sample as previously mentioned viewed by SEM. In this case, SEM showed surface related

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features which were not observed in TEM. Figure 8.6A shows the appearance of fractured crystals at the oil-water interface, which were also aligned. At higher magnification, Figure 8.6B, the appearance of sheeted layers may have been an indication that grafted CNCs not only aggregated laterally with adjacent CNCs, but also aggregated into sheets forming larger structures with lateral dimensions >1.5 µm at the oil-water interface.

![SEM images of (A) fractured poly(NiPAAm)-g-CNCs at oil-water interface, and (B) layers of broken poly(NiPAAm)-g-CNCs after freeze-fracture.](image)

**Figure 8.6** SEM images of (A) fractured poly(NiPAAm)-g-CNCs at oil-water interface, and (B) layers of broken poly(NiPAAm)-g-CNCs after freeze-fracture.

**Temperature.** The stability of Pickering emulsions with nanoparticles carrying thermo-responsive polymer brushes were expected to be affected by temperature. Emulsions prepared were stable >3 months at the time of writing this manuscript, however after heating above the LCST of poly(NiPAAm) for approximately one minute, all emulsions were broken. Figure 8.7 shows the appearance of Pickering emulsions prepared with 0.25 wt % poly(NiPAAm)-g-CNCs before and after exposure to heat above the LCST for one minute. After the three phase system was heated above ca. 35 °C, poly(NiPAAm)-g-CNCs desorbed
from the oil-water interface, thus producing heptane droplet coalescence. In addition, the lower aqueous phase in the test tubes after became more turbid to the naked eye (seen in Figure 8.7), due to grafted nanoparticle aggregation.

![Image of Pickering emulsions](image)

**Figure 8.7** Appearance of Pickering emulsions with 0.25 % poly(NiPAAm)-g-CNCs before (left) and after (right) heat for one minute above the LCST.

To more accurately determine the effect of temperature on Pickering emulsion stability, rheological measurements were performed. Emulsion samples were sheared at a constant rate of 100 s\(^{-1}\) for a temperature ramp of 25 to 45 °C. Shown in Figure 8.8 are the resulting viscosity versus temperature curves of emulsions prepared from four concentrations of poly(NiPAAm)-g-CNCs without the addition of salt. The lowest concentration of 0.05 wt % (Figure 8.8A) showed drastic instability after reaching the LCST at ca. 33 °C due to emulsions being broken and phase separation. When higher concentrations were sheared with temperature, apparent increases in viscosities were noted at the LCST, seen in Figure
8.8B and 8.8C. Interestingly, the peak in viscosity for emulsions prepared with 0.1 wt %
grafted nanoparticles were 1-2 °C lower than others (shown more clearly in the graph insert
of Figure 8.8). Also, the viscosity after the LCST was clearly more stable with
concentrations at 0.1 and 0.25 wt %, which may have been an indication of less significant
phase separation (Figure 8.8B-C). At concentrations of 0.5 wt % grafted nanoparticles, seen
in Figure 8.8D, the initial viscosity was significantly higher (~10x) than for lower
concentrations most likely due to smaller particle sizes. Additionally, a broader transition in
increased viscosity was observed at the LCST from ~32.5 to 36.5 °C, followed by a steady
decrease to a similar value as the other emulsion samples. In the case of unmodified CNCs
in water, the viscosity only slightly decreased with temperature and did not show thermo-
responsiveness, as reported in a previous publication.207 The observed changes in viscosity
for all samples were clear indications of emulsions being broken and oil droplet coalescence
due to collapse of poly(NiPAAm) brushes grafted from CNCs.
The effect of ionic strength on viscosity curves with temperature was also undertaken with Pickering emulsions prepared with 0.05 wt % poly(NiPAAm)-g-CNCs (see Figure 8.9). As previously mentioned, emulsions samples prepared with 0.05 wt % grafted nanoparticles without salt showed instability after the LCST (Figure 8.9A). Upon the addition of 1 mM NaCl to the aqueous phase before emulsification, viscosity curves were more stable after the LCST (Figure 8.9B-C), most likely explained by decreased steric and electrostatic repulsion between poly(NiPAAm)-g-CNCs at the surfaces of oil droplets. Although a clear transition
of the LCST was not observed with 1 mM NaCl due to very low concentration of grafted nanoparticles, a clear peak in viscosity was observed with the addition of 100 mM NaCl at ~32 °C, shown in Figure 8.9C.

Figure 8.9 Viscosity versus temperature for Pickering emulsions prepared with 0.05 wt % poly(NiPAAm)-g-CNCs at (A) 0 mM, (B) 1 mM, and (C) 100 mM NaCl (note log scale).

8.4 Conclusions

Thermally responsive heptane-in-water Pickering emulsions were successfully prepared with cellulose nanocrystals grafted with thermo-responsive poly(NiPAAm) brushes. Emulsions prepared with 0.05 to 0.5 wt % poly(NiPAAm)-g-CNCs were observed to be stable more than 3 months at the time of writing this manuscript. Depending on the concentration
of grafted nanoparticles, heptane droplets obtained ranged from 30 to >100 µm and showed polydisperse, however uninodal particle size distributions. All emulsions were broken when heated above the LCST of poly(NiPAAm) for one minute. This phenomenon was further elucidated via rheological measurements, in which viscosities of emulsion samples increased on approach of the LCST, and smaller particles sizes showed significantly higher viscosities. The ionic strength of emulsions showed a stabilization effect on the viscosity explained by decreased electrostatic and steric repulsion between poly(NiPAAm)-g-CNCs at the oil-water interface. The anisotropy of grafted nanoparticles at the oil-water interface was observed via Freeze-fracture TEM. In addition, larger layered sheet assemblies (>1.5 µm) were observed as an indication of grafted nanoparticle aggregation during emulsification at the oil-water interface. It is expected that the development of thermally responsive Pickering emulsions utilizing naturally abundant substrates with controllable stability will allow significant advances for biomedical and cosmetics applications.

8.5 REFERENCES


9 OVERALL CONCLUSIONS AND FUTURE WORK

In the present work, it was shown that the ‘grafting onto’ approach of CNCs led to less efficient polymer grafting as compared to ‘grafting from’ via SI-SET-LRP. This was expected due to decreased reaction solution viscosities and steric hindrance in the latter case. By modifying the SI-SET-LRP grafting conditions, a variety of molecular weights of grafted polymer brushes was obtained at CNCs surfaces. Aqueous dispersions of poly(NiPAAm)-g-CNCs displayed controlled colloidal stability with temperature and ionic strength while unmodified CNCs displayed no response. Spin-coated films of unmodified CNCs showed an electrostatic repulsion behavior while poly(NiPAAm)-g-CNCs showed a steric repulsion behavior. Poly(NiPAAm) brushes grafted from CNCs showed responsiveness to ionic strength and controllable surface adhesion due to brush compression.

Due to the surface activity of poly(NiPAAm)-g-CNCs, Pickering emulsions could be formed (stability >3 months), while unmodified CNCs did not stabilize emulsions. The preferred emulsion type was oil-in-water for all cases due to insolubility of poly(NiPAAm) brushes in heptane. Increased ionic strength (up to 100 mM [NaCl]) had a positive impact on emulsion stability. Higher concentrations of grafted nanoparticles gave smaller oil droplet size (<40 µm). Poly(NiPAAm)-g-CNCs appeared to aggregate into anisotropic structures to form thick layers (>1 µm) at the oil-water interface. All Pickering emulsions were broken within one minute after heating above the lower critical solution temperature.
Most importantly, this work has demonstrated a variety of synthetic pathways to modify the surface of nanocelluloses to ultimately produce novel biomaterials with advanced functionalities. The future prospects of modified nanocelluloses were demonstrated in such areas as high-strength, biodegradable nanocomposites, nanosensing, biomedical, and cosmetics applications. It is expected that these studies will provide the basis for the development of novel nanomaterials derived from abundant natural resources and bring forth new and advanced applications of cellulosic nanoparticles.

Future work focused on ‘grafting from’ CNCs via LRP should be focused on better control of graft densities of polymer grafts via the use of ‘dummy’ initiators. In this case, mixtures of active initiator and similar small molecules without the LRP active halide functionality could be used to provide more accurate control over graft density. In improved determinations of polymer graft densities, elemental analysis could be performed to determine halide content. This information coupled with saponification of polymer grafts from CNCs surfaces and subsequent SEC analysis will lead to a full characterization of polymerization reactions. To improve control over living radical polymerizations, the initial addition of deactivator (i.e. Cu(II)Br) may yield lower polydispersity indeces of grafted polymers. In addition, in depth studies on the effect of initiator graft density on the obtained molecular masses and polydispersity indeces, and thus rates of polymerization should be performed. In this case, local heterogeneities in active and inactive catalyst complex that may occur close to colloidal surfaces could be probed via UV-Visible spectroscopy studies. An additionally variable could be the ligand used for copper complexation. More active
ligands, such as tris[2-(dimethylamino)ethyl]amine (Me₆TREN) could be utilized to yield higher rates of polymerization. To obtain a larger variety of stimuli-responsiveness, block and/or random copolymers containing responsive and non-responsive monomers could be grafted from CNCs surfaces. Such elements could give great improvement for the development of naturally abundant cellulosic substrates carrying stimuli-responsive functionalities.
APPENDIX
APPENDIX A

X-ray Diffraction profiles of initiator-g-CNCs and preliminary rheological measurements of the thermo-responsive behavior of poly(NiPAAm)-g-CNCs.

Experimental

Wide-angle X-ray diffraction (WAXS). WAXS experiments were carried out at ambient temperature on dried pellets of unmodified or modified CNCs. A Philips (XLF, ATPS XRD 1000) diffractometer operated with a CuKα anode (λ = 0.15406 nm) was used with a 2θ range from 5° to 40° with steps of 0.1° and a counting time of 60 s.

Rheometry. Viscosity measurements of aqueous suspensions of unmodified CNCs, poly(NiPAAm)-g-CNCs, and free poly(NiPAAm) were undertaken using a TA Instruments AR 2000 Rheometer. A Peltier plate and 25 mm 1.59° cone geometry was used at a constant shear rate of 150 s⁻¹. Viscosity was monitored at a temperature ramp range of 25 to 40 °C.

Discussion

To determine the effect of the initiator esterification reaction on the crystalline structure of CNCs, freeze-dried samples were exposed to X-ray diffraction (XRD) analysis. The WAXD analyses of both unmodified (Figure A.1 A) and initiator-modified CNCs (Figure A.1 B-D) showed typical patterns of the native crystalline form of cellulose (cellulose I). They displayed 2θ diffraction peaks at 14.8° (0.60 nm), 16.5 (0.54 nm), 22.6 (0.39) nm and 34.5
(0.258 nm). This fact confirmed that the crystalline core of the CNCs was not affected by the immobilization of the initiator, which is therefore limited only to the surfaces of the nanocrystals.

To confirm the thermo-responsive behavior of poly(NiPAAm)-g-CNCs, rheological measurements were performed. After a desired concentration of aqueous suspension was equilibrated on the Peltier plate at 25 °C, viscosity was monitored over a temperature ramp to 40 °C at a constant shear rate. The results of the rheological measurements are summarized in Figure A.2. Typically, the viscosity of colloidal suspensions in a Newtonian fluid will decrease as temperature is raised due to increased fluidity most simply explained by the Arrhenius equation for molecular kinetics. Thus, a suspension of unmodified CNCs showed this behavior as seen in Figure A.2. Free poly(NiPAAm) was also synthesized and viscosity measured to confirm the LCST of ca. 32 °C. MD poly(NiPAAm)-g-CNCs showed significant increases in viscosity on approach of the LCST of grafted polymer brushes. Below the LCST of grafted poly(NiPAAm) brushes, poly(NiPAAm)-g-CNCs were well dispersed with viscosity values similar to water, however as the LCST was reached, the collapse of polymer brushes on the surfaces of CNCs created a more hydrophobic dispersed phase, and thus colloidal instability yielding a ca. two-fold increase in viscosity.
Figure A.1  X-ray Diffraction profiles of (A) unmodified CNCs, (B) very high density (VHD) (C) medium density (MD) and (D) low density (LD) initiator-g-CNCs.
Figure A.2  Normalized viscosity versus temperature of unmodified CNCs, poly(NiPAAm)-g-CNCs, and free poly(NiPAAm). (lines to guide the eye)