NRC Publications Archive
Archives des publications du CNRC

Characteristics and Properties of Carboxylated Cellulose Nanocrystals Prepared from a Novel One-Step Procedure

Leung, Alfred C. W.; Hrapovic, Sabahudin; Lam, Edmond; Liu, Yali; Male, Keith B.; Mahmoud, Khaled A.; Luong, John H. T.

National Research Council Canada   Conseil national de recherches Canada

Canada

Characteristics and Properties of Carboxylated Cellulose Nanocrystals Prepared from a Novel One-Step Procedure

Alfred C. W. Leung, Sabahudin Hrapovic, Edmond Lam, Yali Liu, Keith B. Male, Khaled A. Mahmoud, and John H. T. Luong*

Cellulose nanocrystals (CNCs) have emerged as a new class of nanomaterials for polymer reinforcement and nanocomposite formulation owing to their exceptionally high mechanical strength (modulus of 100–140 GPa), low density (1.6 g cm^{-3}), chemical tunability, environmental sustainability, and anticipated low cost.[1] CNCs have also been fostered for a myriad of applications including enzyme immobilization,[2] drug delivery,[3] and biomedical applications.[4] In order to obtain CNCs, native semicrystalline cellulose is broken down into its elementary crystalline domains, removing amorphous cellulose segments with inferior mechanical and chemical characteristics. Wood and plant fibers represent the most abundant source of cellulose, but the extraction of CNCs from these natural sources requires prior removal of their noncellulosic constituents such as lignin, pectin, hemicellulose, and wax. Existing methods to prepare and isolate CNCs use acids, enzymes, oxidizers, mechanical means, or a combination of these in multiple steps.[5] These procedures require relatively pure cellulosic starting materials such as steam-exploded wood pulp and microcrystalline cellulose, or alkaline and bleaching agents as pretreatments. The use of highly corrosive mineral acids (requiring expensive corrosion resistant equipment), multiple treatment steps or tedious isolation techniques impedes large-scale production and real-world applications of CNCs. Herein, a simple and versatile one-step procedure to produce highly crystalline carboxylated CNCs is reported. This method is amenable to scale-up and broadly applicable to a variety of native plant fibers and other cellulose sources, simultaneously removing noncellulosics and amorphous cellulose to yield high-quality CNCs.

Ammonium persulfate (APS), an oxidant with low long-term toxicity, high water solubility and low cost, was favored over its sodium and potassium counterparts. CNCs were prepared by heating cellulosic materials at 60 °C in 1 M APS for 16 h with vigorous stirring. The reaction time was substrate-dependent, ranging from 3 h for bacterial cellulose and 16 h for complex substrates such as hemp, flax,

and triticale. HPLC analysis confirmed no detectable furfural or 2-hydroxymethylfurfural (HMF) in the resulting liquid. For comparison, acid hydrolysis was also conducted to confirm the presence of these two hazardous chemicals, which are often encountered when carbohydrates are hydrolyzed using strong acids.[6] Atomic force microscopy (AFM) and transmission electron microscopy (TEM) micrographs confirmed the production of highly uniform CNCs with significantly smaller diameters compared to those obtained by acid treatment (**Figure 1**a). CNCs produced from flax and hemp using a combination of acid hydrolysis and bleaching have average diameters of 16–28 nm and 20–40 nm, respectively.[7] In comparison, the cross-section dimensions of CNCs prepared from APS treatment of hemp, flax, and triticale centered at ≈3–6 nm, reflecting elementary fibrils (≈3–7 nm in diameter) of cellulose. CNC samples prepared from different cellulosics also showed a similar mean particle length and length polydispersity (**Table 1**). This was a very important finding, since the uniformity, small diameter, and high aspect ratio of such nanocrystals are critical for their intended applications as nanofillers or for drug delivery. CNCs with high uniformity greatly facilitated their subsequent recovery and purification as the resulting liquid was easily processed using centrifugation or ultrafiltration with a 50 kDa cut-off membrane filter. Owing to the corrosiveness of strong acids, the CNC-containing liquid obtained by acid hydrolysis must be diluted at least five-fold, followed by differential centrifugation to obtain CNCs with a narrow size distribution.[8] Of further importance is a direct comparison of the yield obtained by our procedure with the widely used acid hydrolysis for the preparation of CNCs from microcrystalline cellulose (MCC). CNCs produced by acid hydrolysis of MCC consist of six major size fractions with a total yield of 21% compared to 65% with our procedure as shown in Table 1.[8] Similarly, only ≈5% CNC yield was attainable from acid hydrolysis and bleaching of flax and hemp fibers, compared to 28–36% with the APS treatment.

Scanning electron microscopy (SEM) imaging shows multiple morphological changes on the surface of the fibers after treatment with APS for 1 h, indicating the onset of CNC formation (Figure 1b). In contrast, the fibers remained intact when subjected to heating without APS. Evidently, APS was able to produce CNCs in situ by removing lignin, hemicellulose, pectin, and other plant contents (Figure 1c). Free radicals are formed when the solution containing persulfate is heated ($S_2O_8^{2-} + heat \rightarrow 2SO_4^{-}$). Under the acidic conditions used

Dr. A. C. W. Leung, S. Hrapovic, Dr. E. Lam, Y. Liu, K. B. Male, Dr. K. A. Mahmoud, Prof. J. H. T. Luong
Biotechnology Research Institute
National Research Council of Canada
6100 Royalmount Avenue, Montreal H4P 2R2, Canada
E-mail: john.luong@cnrc-nrc.gc.ca
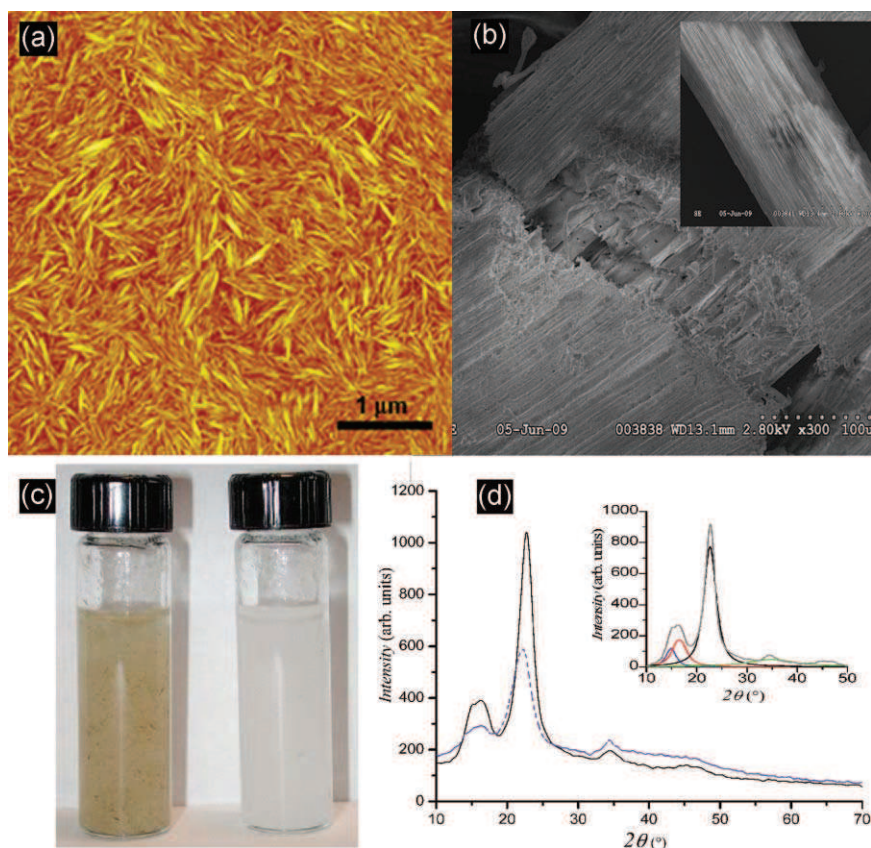
Figure 1. a) Height mode AFM images of CNCs from flax (5 μm). b) SEM image of flax fibers after 1 h of APS treatment (inset: the fibers were treated without APS). c) Aqueous suspensions of ground flax fibers before treatment (left) and cellulose nanocrystals (1 wt%) after treatment with 1 M APS. d) XRD spectra of CNCs produced from hemp before (blue) and after (black) treatment with 1 M APS: inset shows the deconvoluted cellulose peaks.

pretreatments with alkaline or bleaching agents to remove other fiber contents, and this treatment with alkalines often affects the crystallinity and structure of cellulose (cellulose I to II).[10]

CNCs prepared from various biomass sources were characterized by X-ray diffraction (XRD), solid-state NMR, and IR spectroscopy. The CNC diffractograms exhibited the most intense peak (002) with a shoulder (021) and two lower peaks (101 and $10\bar{1}$). Such features, including the d-spacing and average crystallite size as determined by the Debye–Scherrer formula,[11] resembled the diffraction pattern of cellulose I and confirmed the integrity of the material during the course of treatment with APS. The crystallinity index (CRI) of CNCs was then estimated using an integral method based on the ratio of the areas of crystallines to total scattered intensity, with the results summarized in Table 1.[12] In general, the CRI of CNCs was noticeably higher than that of its parental counterparts except for CNCs prepared from MCC and Whatman CF1. With very high initial crystallinity, the treatment of MCC and Whatman CF1 with APS resulted in nanosized fibers, whereas CRI only increased slightly for CNC samples produced from MCC and remained

in this study (pH 1.0), hydrogen peroxide was also formed $(S_2O_8^{2-} + 2H_2O \rightarrow 2HSO_4^- + H_2O_2)$.[9] Collectively, these free radicals and H_2O_2 are capable of penetrating the amorphous regions to break down amorphous cellulose to form CNCs. Both free radicals $SO_4^{\cdot-}$ and H_2O_2 also open the aromatic rings of lignin to decolorize this material. Our data confirmed that APS was capable of producing CNCs from cellulosics containing up to 20% lignin. In contrast, acid hydrolysis requires

similar for Whatman CF1. CNC thermostability was investigated with thermogravimetric analysis (TGA), indicating that the CNCs were stable to ≈220–270 °C with <5% mass loss below this temperature (T_{d5}). In comparison, CNCs obtained from sulfuric acid hydrolysis have lower thermal stability (≈150–200 °C) due to the presence of sulfate groups.[13] Notice that T_{d5} directly correlates with the CRI of the CNCs (Supporting Information).

Table 1. Yield, degree of oxidation, crystallinity index (CRI), and dimensions of CNCs from various cellulosic sources. CRI is estimated using the integral method. Length and diameter are reported at 95% confidence intervals (S.M. denotes starting materials, SD denotes the standard deviation).

Cellulosic Material	Yield [%]	Degree of Oxidation	CRI [%] S.M.	CRI [%] CNC	Length [nm]	Diameter [nm]
Flax	28	0.18	69	75	144 ± 5 (n = 138, SD = 53)	3.8 ± 0.1 (n = 1653, SD = 1.9)
Flax Shives	22	0.19	48	64	296 ± 16 (n = 445, SD = 194)	5.1 ± 0.1 (n = 1350, SD = 1.7)
Hemp	36	0.17	56	73	148 ± 3 (n = 292, SD = 52)	5.8 ± 0.1 (n = 906, SD = 1.8)
Triticale	31	0.11	61	73	134 ± 5 (n = 118, SD = 54)	4.2 ± 0.1 (n = 266, SD = 1.8)
MCC	65	0.19	79	83	128 ± 4 (n = 100, SD = 38)	5.5 ± 0.1 (n = 2291, SD = 1.5)
Whatman CF1	81	0.12	91	91	121 ± 3 (n = 129, SD = 39)	6.7 ± 0.3 (n = 207, SD = 2.2)
Wood Pulp	36	0.19	73	81	124 ± 6 (n = 200, SD = 41)	6.0 ± 0.2 (n = 253, SD = 1.7)
Bacterial Cellulose	14	0.14	59	70	88 ± 5 (n = 500, SD = 55)	6.5 ± 0.2 (n = 497, SD = 2.3)

IR spectra of the CNCs show absorption bands that are typical for cellulosic materials. The presence of signals at 1429, 1163, 1111, and 897 cm^{-1} indicated that the CNCs are primarily in the form of cellulose I$_\beta$, except for APS-treated bacterial cellulose in which characteristic absorption bands at 3241 and 753 cm^{-1} confirm the high content of cellulose type I$_\alpha$ in bacterial cellulose. The CNC absorption patterns remained unchanged after treatment with APS, indicating no significant changes of the conformation of the cellulose structure, i.e., mercerization did not occur. The IR spectra also displayed a signal at 1735 cm^{-1}, attesting the presence of carboxylic acid groups. The degree of oxidation (DO) of the CNCs was determined using a conductometric titration method to fall in the range of 0.11–0.19.[14] In agreement with literature data, CP-MAS ^{13}C-NMR spectra of the CNCs show the expected signals corresponding to the presence of high purity and crystallinity cellulose I (Supporting Information). The appearance of a signal at δ = 175 ppm confirms the presence of carboxylic acid groups.[5c] Of importance to note was that the conversion of the carboxylic acid groups to their carboxylate form (with the addition of a small amount of NaOH to achieve ≈pH 7) significantly improved the ease of CNC redispersion after lyophilization. Nonflocculate and stable suspensions were prepared simply by stirring freeze-dried CNC powders in water.

The synthesis of carboxylated CNCs was reported by Montanari et al. using a 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) radical-catalyzed oxidation method.[5d] With this procedure, the C6 primary alcohol of CNCs prepared by acid hydrolysis can be selectively oxidized to generate carboxylated CNCs. Our experimental data attested that without prior acid treatment, the TEMPO procedure produced longer microfibrils, suggesting that TEMPO was only effective in fiber defibrillation, i.e., it did not completely break down the amorphous domains, in agreement with Saito et al.[15] In comparison, APS defibrillated and removed amorphous cellulose effectively, resulting in uniform CNCs with nanoscale dimensions. Both FTIR and NMR data also suggested that oxidation occurred preferentially at the C6 primary alcohol of crystalline cellulose. The oxidation of C2 and C3 secondary alcohols is known to induce the cleavage of the glucopyranose ring, resulting in detrimental lowering of the CRI.[16] The absence of an IR signal relating to hemiacetal formation (880 cm^{-1}) or any significant changes in the respective chemical shifts of the CP-MAS ^{13}C-NMR signals confirmed the intact crystalline structure of CNCs prepared by APS.

Carboxylated CNCs can be activated by a number of strategies for coupling amine-containing molecules to form stable amide linkages.[17] The presence of carboxylic acid groups offers active sites for surface modification and protein/enzyme immobilization, as well as templates for the synthesis of nanoparticles.[18] To highlight the chemical tunability of the carboxylated CNCs, decylamine, dioctylamine, 2-aminoanthracene, and avidin were attached to CNCs using standard peptide-coupling protocols (**Scheme 1**). Modified CNCs with long alkyl chains imparted hydrophobicity for compatibility with hydrophobic polymer matrices. Conjugation of CNCs with aminoanthracene resulted in a novel fluorescent
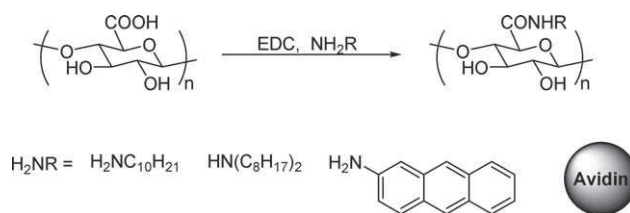
Scheme 1. Surface modification of carboxylated CNCs. EDC = N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride.

nanomaterial (excitation = 370 nm, emission = 422 nm), which might be used for cell imaging and biosensing. Futhermore, we were able to attach avidin onto the surface of the carboxylated CNCs, and these may serve as platforms for the immobilization of biotinylated-enzymes.[19] Beside their potential application as polymer nanofillers, carboxylated CNCs may serve as useful platforms for biosensing, enzyme immobilization, affinity chromatography, and drug targeting/delivery.[20] A recent report revealed that CNCs have low toxicity potential and environmental risk.[21]

In brief, we have described a one-step procedure for producing CNCs from cellulosic biomass that would carry significant benefits in terms of sustainability and low costs. Our approach could be considered as a chemical nanoscissor effective in processing raw cellulosics, in contrast to acid hydrolysis which requires pretreatment steps for the isolation of cellulose. Our facile procedure is amenable to the large-scale production of CNCs with enhanced uniformity and crystallinity. Tunable hydropilicity or hydrophobicity can be easily attained by conjugation of the carboxyl group with pertinent molecules.

Experimental Section

Preparation of Cellulose Nanocrystals: The starting biomass material (10 g) was added to 1 L of 1 M ammonium persulfate solution. The mixture was heated to 60 °C for 16 h (only 3 h for bacterial cellulose) to give a suspension of CNCs. The suspension was centrifuged (12 000 rpm, relative centrifugal force (RCF) = 22 100) for 10 min. The solution was decanted, and ≈200 mL of water was added to the CNC pellet, followed by 5 min of mixing and repeated centrifugation. The centrifugation/washing cycles were repeated 4 times until the solution conductivity was ≈5 µS cm^{-1} (pH 4), close to that of deionized water. The product was lyophilized to yield a white powder. CNCs in their sodium form were prepared by adding 1 M NaOH until the suspension reaches pH 7, followed by washing/centrifugation with deionized water.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

[1] a) S. J. Eichhorn, A. Dufresne, M. Aranguren, N. E. Marcovich, J. R. Capadona, S. J. Rowan, C. Weder, W. Thielemans, M. Roman, S. Renneckar, W. Gindl, S. Veigel, J. Keckes, H. Yano, K. Abe, M. Nogi, A. N. Nakagaito, A. Mangalam, J. Simonsen, S. Benight, A. Bismarck, L. A. Berglund, T. Peijs, *J. Mater. Sci.* **2010**, *45*, 1; b) Y. Habibi, L. A. Lucia, O. J. Rojas, *Chem. Rev.* **2010**, *110*, 3479; c) J. R. Capadona, O. van den Berg, L. A. Capadona, M. Schroeter, S. J. Rowan, D. J. Tyler, C. Weder, *Nature Nanotech.* **2007**, *2*, 765.

[2] K. A. Mahmoud, K. B. Male, S. Hrapovic, J. H. T. Luong, *Appl. Mat. Interfaces* **2009**, *1*, 1383.

[3] K. J. Edgar, T. Heinze, C. M. Buchanan in *Polysaccharide Materials: Performance by Design*, American Chemical Society, Washington DC, USA **2009**, Ch. 4.

[4] S. Dong, M. Roman, *J. Am. Chem. Soc.* **2007**, *129*, 13810.

[5] a) J.-F. Revol, H. Bradford, J. Giasson, R. H. Marchessault, D. G. Gray, *Int. J. Biol. Macromol.* **1992**, *14*, 170; b) X. Cao, H. Dong, C. M. Li, *Biomacromolecules* **2007**, *8*, 899; c) P. B. Filson, B. E. Dawson-Andoh, D. Schwegler-Berry, *Green Chem.* **2009**, *11*, 1808; d) S. Montanari, M. Roumani, L. Heux, M. R. Vignon, *Macromolecules* **2005**, *38*, 1665.

[6] CNCs were prepared from acid hydrolysis of MCC according to: S. Beck-Candanedo, M. Roman, D. G. Gray, *Biomacromolecules* **2005**, *6*, 1048. The spent acid solutions were analyzed using HPLC and were found to contain 0.21 mM furfural and 0.38 mM HMF.

[7] X. Cao, Y. Chen, P. R. Chang, M. Stumborg, M. A. Huneault, *J. Appl. Polym. Sci.* **2008**, *109*, 3804.

[8] W. Bai, J. Holbery, K. Li, *Cellulose* **2009**, *16*, 455.

[9] S. C. Hsu, T.-M. Don, W.-Y. Chiu, *Polym. Degrad. Stab.* **2002**, *75*, 73.

[10] H. A. Krassig, *Cellulose: Structure, Accessibility and Reactivity*; Gordon & Breach Sci. Publishers, Amsterdam, Netherlands **1996**.

[11] P. Debye, *Ann. Physik* **1915**, *46*, 809.

[12] G. Jayme, H. Knolle, *Papier* **1964**, *19*, 106.

[13] M. Roman, W. T. Winter, *Biomacromolecules* **2004**, *5*, 1671.

[14] D. da Silva Perez, S. Montanari, M. R. Vignon, *Biomacromolecules* **2003**, *4*, 1417.

[15] a) T. Saito, Y. Okita, T. T. Nge, J. Sugiyama, A. Isogai, *Carbohyd. Polym.* **2006**, *65*, 435; b) T. Saito, Y. Nishiyama, J.-L. Putaux, M. Vignon, A. Isogai, *Biomacromolecules* **2006**, *7*, 1687.

[16] a) U.-J. Kim, S. Kuga, M. Wada, T. Okano, T. Kondo, *Biomacromolecules* **2000**, *1*, 488; b) A. Potthast, T. Rosenau, P. Kosma, *Adv. Polym. Sci.* **2006**, *205*, 1.

[17] A. P. Mangalam, J. Simonsen, A. S. Benight, *Biomacromolecules* **2009**, *10*, 497.

[18] S. Ifuku, M. Tsuji, M. Morimoto, H. Saimoto, H. Yano, *Biomacromolecules* **2009**, *10*, 2714.

[19] H. Brumer III, Q. Zhou, M. J. Baumann, K. Carlsson, T. T. Teeri, *J. Am. Chem. Soc.* **2004**, *126*, 5715.

[20] Z. Zhong, S. Patskovskyy, P. Bouvrette, J. H. T. Luong, A. Gedanken, *J. Phys. Chem. B* **2004**, *108*, 4046.

[21] T. Kovacs, V. Naish, B. O'Connor, C. Blaise, F. Gagné, L. Hall, V. Trudeau, P. Martel, *Nanotoxicology* **2010**, *4*, 255.