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Poly(N-isopropylacrylamide) brushes grafted from Cellulose Nanocrystals via Surface-Initiated **Single- Electron Transfer Living** Radical Polymerization

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ABSTRACT

Never-dried cellulose nanocrystals (CNCs) or nanowhiskers produced from sulfuric acid hydrolysis of ramie fibers were used as substrates for surface chemical functionalization with thermo-responsive macromolecules. The CNCs were grafted with poly(N-isopropylacrylamide) brushes via surface-initiated single-electron transfer living radical polymerization (SI-SET-LRP) under various conditions at room temperature. The grafting process was confirmed via Fourier transform IR spectroscopy and X-ray photoelectron spectroscopy and the different molecular masses of the grafts were quantified and found to depend 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. It is expected that suspension stability, interfacial interactions, friction and other properties of grafted CNCs can be controlled by changes in temperature and provide a unique platform for further development of stimuli-responsive nanomaterials.

KEYWORDS. Cellulose nanocrystals, cellulose whiskers, SI-SET-LRP, SI-ATRP, grafting-from, thermo-responsive, poly(N-isopropylacrylamide).

Introduction

Recent advances in the field of lignocellulosics have stream-lined the development of renewable biomaterials for advanced applications.^{1, 2} 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. 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.^{3, 4} 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.⁵ 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 .⁶ However, electrostatic forces affect colloidal stability, making suspensions of CNCs sensitive to the ionic strength of the medium.⁷ In order to prevent aggregation in aqueous and organic media, steric stabilization could be achieved via polymer brushes grafted from CNCs.⁸ Importantly, the destabilization effect brought about by the presence of electrolytes can be minimized when nonionic polymer chains are grafted.⁹

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.¹⁰⁻¹² 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 $^{\circ}\text{C}$, 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 $^{\circ}\text{C}$, it has been

extensively used in applications involving controlled drug release.^{13, 14} The utility of nanocellulosic substrates as nanocarriers of responsive functionalities for such applications has an obvious advantage related to their biocompatibility and cost.

Polymer grafting on cellulose can be accomplished by two main strategies, namely, the “grafting-onto” and “grafting-from”.¹⁵ 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.¹⁶

Since the mid 1990s,¹⁷ 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.¹⁸ 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.^{19, 20} Most recently, the discovery of a Cu(0) mediated single-electron transfer living radical polymerization (SET-LRP)^{21, 22} process has streamed great advances in ultrafast polymerizations with high molecular weights at or below ambient temperature with small amounts of catalyst.²³ 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. Within the last year, it has been shown that Cu(I)-mediated ATRP of NiPAAm actually proceeds via a Cu(0)-mediated SET-LRP,²⁴ and thus more recent efforts in polymerizations of thermo-responsive graft copolymers via sequential SET-LRP and ATRP have surfaced.^{25, 26} In addition, Nguyen et al.²⁷ reported on the SET-LRP of NiPAAm initiated with 2-

1 methylchloropropionate (MCP) in protic and dipolar aprotic solvents. In consequence to previous
2 efforts, a comparison of surface-initiated ATRP and SET-LRP has been carried out.²⁸
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5 Lindqvist et al.²⁹ have reported the development of novel dual-responsive surfaces via SI-ATRP, now
6 known to have proceeded via SI-SET-LRP,²⁴ from cellulose fibers in filter paper. In their work,
7 NiPAAm as well as 4-vinylpyridine were utilized to produce thermo- and pH- responsive materials after
8 grafting the respective block copolymer brushes on the fiber surface. A large increase in the water
9 contact angle ($\sim 120^\circ$) of the functionalized fibers was observed when increasing the temperature above
10 the LCST of poly(NiPAAm). However, few reports are available on the use of surface-initiated LRP to
11 modify CNCs. Yi et al. investigated the liquid crystalline phase behavior of CNCs resulting from
12 grafting poly(styrene) (PS),³⁰ poly(6-[4-(4-methoxyphenylazo)phenoxy]hexyl methacrylate (PMMAZO),
13 and poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA)³¹ from the surface of CNCs via SI-
14 ATRP, however the effects of reaction conditions were not undertaken. On the other hand, Morandi et
15 al.³² used various grafting conditions to control grafting density of polystyrene brushes on CNCs using
16 excess concentrations of initiator during modifications. In these three reports, freeze-dried CNCs were
17 utilized as the substrate for surface modification. Since drying of the CNCs can lead to aggregation, it
18 can be argued that surface modification was limited due to the reduced available surface area. To our
19 knowledge, grafting from never-dried CNCs via LRP has not been reported. Therefore, this study
20 addresses the important issue of grafting poly(NiPAAm) from CNCs to develop brushes with various
21 surface densities and degrees of polymerization. Such systems are expected to not only provide steric
22 stabilization of colloidal suspensions of CNCs, but also provide a bio-based platform for
23 nanotechnological systems that may benefit from thermo-responsive functionalities.
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49 Experimental

50 *Materials.* Pure ramie fibers were obtained from Stucken Melchers GmbH & Co. (Germany). 2-
51 bromoisobutyryl bromide (BriB), 2-dimethylaminopyridine (DMAP), tetrahydrofuran (THF, 99%), N-
52 isopropylacrylamide (NiPAAm), copper (I) bromide and *N,N,N',N'',N'''*-pentamethyldiethylenetriamine
53 (PMDETA), were all obtained from Sigma-Aldrich. Sulfuric acid (95%), acetic acid (glacial),
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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.³³

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 **anyhydroglucose 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 1. Scheme 1 shows a summary of the key steps involved in the production of poly(NiPAAm)-g-CNCs.

Table 1. Summary of reaction conditions for initiator-g-CNCs and poly(NiPAAm)-g-CNCs.

Molar ratios	amount of initiator, mmol [mM]	amount of monomer, mmol [mM]
[Br]:[AGU][§]		
5:12 (LD)	2.5 [25 mM]	100 [667 mM]
5:6 (MD)	5.0 [50 mM]	100 [667 mM]
5:4 (HD)	7.5 [75 mM]	100 [667 mM]
5:3 (VHD)	10 [100 mM]	100 [667 mM]
[NiPAAm]:[AGU][†]		

50:3 (LDP)	5.0 [50 mM]	100 [667 mM]
75:3 (MDP)	5.0 [50 mM]	150 [1 M]
100:3 (HDP)	5.0 [50 mM]	200 [1.33 M]

[§] 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

Step 1:

Step 2:

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 $\text{CuK}\alpha$ anode ($\lambda = 0.15406\text{ nm}$) was used with a 2θ range from 5° to 40° with steps of

0.1° and a counting time of 60 s. The crystallinity index (CrI) was calculated by the method of Segal et al.³⁴ according to:

$$CrI = 1 - \frac{I_{am}}{I_{002}},$$

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 formula³⁵:

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

where θ is the diffraction angle, λ 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 mm²) 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.^{36, 37} The binding energy scale was shifted using the C-C binding energy at 285.0 eV.³⁸ Cellulose content was calculated by the following equation:

$$\% \text{ cellulose} = \frac{(\% \text{ C1s})(\% \text{ CO})(11)(0.01)}{5}$$

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_2O at 25°C in 5 mm o.d. tube (internal acetone ^{13}C (CH_3) at 31.5 ppm relative to Me_4Si), 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 ($5\ \mu\text{m}$; 10^3 and $10^5\ \text{\AA}$, 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 ($K = 5.754 \times 10^{-3}\ \text{mL g}^{-1}$, $\alpha = 0.78$) were used to establish the number-average molecular weight (M_n) and polydispersity index (PDI).³⁹

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-bromoisobutryl 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-bromoisobutryl-CNCs ester, or initiator-modified CNCs, was synthesized by surface esterification of CNCs with 2-bromoisobutryl 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 1, indicated the appearance of a carbonyl ester stretch at $1735\ \text{cm}^{-1}$, 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.³²

Figure 1. FTIR spectra of unmodified CNCs (A), initiator-modified CNCs (B) and poly(NiPAAm)-g-CNCs (C).

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.³⁶ Figure 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 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 supporting information) of the native crystalline form of cellulose (cellulose I). They display 2 θ 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 confirms 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 Scherrer's formula and a shape constant of 0.9. These parameters are summarized in Table 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 2. Width and crystallinity index of unmodified CNCs and initiator-g-CNCs for different [Br]:[AGU] ratios.

Sample	Average crystallite width*, nm	Crystallinity Index, %
Unmodified ramie CNCs	6.2	87.8
Initiator-g-CNCs		
VHD	6.1	86.0
MD	5.7	85.4
LD	5.9	87.4

*calculated by Scherrer'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 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 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 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 1.

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 2 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.⁴⁰ 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,⁴¹ 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 investigations²³ 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 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 3. Atomic percentages (XPS) and molar % content of Poly(NiPAAm) and cellulose for unmodified CNCs and poly(NiPAAm)-g-CNCs.

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

The ratio of oxygen to carbon atomic percentages shown in Table 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 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 4).

Table 4. Carbon deconvolution of unmodified CNCs and poly(NiPAAm)-g-CNCs determined by high resolution XPS of C 1s.

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

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.³⁶

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 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.⁴² 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 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 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.⁴³ 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.

Figure 5. DSC thermograms of unmodified CNCs, bulk poly(NiPAAm) and poly(NiPAAm)-g-CNCs (VHD).

From the DSC profiles, ΔC_p was also determined for each glass transition; and when compared to bulk polymer, the ΔC_p of grafted chains was used to estimate the relative quantities of poly(NiPAAm) grafts present in each sample (see Table 5). A similar trend from the XPS results (Table 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 5. DSC results of poly(NiPAAm)-g-CNCs and bulk poly(NiPAAm).

Molar ratio	T_g , °C	ΔC_p , J/g °C	% poly(NiPAAm)
LD	142.7	0.31	62
MD	142.3	0.31	62
HD	143.0	0.37	74
VHD	141.7	0.40	80
LDP	142.3	0.31	62

MDP	142.1	0.39	78
HDP	142.2	0.43	86
Bulk poly(NiPAAm)	130.1	0.50	100

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.¹⁹ 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. 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 ¹³C-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 (left) depicts the ¹³C-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,⁴⁴ 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 CH₃ 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 CH₂ groups of the polymer backbone that relaxed much more slowly because of their restricted mobility. Figure 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. ¹³C-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 7 and the corresponding data are reported in Table 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.⁴⁵ 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.

1 The explanation proposed was that local concentration heterogeneities shifted the ATRP equilibrium to
2 the active state. To the best of our knowledge, a corresponding study has not yet been undertaken for
3 the case of SET-LRP, however a similar argument may apply. Near the surface of suspended CNCs
4 during polymerization, heterogeneities in the local concentrations of activator and deactivator ligand
5 complexes may have caused an increase in the activation rate and thus an increased monomer
6 conversion (up to 17% for VHD).
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14 In all cases, moderate polydispersity indices (PDIs) were obtained for poly(NiPAAm) brushes grafted
15 from CNCs. The PDI of bulk poly(NiPAAm) synthesized in the same fashion as grafted CNCs with a
16 target molecular weight of 11,300 Da was much higher than that of detached polymers from CNCs. This
17 also supported the previous hypothesis of local heterogeneities yielding higher rates of polymerization,
18 and thus more control on the PDI. In this case, it was inferred that the polymerization from the surface
19 of the dispersed phase gave better control without the addition of deactivator when compared to the bulk
20 polymerization from free initiator.
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30 In the case of a fixed initiator content and increased monomer concentrations, the molecular masses of
31 the grafts also showed a distinct increase, as expected. However, monomer conversion was only
32 between 12-13% for these cases, thus monomer concentration did not have a significant effect on the
33 rate of polymerization. Addition of small amounts of deactivator, CuBr₂, during polymerization is
34 expected to yield better control according to Rosen et al.²³ In future work, these methodologies will be
35 attempted in order to achieve lower polydispersity indices for the polymer brushes grafted from CNCs.
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Figure 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. Relative molecular masses (M_n and M_w) and polydispersity indices of cleaved poly(NiPAAm) chains from CNCs surface.

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

[^]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.³² In this case, up to ca. 50 mL of 2-bromoisobutryl 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.,³⁰ 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); 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.

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.

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Supporting information available

Supporting Information is available free of charge via the Internet at <http://pubs.acs.org/>

References

- (1) Hamad, W., *Can. J. Chem. Eng.* **2006**, 84 (5), 513-519.
- (2) Kamel, S., *eXPRESS Polym. Lett.* **2007**, 1 (9), 546-575.
- (3) Dufresne, A., *Can. J. Chem.* **2008**, 86 (6), 484-494.
- (4) Habibi, Y.; Lucia, L. A.; Rojas, O. J., *Chem. Rev.* **2010**, 110 (6), 3479-3500.
- (5) Dong, X. M.; Revol, J.-F.; Gray, D. G., *Cellulose* **1998**, 5 (1), 19-32.

- (6) Elazzouzi, S. PhD thesis, Joseph Fourier University, Grenoble, 2006.
- (7) Dong, X. M.; Kimura, T.; Revol, J.-F.; Gray, D. G., *Langmuir* **1996**, 12 (8), 2076-82.
- (8) Araki, J.; Wada, M.; Kuga, S., *Langmuir* **2001**, 17 (1), 21 -27.
- (9) Napper, D. H., *J. Colloid Interface Sci.* **1977**, 58 (2), 390-407.
- (10) Mauricio, M. R.; Carvalho, G. M.; Radovanovic, E.; Muniz, E. C.; Rubira, A. F., *Mater. Sci. Eng., C* **2009**, 29 (2), 594-598.
- (11) Wang, W.; Chen, L., *J. Appl. Polym. Sci.* **2007**, 104 (3), 1482-1486.
- (12) Yin, J.; Ge, Z.; Liu, H.; Liu, S., *J. Polym. Sci., Part A: Polym. Chem.* **2009**, 47 (10), 2608-2619.
- (13) Hamidi, M.; Azadi, A.; Rafiei, P., *Adv. Drug Delivery Rev.* **2008**, 60 (15), 1638-1649.
- (14) Schild, H. G., *Prog. Polym. Sci.* **1992**, 17 (2), 163-249.
- (15) Roy, D.; Semsarilar, M.; Guthrie, J. T.; Perrier, S., *Chem. Soc. Rev.* **2009**, 38, 2046-2064.
- (16) Zhao, B.; Brittain, W. J., *Prog. Polym. Sci.* **2000**, 25 (5), 677-710.
- (17) Wang, J.-S.; Matyjaszewski, K., *J. Am. Chem. Soc.* **1995**, 117 (20), 5614-5615.
- (18) Matyjaszewski, K.; Tsarevsky, N. V., *Nat. Chem.* **2009**, 1 (4), 276-288.
- (19) Edmondson, S.; Osborne, V. L.; Huck, W. T., *ChemInform* **2004**, 35 (16).
- (20) Xie, M. R.; Dang, J. Y.; Han, H. J.; Wang, W. Z.; Liu, J. W.; He, X. H.; Zhang, Y. Q., *Macromolecules* **2008**, 41 (23), 9004-9010.
- (21) Percec, V.; Guliashvili, T.; Ladislav, J. S.; Wistrand, A.; Stjerndahl, A.; Sienkowska, M. J.; Monteiro, M. J.; Sahoo, S., *J. Am. Chem. Soc.* **2006**, 128 (43), 14156-14165.
- (22) Percec, V.; Popov, A. V.; Ramirez-Castillo, E.; Monteiro, M.; Barboiu, B.; Weichold, O.; Asandei, A. D.; Mitchell, C. M., *J. Am. Chem. Soc.* **2002**, 124 (18), 4940-4941.
- (23) Rosen, B. M.; Percec, V., *Chem. Rev. (Washington, DC, U. S.)* **2009**, 109 (11), 5069-5119.
- (24) Feng, C.; Shen, Z.; Li, Y.; Gu, L.; Zhang, Y.; Lu, G.; Huang, X., *J. Polym. Sci., Part A Polym. Chem.* **2009**, 47 (7), 1811-1824.
- (25) Feng, C.; Li, Y.; Yang, D.; Li, Y.; Hu, J.; Zhai, S.; Lu, G.; Huang, X., *J. Polym. Sci., Part A Polym. Chem.* **2010**, 48 (1), 15-23.

- (26) Feng, C.; Shen, Z.; Yang, D.; Li, Y.; Hu, J.; Lu, G.; Huang, X., *J. Polym. Sci., Part A Polym. Chem.* **2009**, 47 (17), 4346-4357.
- (27) Nguyen, N. H.; Rosen, B. M.; Percec, V., *J. Polym. Sci., Part A Polym. Chem.* **2010**, 48 (8), 1752-1763.
- (28) Ding, S.; Floyd, J. A.; Walters, K. B., *J. Polym. Sci., Part A Polym. Chem.* **2009**, 47 (23), 6552-6560.
- (29) Lindqvist, J.; Nystroem, D.; Oestmark, E.; Antoni, P.; Carlmark, A.; Johansson, M.; Hult, A.; Malmström, E., *Biomacromolecules* **2008**, 9 (8), 2139-2145.
- (30) Yi, J.; Xu, Q.; Zhang, X.; Zhang, H., *Polymer* **2008**, 49 (20), 4406-4412.
- (31) Yi, J.; Xu, Q.; Zhang, X.; Zhang, H., *Cellulose* **2009**, 16 (6), 989-997.
- (32) Morandi, G.; Heath, L.; Thielemans, W., *Langmuir* **2009**, 25 (14), 8280-8286.
- (33) Habibi, Y.; Foulon, L.; Aguié-Beghin, V.; Molinari, M.; Douillard, R., *J. Colloid Interface Sci.* **2007**, 316 (2), 388-397.
- (34) Segal, L.; Creely, J. J.; Martin, A. E.; Conrad, C. M., *Text. Res. J.* **1959**, 29, 786-94.
- (35) Klug, H. P.; Alexander, L. E., Wiley Interscience: New York, 1954.
- (36) Johansson, L.-S.; Campbell, J. M., *Surf. Interface Anal.* **2004**, 36 (8), 1018-1022.
- (37) Johansson, L.-S.; Campbell, J. M.; Koljonen, K.; Stenius, P., *Appl. Surf. Sci.* **1999**, 144-145, 92-95.
- (38) Beamson, G.; Briggs, D., Wiley & Sons: Chichester, England, 1992.
- (39) Ganachaud, F.; Monteiro, M. J.; Gilbert, R. G.; Dourges, M.-A.; Thang, S. H.; Rizzardo, E., *Macromolecules* **2000**, 33 (18), 6738-6745.
- (40) Wang, N.; Ding, E.; Cheng, R., *Polymer* **2007**, 48 (12), 3486-3493.
- (41) Habibi, Y.; Chanzy, H.; Vignon, M. R., *Cellulose* **2006**, 13, 679-687.
- (42) Roman, M.; Winter, W. T., *Biomacromolecules* **2004**, 5 (5), 1671-1677.
- (43) Yin, W.; Yang, H.; Cheng, R., *Eur. Phys. J., E: Soft Matter* **2005**, 17 (1), 1-5.
- (44) Zeng, F.; Tong, Z.; Feng, H., *Polymer* **1997**, 38 (22), 5539-5544.

- (45) Behling, R. E.; Williams, B. A.; Staade, B. L.; Wolf, L. M.; Cochran, E. W., *Macromolecules* **2009**, 42 (6), 1867-1872.

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