

**Nanoparticle emulsifiers based on bifunctionalized cellulose nanocrystals as marine diesel
oil-water emulsion stabilizers**

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Abstract

The preparation and properties of marine diesel oil-in-water (o/w) emulsion stabilized by bifunctionalized cellulose nanocrystals (*But*-CNCs) were investigated. Bifunctional *But*-CNCs containing both carboxylic and *n*-butylamino groups were obtained using partial, sequential periodate-chlorite oxidation and reductive amination, followed by a homogenization treatment to liberate individualized nanocrystals with amphiphilic characteristics. The fabricated *But*-CNC suspensions were optically transparent, and the nanocrystals that were isolated were rod-like, with lengths ranging between 35–120 nm and with lateral dimensions varying from 2–4 nm. Bifunctionalized CNCs at low concentrations (up to 0.45 wt %) were investigated as possible surface-active stabilizers in o/w emulsions. In particular, their ability to enhance the emulsification of marine diesel oil in an aqueous environment was addressed in order to evaluate their potential to be used as “green” oil spill response agents. *But*-CNCs at 0.1wt % concentration in dispersion reduced the oil particle size from 50 μm to 9–16 μm , and the stability against coalescence was improved in emulsions where bifunctionalized CNCs were used compared to plain o/w emulsion.

In addition, the influence of *But*-CNC dosage and emulsion salinity (0–5% NaCl by weight) was investigated. The background salt concentration only had a minor effect on droplet size, and the stabilization effect was still apparent, with high electrolyte concentration. These results demonstrate the potential of bifunctionalized cellulose (with surface-active groups attached) to act as a nanoparticle emulsifier in oil-water emulsions, thus enabling its utilization in oil-destruction activities.

Keywords: Dispersing agent; Nanocellulose; Nanoparticle, Oil spill; o/w emulsion

1. Introduction

As a renewable and abundant raw material, plant celluloses have become an attractive resource in the development of novel “green” materials and chemicals for the emerging bio-based economy. In addition to the utilization of celluloses in the form of fibers, researchers have paid a great deal of attention to the fabrication and exploitation of micro- and nano-sized cellulose constituents, such as cellulose nanocrystals (CNCs) and cellulose nanofibrils, in multiple applications [1–7]. CNCs possess many advantages like unique optical properties, and higher strength properties such as stiffness and modulus [8]. A typical individual cellulose crystal from a wood source is approximately 3–5 nm in diameter, with a length of 200 nm or less [3]. Depending on the cellulose source, the aspect ratios (length to width [L/w]) of CNCs range from 10–100 [1]. The presence of several hydroxyl groups on the surfaces of CNCs makes them inherently hydrophilic, and enhances their interaction with water [9, 10].

One traditional way to produce CNCs (also known as nanocrystalline cellulose or cellulose “nanowhiskers”) is to conduct strong acid hydrolysis, which dissolves the amorphous regions from the cellulose structure and yields pure, nano-sized crystalline cellulose particulates [1]. Today, other chemical, enzymatic, or mechanical methods (or a combination of these methods) are also used in CNC fabrication [3, 9]. The first non-acidic treatment to produce CNCs was the APS (ammoniumpersulfate extraction) method [11], although researchers have recently developed another acid-free oxidative pre-treatment to produce nanocrystals with tuned surface properties [9, 10]. In this approach, the surface modifications can be conducted during the pre-treatment step before liberating the individual CNCs, which reduces the need for difficult and time-consuming post-treatments [10].

Several different chemicals are used to promote the dispersion and stabilization of o/w emulsions. These dispersants or emulsifying agents (which are typically soluble compounds with amphiphilic characteristics) minimize interfacial energy, thus enhancing emulsions’

long-term stability [12]. Dispersants can also be used in the form of nanoparticles for the stabilization of o/w emulsions that will result in so-called Pickering emulsions [13]. The solid particles are able to adsorb irreversibly at the o/w interface by the action of capillary forces [14] to form a densely packed steric barrier layer [15]. This effect can, in turn, efficiently prevent drop coalescence and can result in very stable emulsions with low particle dosages [15, 16]. The particles that are used for Pickering emulsions typically have amphiphilic characteristics, i.e. they have an affinity for the two phases of the emulsion [17].

Because of bio-based nanoparticles' potential inherent biodegradability, researchers have considered such nanoparticles to be especially beneficial as oil-spill response agents for promoting oil dispersion; many of the currently available soluble synthetic polymeric dispersants, in contrast, have been associated with negative health and environmental impacts [18–20]. Certain nanoparticles have been reported to possess low toxicity and reduced negative impact on marine organisms, with good stabilization performance [21]. Previously, nanoparticles that contain silica, clay, iron oxide, and carbon black, for example, have been tested in o/w emulsions, with the aim of enhancing preparedness for oil spills in deep-water seas [22]. These emulsifying particles can improve the natural biodegradability of oil by marine organisms when mechanical oil recovery is not feasible (for example, due to harsh climate conditions).

Researchers have previously used CNCs for successful Pickering emulsion stabilization in o/w interfaces in model suspensions [15–17, 23–27]. CNCs' dimensions are considered to be in the optimal range for providing stable Pickering emulsions by the action of stabilizing capillary forces [28], although studies have found that their performance depends on cellulose surface characteristics [17, 25]. Because CNCs typically possess mainly hydrophilic surface characteristics, they are especially viable for forming oil-in-water emulsions (water as dispersing phase), as stated by the Bancroft rule [29]. We have also

reported the use of hydrophobized nanocrystals to increase the stabilization of soybean oil emulsions [10]. Because the use of functionalized CNCs as dispersants for petroleum oil spills has not yet been reported in the literature, there is significant potential in this field. The performance of CNCs in petroleum oil stabilization could potentially be further enhanced by the introduction of hydrophilic and hydrophobic functional groups, thus resulting in bifunctional structures.

In this work, the preparation and properties of marine diesel o/w emulsion stabilized by bifunctionalized cellulose nanocrystals were investigated. First, celluloses with amphiphilic characteristics were synthesized using partial, sequential periodate-chlorite oxidation and a reductive amination treatment, followed by a homogenization treatment to liberate the individual CNCs. Bifunctional CNCs with variable carboxylic and amino group contents were obtained and further characterized with transmission electron microscopy (TEM), diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, and optical transmittance measurement. The potential of differently synthesized CNCs to act as dispersing agents in o/w emulsions was screened, and two products (*But-CNC/A* and *But-CNC/D*) were chosen for more in-depth analysis. The effects of CNC and electrolyte concentrations on emulsion stability and average oil-droplet size were studied using laser diffractometry and analytical centrifugation.

2. Materials and methods

2.1. Raw materials and chemicals

Chemical birch pulp (*Betula pendula*) was received as dry sheets that were disintegrated in deionized water according to standard procedure and used as the raw material for CNC fabrication. NaIO₄ (India; purity ≥ 99.0%) and LiCl (Germany; purity ≥ 98.0%) were obtained from Sigma-Aldrich for the fabrication of dialdehyde cellulose (DAC) by periodate oxidation.

The chemicals used in the chlorite oxidation (NaClO_2 and CH_3COOH) were also purchased from Sigma-Aldrich. In the reductive amination reaction, 2-picoline borane (Sigma-Aldrich, USA [95%]) and *n*-butylamine hydrochloride (Tokyo Chemical Industry, Belgium [$> 98\%$]) were used. Ethanol (96%), obtained from VWR (Finland), was used in the washing step after the amination process. All chemicals were pro analysis grade and were used without further purification. Deionized water was used throughout the experiments in the dilutions and disintegrations; lightweight marine diesel oil was used for all o/w emulsions. The diesel oil was a sulfur-free winter-grade oil with a density of 828 kg/m^3 at 15°C and viscosity of $1.846 \text{ mm}^2/\text{s}$ at 40°C (Neste Oyj, Finland).

2.2. Chemical bifunctionalization of cellulose

A sequential oxidation route, followed by reductive amination, was performed to fabricate bifunctionalized CNCs. The periodate oxidation method was initially used to modify the disintegrated birch cellulose to DAC. Oxidation was performed with two different procedures, and two diverse aldehyde contents were obtained (DAC1 and DAC2). Disintegrated cellulose (15 grams oven-dry weight) was oxidized using 12.3 g of sodium periodate (NaIO_4) in water (1,500 ml) at 65°C for 180 min to attain DAC1 with an aldehyde content of 2.20 mmol g^{-1} . The oxidation of 15 g cellulose with 12.3 g of sodium periodate (NaIO_4) and 27 g of lithium chloride (LiCl) in water (1,500 ml) resulted in DAC2, with an aldehyde content of 3.86 mmol g^{-1} . The determination of the aldehyde content of the DAC was based on an oxime reaction between the aldehyde group and $\text{NH}_2\text{OH}\cdot\text{HCl}$ [30].

Both DAC1 and DAC2 were further oxidized with a similar procedure, using sodium chlorite (NaClO_2) to obtain partially oxidized dicarboxylic acid cellulose (PO-DAC). Three different sodium chlorite amounts were used in the oxidation of DAC1. In this procedure, 0.497 g, 0.994 g, and 1.243 g of sodium chlorite were each diluted with 17.78 g of deionized

water, then poured into three beakers with 26.65 ml of 20 vol % acetic acid. These solutions were then mixed with 2 g of DAC1 diluted in 44.4 ml of water to attain carboxylic acid contents of 0.50 mmol g⁻¹, 1.13 mmol g⁻¹, and 1.58 mmol g⁻¹, respectively. The carboxyl content was then analyzed by conductometric titration using a procedure described by Katz et al. [31] and Rattaz et al. [32].

The oxidation reaction was completed under a fume hood with continuous mixing for 8 min. The three cellulose products that were obtained were labeled PO-DAC/A, PO-DAC/B, and PO-DAC/C. The oxidation of the DAC2 was completed with two different sodium chlorite amounts—0.87 g and 2.18 g—in 17.78 g of deionized water. An amount of 26.65 ml of 20 vol % acetic acid was mixed with a sodium chlorite solution. After 8 min. of reaction time, two products (PO-DAC/D and PO-DAC/E), with carboxylic acid contents of 1.08 mmol g⁻¹ and 1.90 mmol g⁻¹, respectively, were obtained.

Reductive amination was then used to introduce *n*-butylamino groups to the oxidized cellulose. The amination was conducted by weighing the *n*-butylamine hydrochloride into 200 ml of deionized water and adjusting the pH to 4.5 with a diluted HCl solution. Next, a 2-picoline borane solution (where twofold excess of 2-picoline borane, based on the assessed amount of the aldehyde groups, had been diluted in 200 ml deionized water) was introduced to the *n*-butylamine hydrochloride solution, where 4 g of each PO-DAC fiber sample was also weighed.

After 72 h of continuous mixing at room temperature, the samples were filtered to stop the reaction and were washed with water ($V = 200$ ml) and ethanol ($V = 300$ ml). Finally, the solid was collected into a beaker, and 200 ml 0.1 M HCl solution was added. After 5 min. of mixing, the suspension was filtered and washed with 500 ml of deionized water. This procedure resulted in five different bifunctionalized samples with butylamino groups attached,

which were labeled *But/A*, *But/B*, *But/C*, *But/D*, and *But/E*. The chemical reaction route of the sequential periodate and chlorite oxidation and reductive amination is presented in Figure 1.

2.3. Liberation of nanocrystals from bifunctionalized cellulose

The modified cellulose materials were converted to individual nanocrystals at a solids content of 0.5 wt % and a pH of 10 (the pH was adjusted using NaOH) using a homogenization treatment with M-110EH-30 microfluidizer (Microfluidics, USA). All suspensions were passed once through a 400 μm auxiliary processing module (APM) and a 200 μm interaction chamber (IXC) at a pressure of 1300 bars. Afterward, the suspensions were passed once through a combination of 400 μm APM and 100 μm IXC at a pressure of 2000 bars. The CNC suspensions that were obtained were clear and only slightly viscous. The samples were labeled *But-CNCs A to E*.

2.4. Characterization of bifunctionalized cellulose nanocrystals

2.4.1. Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy

Chemical characterization of the pure cellulose and chemically modified celluloses were performed by DRIFT spectroscopy. The spectra were collected with a Bruker Vertex 80v spectrometer (USA) from freeze-dried samples; the spectra were obtained in the 600–4,000 cm^{-1} range; 40 scans were taken for each sample, at a resolution of 2 cm^{-1} .

2.4.2. Transmission electron microscopy (TEM)

A transmission electron microscope (Tecnai G2 Spirit, FEI Europe, The Netherlands) was used to determine the morphological characteristics of the CNCs. Preparation of the samples was performed by first adding a small droplet of diluted CNC solution on the Butvar-coated copper grid. Excess water in the sample was then absorbed with a small piece of filter paper.

All samples were negatively stained with uranyl acetate (2% w/v) and were allowed to dry at room temperature before the measurements were conducted. The standard conditions with 100 kV were used during the TEM analysis. A Quemesa CCD camera and iTEM image analysis software (Olympus Soft Imaging Solutions GmbH, Munster, Germany) were used in the capturing and processing images of the CNC samples. CNC width and length were measured from 70 individual CNCs, and the averages (as well as standard deviations) were calculated for each sample that was studied.

2.4.3. Optical transmittance of nanocrystal suspensions

Optical transmittance was measured with a Hach DR 2800 spectrophotometer (USA) from 0.1% (w/w) CNC solutions. Samples were analyzed in multiple wavelengths (340–800 nm) as duplicates. The results that were achieved were then averaged.

2.4.4. The Amino group amount of the But-CNC suspensions

The samples were freeze-dried (Scanvac, CoolSafe, Denmark) at a solids content of 0.5 wt % for 54 h prior to elemental analysis. A CHN/O 2400 organic elemental analyzer (PerkinElmer, USA) was used to determine the nitrogen content of the samples, from which the amino group amounts were calculated.

2.4.5. X-ray diffraction (XRD)

The crystallinity of the butylamino-functionalized CNCs was analyzed using an X-ray diffractometer, and was determined based on the Segal method. [33] The samples were prepared by freeze-drying the homogenized *But*-CNC suspensions and pressing dry samples into tablets with a thickness of approximately 1 mm. Measurements were conducted on a Siemens D5000 diffractometer (Germany) equipped with a Cu K α radiation source ($\lambda =$

0.1542 nm). Scans were taken over a 2θ (Bragg angle) range from 5 to 50° at a scanning speed of 0.1° s^{-1} using a step time of 5 s. The degree of the peak intensity of the main crystalline plane (002) diffraction (I_{002}) was located at 22.5° . I_{200} represents both the crystalline and amorphous contents of the specimen. The peak intensity associated with the amorphous fraction of the cellulose (I_{am}) was observed at 18.0° . The degree of crystallinity is given by equation 1:

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100\% \quad (1)$$

2.5. Preparation of o/w emulsion

The diesel o/w emulsion was prepared using an UltraTurrax mixer (IKA T25, Germany) at 7,000 rpm for 15 min. The ratio of oil and water was kept at 1:10 throughout the study. The 0.5% (wt) *But*-CNC-solution was diluted to the water phase before oil was added and dispersed. The final *But*-CNC concentrations in the emulsions varied between 0–0.45% (w/w).

In addition, different background salt concentrations were used to illustrate the effect of the electrolytes on dispersion stability and oil-droplet size. Sodium chloride (NaCl) was used at concentrations of 0–5% (by weight), which was equivalent to the salinity of 0–880 mmol l^{-1} . Emulsions with 0.1% (w/w) of CNC (*But*-CNC/A) and 0.05% (w/w) (*But*-CNC/D) in four different salt concentrations (0.5%, 1%, 3.5%, and 5% [w/v]) were prepared with the UltraTurrax mixer at 7,000 rpm for 15 min. As a reference, plain o/w emulsion without the CNC addition was used.

The changes in oil-droplet size were analyzed with a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, USA). Each sample was measured directly after dispersing, and three parallel measurements were performed.

2.6. Stability measurement of o/w emulsions with butylamino-functionalized cellulose as a dispersant

Emulsion stability was evaluated by measuring the o/w phase separation under centrifugal force with an analytical centrifuge (LUMiFuge, L. U. M. GmbH, Germany) at 20° C; a 1,200 rpm rotational speed, which corresponds to the centrifugal force of 205 G, was used. During the centrifugation, the near-infrared sensor measured the light transmission at the 800 nm wavelength through sample cells that were positioned horizontally. Changes in transmission could then be used to indicate the formation of an oil-water interface; continuous measurement for 1 h revealed the separation process as a function of time. Two parallel measurements of each sample were analyzed.

3. Results and Discussion

3.1. Chemical and physical properties of bifunctionalized celluloses

In this study, birch cellulose pulp fibers were chemically modified with sequential periodate and chlorite oxidations, followed by reductive amination to obtain bifunctionalized celluloses. These samples were easily homogenized at high pressure without clogging to liberate the individual CNCs. Five aqueous CNC dispersions, which were visually transparent and non-viscous solutions, were fabricated. In this procedure, surface functionalities were introduced prior to mechanical homogenization; any supplementary treatments after the liberation of the CNCs were unnecessary. The aldehyde and carboxyl contents of the oxidized cellulose samples, and the amount of the amino groups of the CNCs, are presented in Table 1. The initial aldehyde content of the cellulose was relatively high in order to enable the liberation of nanocrystals instead of nanofibrils. In addition, the carboxyl content had to be high enough (≥ 0.50 mmol/g) to promote the liberation of the CNCs. The hydrophobicity of the CNCs was altered by modifying the amino group content from 0.1 to 0.73 mmol/g.

Based on the elemental analysis, the amino substituent content of the CNCs varied from 0.53 mmol g⁻¹ to 0.73 mmol g⁻¹, while the carboxyl content ranged from 0.50 mmol g⁻¹ to 1.95 mmol g⁻¹. These corresponded to percentages of 3.8–24.0% and 22.7–71.8% in the conversion of amination and carboxylation reactions, respectively, since the aldehyde contents were 2.20 mmol g⁻¹ and 3.86 mmol g⁻¹, respectively. The residual aldehydes (23.6–53.4%) were converted back to alcohols by the reductive agent that had been used in the amination reaction.

The success of the chemical modification was assessed by DRIFT spectroscopy; Figure 2 shows the DRIFT spectra of pure and chemically modified celluloses. In addition to the typical cellulose spectrum, a new peak was observed at 1730 cm⁻¹ for periodate oxidized cellulose, representing the C=O stretching of aldehyde. After chlorite oxidation, a small aldehyde band was still observable, along with the appearance of a C=O stretching band at 1614 cm⁻¹, thus confirming that aldehydes were partially oxidized to corresponding carboxylic acids. The disappearance of the aldehyde band could be seen from the *But-CNC/D* spectrum, indicating that the aldehydes were converted to secondary amines or were reduced to hydroxyl groups. Due to the overlapping, however, the appearance of an amine bond could not be directly confirmed by DRIFT analysis.

3.2. Crystallinity

The X-ray diffraction patterns showed the characteristic peaks for cellulose I, indicating that no rearrangement had occurred to other allomorphs of the cellulose. The crystallinity indices calculated according to Eq. 1 for butylamino-functionalized CNC/A, CNC/B, CNC/C, CNC/D, and CNC/E were 53.3%, 54.6%, 50.1%, 38.6%, and 45.3%, respectively.

As Table 1 illustrates, *But-CNC/A*, *But-CNC/B*, and *But-CNC/C* had an initial aldehyde content of 2.20 mmol g⁻¹, and *But-CNC/D* and *But-CNC/E* had initial aldehyde contents of 3.86 mmol

g^{-1} . The high initial aldehyde content normally destroys more of the cellulose crystalline structure. The crystallinity index is typically lower when oxidation is prolonged and more carboxyl groups are formed. The periodate oxidation step seems to be the most significant phase of oxidation, and it also affects crystallinity. It is possible that during the chemical synthesis, the amorphous parts of the cellulose were dissolved, thus resulting in relative changes in the crystallinity index.

3.3. Morphology and optical properties of functionalized CNCs

The morphology of bifunctionalized CNCs was visualized with TEM, and image analysis was used into average the length (L), width (w), and aspect ratio (L/w) of the CNCs. The TEM images showed that the samples contained isolated, individual rod-shaped CNCs. A typical TEM image of *But-CNC/A* is shown in Figure 3. The lateral dimensions of CNCs were approximately 3 nm, while the lengths varied widely from 66–89 nm. Overall, the morphology of individualized crystals was similar to that reported by Visanko et al. [34]. However, the dimensions of the produced CNCs were smaller than is typical for hardwood, as lengths of 140–150 nm and widths of 4–5 nm have previously been reported [3]. Table 2 summarizes the average lengths and widths of the two butylamino-functionalized cellulose products that were studied, *But-CNC/A* and *But-CNC/D*. It is likely that the harsher reaction conditions during the periodate oxidation that we used for the *But-CNC/D* exposed the cellulose to chain scissions, which reduced the crystal length compared to *But-CNC/A*.

The bifunctionalized CNC suspensions had high optical transmittance values, at visible wavelengths of 340–800 nm (Figure 4). This indicates that the cellulosic fibers had effectively disintegrated, and the presence of nano-scale cellulose crystals was clear. The highest transmission was achieved with *But-CNC/D*, which had values ranging from 85–95% at all studied wavelengths. This indicates that suspension was successfully homogenized into the

individualized CNCs. *But*-CNC/A had lower transmission values (54–81%), which may have been due to the presence of larger fiber cell-wall fragments.

3.4. *O/w emulsions stabilized by bifunctionalized CNCs*

A laser diffraction particle size analyzer was used to determine the average oil-droplet particle size in the marine diesel o/w emulsions stabilized by CNCs. Figure 5 presents the average particle sizes of the emulsions that were stabilized with bifunctionalized cellulose products.

The dispersal of oil and emulsion stabilization with bifunctionalized CNCs resulted in a significant reduction in oil-droplet sizes compared to o/w emulsion without any dispersant. The particle size of the o/w reference after dispersal was approximately 48.2 μm ; this emulsion was not stable, and separation of the oil phase on top of the water occurred soon after dispersal. All CNC-stabilized emulsions had an average droplet size of approximately 15 μm except the *But*-CNC/D, which had a droplet size of approximately 10 μm . Although the small droplet sizes in emulsion were emphasized the most in *But*-CNC/A, that product was also chosen for further study because it achieved the best initial emulsion, with the lowest chemical consumption in the mildest conditions.

3.5. *Optimization of CNC dosage*

Figure 6 shows the influence of different concentrations of *But*-CNC/A and *But*-CNC/D on oil-droplet size. Only the addition of 0.05% (w/w) of both CNCs significantly reduced the oil particle size, and there were only small changes in droplet size as a function of CNC dosage. The *But*-CNC/D, however, resulted in a smaller droplet size than the *But*-CNC/A (approximately 10 μm vs. 20 μm). *But*-CNC/A and *But*-CNC/D differed from each other by their amino group content, as shown in Table 1. It is generally assumed that the hydrophobic

nature of CNCs increases as a function of amino group content. *But*-CNC/D is therefore more likely to result in smaller particle sizes in emulsions at lower concentrations.

3.6. Effect of water salinity on oil-droplet size in o/w emulsion

Dispersal efficiency in o/w emulsification is affected by temperature, mixing energy, and water electrolyte concentrations (salinity) [35]. Background electrolytes, in particular, can affect electrosterically stabilized emulsions, since the counter ions screen the electrostatic repulsion between surface-charged oil droplets, which in turn promote droplet coalescence and phase separation. The extent of this effect is associated with the valence of electrolytes, as stated by the Schulze-Hardy rule [36].

The effect of NaCl dosage on marine diesel oil-droplet size can be seen in Figure 7. The droplet size increased in low salt concentrations with both *But*-CNC/D- and *But*-CNC/A-stabilized emulsions. At the highest NaCl concentration (5% by weight), the droplet sizes were approximately 18 μm and 23 μm , respectively. The droplet size was still over 50% smaller than in the reference sample in the absence of electrolytes. This phenomenon indicates that the stabilization effect of bifunctionalized CNCs can be attributed to a combined electrosterical mechanism, since the electrolytes increased the droplets' sizes (electrochemical stabilization), but the stabilization effect was maintained despite the high background electrolyte concentration (steric stabilization).

3.7. Stability of o/w emulsions

The stability of o/w emulsions (1:10) in terms of phase separation and droplet coalescence was measured by using an analytical centrifuge, which measures the transmittance of emulsion during centrifugation. The increase in transmittance indicates droplet coalescence and the separation of oil from the water phase. The stability of the reference o/w emulsion without

CNCs was poor, as noted earlier in the droplet-size measurement section, as the de-emulsification had already started during analysis. During the stability testing, for the reference, the phase separation occurred after 750 seconds of centrifugation, after which a constant transmittance of approximately 80% was obtained (Figure 8). The oil separation was remarkably less pronounced for the CNC-stabilized emulsions, as indicated by the lower transmittance of the emulsions; however, there were significant differences in the kinetics of the phase separation as a function of background electrolyte concentration.

The emulsions stabilized by the *But*-CNC/A separated significantly more rapidly than the *But*-CNC/D-stabilized emulsions in the presence of electrolytes. This can probably be attributed to the differences in charge densities and the content of the hydrophobic amino groups and their ratios. For example, the charged carboxyl group content of *But*-CNC/A was approximately half that of *But*-CNC/D, as shown in Table 1. Thus, the smaller effective surface charge of *But*-CNC/A, which affected droplet stability, was likely screened and reduced by the electrolytes below the value required to prevent droplet coalescence. In addition, the emulsions stabilized by *But*-CNC/D seemed to be more stable when the salt concentration was increased. Although the reason for this behavior remains unclear, the electrolytes may affect the conformation of functional groups, which in turn affects CNCs' stabilization effects.

Figure 9 illustrates the appearance of o/w emulsions 30 min. after emulsification for the reference (oil and water only) and for the *But*-CNC-stabilized samples. The stability in the reference emulsions was poor, and oil-droplet coalescence occurred soon after dispersal, which resulted in oil phase separation on the surface of the water. The emulsions with 0.1% (w/w) of *But*-CNCs, in turn, maintained their stability, and no phase separation was observed. CNCs without n-butylamino groups attached were also used as a control sample in LUMIfuge stability analysis; butylamino-functionalized CNCs as well as CNCs without modification

were used at 0.1%. The ratio of oil and water in the emulsion stability test was 1:10. The results showed that butylamino-functionalized CNCs had a better ability to stabilize emulsions than non-functionalized CNCs. The results of these measurements are illustrated in Figure 11 which is provided as supplementary material.

3.8. The mechanism of o/w emulsion stabilization with bifunctionalized CNCs

The introduction of *n*-butylamino groups on the cellulose backbone was used to promote the hydrophobicity of CNCs and to increase their attraction toward oil droplets. In this work, carboxylic groups were also introduced to the cellulose structure, resulting in bifunctionalized CNCs. The carboxylic groups were in turn hydrophilic, and had a pH charge of at least 4 (the pK_a value of the mono-dissociated form of dicarboxylic acid cellulose is 3.66) [37].

It is generally assumed that this bifunctionalized and amphiphilic structure is able to seek the oil-water interface and to interact with the oil phase via *n*-butylamino moieties, while the hydrophilic carboxylic groups mainly protrude toward the aqueous phase. These functional groups may be randomly distributed on nanocrystal surfaces, however, giving them optimal surface energy to be positioned on the oil-water interface. Nanocrystals tend to cover the interface of an oil droplet more densely when shorter crystals are used; longer crystals have more steric hindrance, and therefore more porous and multilayer structures are formed [27]. The rigid crystalline nano-scale cellulose rods act as steric barriers that restrain attractive oil-droplet interactions and stabilize the droplets. This rigid structure is likely less sensitive to the conformational changes that are associated with soluble dispersing agents. The anionic carboxyl groups, in turn, promote droplet stability through repulsive electrostatic interactions, as demonstrated in our study by the o/w emulsion response to the addition of NaCl (Figure 8).

The effectiveness of nanoparticles as stabilizers on o/w emulsions is based on particle size and wettability among particle-to-particle interactions. The o/w emulsion is formed when

the contact angle between the three phases is above 90° [4]. A schematic illustration of the mechanism function of bifunctionalized CNCs in o/w emulsions is shown in Figure 10.

4. Conclusions

The modification route based on sodium periodate and chlorite oxidations, followed by amination with *n*-butylamine, was used to produce bifunctionalized celluloses. The obtained celluloses with an amphiphilic nature were disintegrated to CNCs and utilized as a nanoparticle emulsifier for o/w emulsions. Bifunctionalized CNCs reduced the oil-droplet size in emulsions and acted as a stabilizer. Increasing the salt (NaCl) concentration in the emulsions increased the oil-droplet size, but at the highest concentration (5% [wt]) the droplet sizes were still over 50% smaller than with the reference sample in the absence of electrolytes.

This study found that butylamino-functionalized CNCs can be utilized as an emulsifier in o/w emulsions. The complexity of the interactions between novel cellulose-based nanoparticle emulsifiers and different oil types is clear; further empirical studies on the effects of temperature, oil type, dispersant type, and dispersant mixing energy are needed to address the current lack of statistical data on the subject.

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Tables:**Table 1.** Characteristics of bifunctionalized cellulose samples containing carboxyl and n-butylamine groups.

	Initial aldehyde content (mmol/g)	Carboxyl content (mmol/g)	Content of butylamino groups in CNC (mmol/g)
<i>But-CNC/A</i>	2.20	0.50	0.53
<i>But-CNC/B</i>	2.20	1.13	0.28
<i>But-CNC/C</i>	2.20	1.58	0.10
<i>But-CNC/D</i>	3.86	1.08	0.73
<i>But-CNC/E</i>	3.86	1.95	0.15

Table 2. Average lengths and widths of bifunctionalized CNCs based on iTEM image analysis.

Cellulose derivative	Length [nm]	Width [nm]	L/w
<i>But-CNC/A</i>	89 ± 40	3.0 ± 1.0	29.7
<i>But-CNC/D</i>	66 ± 40	3.3 ± 1.0	20.1

Figure captions:

Figure 1. Sodium periodate and chlorite oxidation followed by reductive amination of cellulose; n-butylamines are introduced to partially oxidized cellulose (PO-DAC).

Figure 2. DRIFT-spectra of the pure birch cellulose, dialdehyde cellulose (DAC), partially oxidized cellulose (PO-DAC), and butylamino-functionalized cellulose (*But-CNC/D*).

Figure 3. TEM image of bifunctionalized cellulose nanocrystals (*But-CNC/A*). The scale bar is 200 nm.

Figure 4. Optical transmittance of bifunctionalized CNC suspensions (0.1 w/w %).

Figure 5. Average particle sizes of o/w emulsion stabilized by bifunctionalized CNCs at concentration 0.1 w/w %.

Figure 6. The effect of bifunctionalized CNC concentration on oil-droplet size in o/w emulsion. The analyses were conducted immediately after emulsification.

Figure 7. The effect of increasing NaCl concentration on oil-droplet size in *But-CNC/A* at 0.1% (w/w) and *But-CNC/D* at 0.05% (w/w) stabilized o/w emulsion immediately after emulsification.

Figure 8. The effect of the addition of background salt (NaCl) on the emulsion stability of 0.1% (w/w) *But-CNC/A*-stabilized and 0.05% (w/w) *But-CNC/D*-stabilized o/w dispersions.

Figure 9. o/w emulsions 30 min. after dispersal. Rapid de-emulsification can be seen in the reference sample, where only oil (red color) is dispersed in the water phase (A), whereas the stabilization effect is clear with the butylamino-functionalized samples *But-CNC/D* (B) and *But-CNC/A* (C).

Figure 10. The dispersal mechanism using bifunctionalized CNCs in o/w emulsions as a schematic illustration.

Highlights

- Bifunctionalized cellulose nanocrystals (*But*-CNCs) were used to stabilize o/w emulsion.
- *But*-CNCs reduced the oil-droplet size in emulsions, and acted as a stabilizer.
- The background salt concentration only had a slight effect on CNC performance.
- Bifunctionalized CNCs can potentially be used as “green” oil spill response agents.