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Cellulose Nanocrystals Incorporating Fluorescent Methylcoumarin Groups

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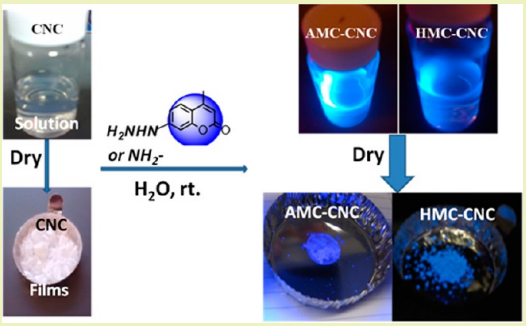
Supporting Information

ABSTRACT: Fluorescent rod-shaped nanoparticles were prepared by attaching hydrazine- or amino-substituted fluorophores onto cellulose nanocrystals (CNC) to form hydrazone and Schiff-base compounds. The products 7-hydrazino-4-methylcoumarin (HMC)−CNC and 7-amino-4-methylcoumarin (AMC)−CNC were examined by ATR-IR, solid-state NMR, ultraviolet−visible absorbance, fluorescence spectroscopy, AFM, TEM, wide-angle XRD, elemental analysis, and X-ray photoelectron spectroscopy (XPS). Estimates of the amount of dye attached to the nanocrystals suggested that substitution was not confined to the reducing end of the nanocrystals. This provides a new route to fluorescent-tagged CNC in an aqueous one-step reaction.



KEYWORDS: *Cellulose nanocrystals (CNC), 7-Hydrazino-4-methylcoumarin, 7-Amino-4-methylcoumarin, One-step synthesis, Fluorescent-tagged CNC*

INTRODUCTION

Nanocelluloses have attracted significant attention because they are renewable, environmentally benign, naturally abundant, biodegrable, and biocompatible materials, with excellent mechanical properties.[1−3] Here, we describe a new way to make fluorescent rod-like particles from nanocrystalline cellulose (CNC), the form of nanocellulose produced by sulfuric acid hydrolysis of natural cellulose fibres. Fluorescent tagging of CNC is expected to have potential applications in determining the location of CNC in biological and sensor systems. The direct fluorescent functionalization of nanocrystalline cellulose is nontrivial because of the difficulties in separating and purifying the nanoparticles and analyzing the relatively minor changes in surface compositions. Furthermore, multistep methods are usually required. For example, Dong and Roman used a three-step method to label CNC with fluorescein-5′-isothiocyanide (FTIC) to the extent of one fluorophore group per 27 nm^2 of a CNC surface.[4] Mahmoud et al. used a similar method to label cellulose nanocrystals with a range of fluorophores, including FTIC.[5] A two-step method was used to attach FTIC and a pyrene-based fluorophore to aminosilane-treated CNC.[6] Filpponen et al. used TEMPO oxidation, carbodiimide amidation, and copper(I)-catalyzed azide−alkyne click chemistry to add coumarin and anthracene fluorophores to CNC surfaces.[7] Nielsen et al. described the modification with responsive fluorophores using one-step and three-step procedures.[8] Here, we present a simple, efficient, and one-step synthetic strategy for fluorescent labeling of nanocrystalline cellulose with 7-hydrazino-4-methylcoumarin (HMC) and 7-amino-4-methylcoumarin (AMC). The crystallinity and properties of CNC are preserved after the fluorescent tagging.

EXPERIMENTAL SECTION

Materials. Cotton from Whatman cellulose filter aid was used as the cellulose source. Deionized water (18.2 MΩ cm, Millipore Milli-Q Purification System) was used throughout. 7-Amino-4-methylcoumarin (AMC), sodium nitrite, solvents, and other reagents were purchased from Sigma−Aldrich chemical company and were used without prior purification.

Preparation of CNC. Suspensions of nanocrystalline cellulose (CNC) were prepared as previously described[9,10] by hydrolysis using 64% w/w sulfuric acid at 45 °C for 45 min with constant stirring. Typically, 20 g of cotton filter-aid was treated with 350 mL of acid. Immediately following the acid hydrolysis, the suspension was diluted 10-fold with deionized water to quench the reaction. The suspension was centrifuged at 10000 rpm for 6 min to concentrate the cellulose and to remove excess water and acid. The resultant precipitate was rinsed, recentrifuged, and dialyzed against water for 6 days until a constant neutral pH was achieved in the effluent. Solid CNC was also obtained by freeze drying overnight or by air drying.

Preparation of HMC-Labeling CNC and AMC-Labeling CNC. 7-Hydrazino-4-methylcoumarin (HMC) was prepared following a modified literature procedure (Supporting Information).[11] HMC-labeled CNC was prepared as follows. 7-Hydrazino-4-methylcoumarin (HMC) was allowed to react with the nanocrystalline cellulose in a water suspension for 24 h at 25 °C in a flask in the dark. When the reaction was completed, the modified nanocrystalline cellulose was isolated by centrifugation and purified by successive Soxhlet extraction with acetone and methanol until no fluorescence was detected in the washing liquor. The nanocrystals were resuspended in water, and the suspension was dialyzed against deionized water and subsequently

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freeze dried. AMC-labeled CNC was prepared in a similar manner by replacing AMC with HMC.

Characterization. Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectra were recorded on a Perkin-Elmer Spectrum 100 FTIR spectrophotometer with a universal ATR sampling accessory (Waltham, MA). The UV−vis absorption spectra were collected with a Varian V-670 UV−vis spectrophotometer (Agilent, U.S.A.). Fluorescence spectra were recorded on a Cary eclipse fluorescence spectrometer (Agilent, U.S.A.). The average particle diameters and morphology were determined by TEM with a Philips CM200 instrument operated at 200 Kv. A MFP-3D atomic force microscope (Asylum Research) was used to image surface morphology. Images were collected in tapping mode using Ultrasharp Si tips (spring constant 5.7 N/m, resonant frequency ca. 160 Hz, NSC14 series, MikroMasch). Surface analysis was performed by X-ray photoelectron spectroscopy (XPS) with aVG Escalab 3 MKII with a power of 206 W. A surface area of 2 mm × 3 mm was analyzed with an escape depth of 5−10 nm. Wide-angle X-ray diffraction (XRD) was performed with a Bruker AXS D8 Discover using Cu Kα radiation at 40 kV and 40 mA.

RESULTS AND DISCUSSION

Suspensions and films of the modified CNC display strong fluorescence when viewed under UV light. The FT-IR spectra in Figure 1 clearly show the successful attachment of the HMC

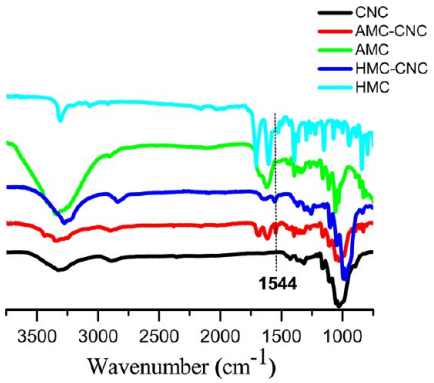

Figure 1. FT-IR spectra of the pure CNC, AMC dyes, HMC dyes, and modified CNC.

dyes and AMC dyes to the CNC by the appearance of a peak at 1544 cm^{-1} corresponding to the ν(C═N) of the imine.[12] The modified CNC not only displayed characteristic peaks for cellulose around 3338 (O−H), 2892 (C−H), 1388 (CH_2), and 1030 (C−O−C) cm^{-1}, but also exhibited the characteristic peaks of the aromatic rings of AMC at 1687 (C═O) and 1604 (C═C) cm^{-1}. The successful modification of CNC was also further confirmed by UV−vis and fluorescence spectroscopy. As shown in Figure 2, the modified CNC show clear absorbance peaks at 325 and 350 nm corresponding to HMC and AMC, respectively, and clear emission peaks at 452 and 440 nm, respectively, whereas the unmodified nanocrystalline cellulose shows no absorption or emission peaks at these wavelengths. The absorption and emission maxima for HMC−CNC were shifted to longer wavelengths relative to the free dye, as would be expected for the formation of a hydrazone.[13] In addition, the solid-state ^{13}C NMR spectra shown in Figure 3 exhibited some new peaks compared with pure CNC. The sharp peak at 65 ppm is attributed to C6, the primary alcohol group of the anhydroglucose units. The resonances for the anhydroglucose carbons C2, C3, and C5 appeared at 70−80 ppm. The resonance peak at 89 ppm corresponds to the carbon C4. The broad peak around 105 ppm is assigned to C1 and to the coumarin carbons 3′, 5′, 7, and 10. Moreover, the upfield shoulder peaks for the C6 and C4 carbons (63 and 85 ppm) are associated with the amorphous and surface regions of the cellulose nanocrystals. The peaks around 120−160 ppm are attributed to aromatic carbons in the dyes ring that are linked to cellulose. The methyl carbon (C11) and hydrazone carbon (C1′) appeared at 19 and 153 ppm, respectively. These shift data are in good accordance with those for model D-glucose−HMC adducts (see experimental details and NMR data in the Supporting Information). The solid-state NMR data is in accord with covalent linkage of the HMC and AMC dyes with CNC.

We need to check that the incorporation of the fluorophores has not destroyed the basic properties of the nanocrystals. Figure 4 shows the AFM images of the CNC before and after modification. As shown in the images, the rod-like morphology of the CNC has been retained; their dimensions are about 10−50 nm wide by 50−200 nm long, which could be further confirmed by the TEM pictures in Figure 5. Figure 6 shows the wide-angle XRD diffraction patterns of the CNC before and after modification. Both before and after modification the pattern shows a clear cellulose Iβ polymorph with the 200 diffraction centered around 23° and a peak signal at 16° for 110 plane.[14] The diffraction patterns were almost the same before and after modification, showing that the crystal structure is essentially not affected by the grafting of the HMC and AMC dyes or the reaction conditions.

Estimation of the amount of dye attached to each nanocrystal is difficult. Results for elemental analyses of CNC and the nitrogen-containing dye adducts are presented in Table 1. The measured elemental analysis for the unmodified CNC shows a somewhat low carbon content relative to the theoretical values for cellulose, $C_6O_5H_{10}$. This is almost certainly due to the presence of hard-to-remove water on the high surface area cellulose nanocrystals. No nitrogen was detected, and the sulfur content results from the presence of sulfate half-ester groups on the nanocrystal surfaces.[15] Nitrogen was detected from the amide and azido linkages on the dye-modified CNC; the values of 0.44 wt % for the hydrazido HMC and 0.23 wt % N for the amido AMC are close to the detection limits of the analysis but do seem real. The reason for the low values is that the reactions are limited to the surfaces of the nanocrystals; the crystalline bulk of the nanocrystals is inaccessible to the dyes. To attempt to increase the sensitivity, surface-sensitive XPS measurements[16] were made on the nanocrystals (Table 1). Compared to the original CNC, HMC−CNC showed a marked increase in the carbon−oxygen ratio, as is expected for the relatively carbon-rich methylcoumarin substituent, and the nitrogen signal of 0.61 wt % is somewhat higher than for the bulk chemical analyses. However, it should be noted that the XPS escape depth for cellulose is many times the thickness of a single nanocrystal, so the analysis is not that of the true nanocrystal surface but is closer to a bulk analysis.

The nitrogen contents are at the limits of detection. However, if the order of magnitude is correct, it implies that the dye incorporation is greater than can be accommodated by reaction at the reducing hemiacetal end of the nanocrystals. The traditional measure of substitution for cellulosic polymers is the degree of substitution (DS) per anhydroglucose unit. The nitrogen content may be converted to DS by

$$N\% = \frac{\mathrm{DS} \times 14n}{162 + (\mathrm{DS} \times M)}$$

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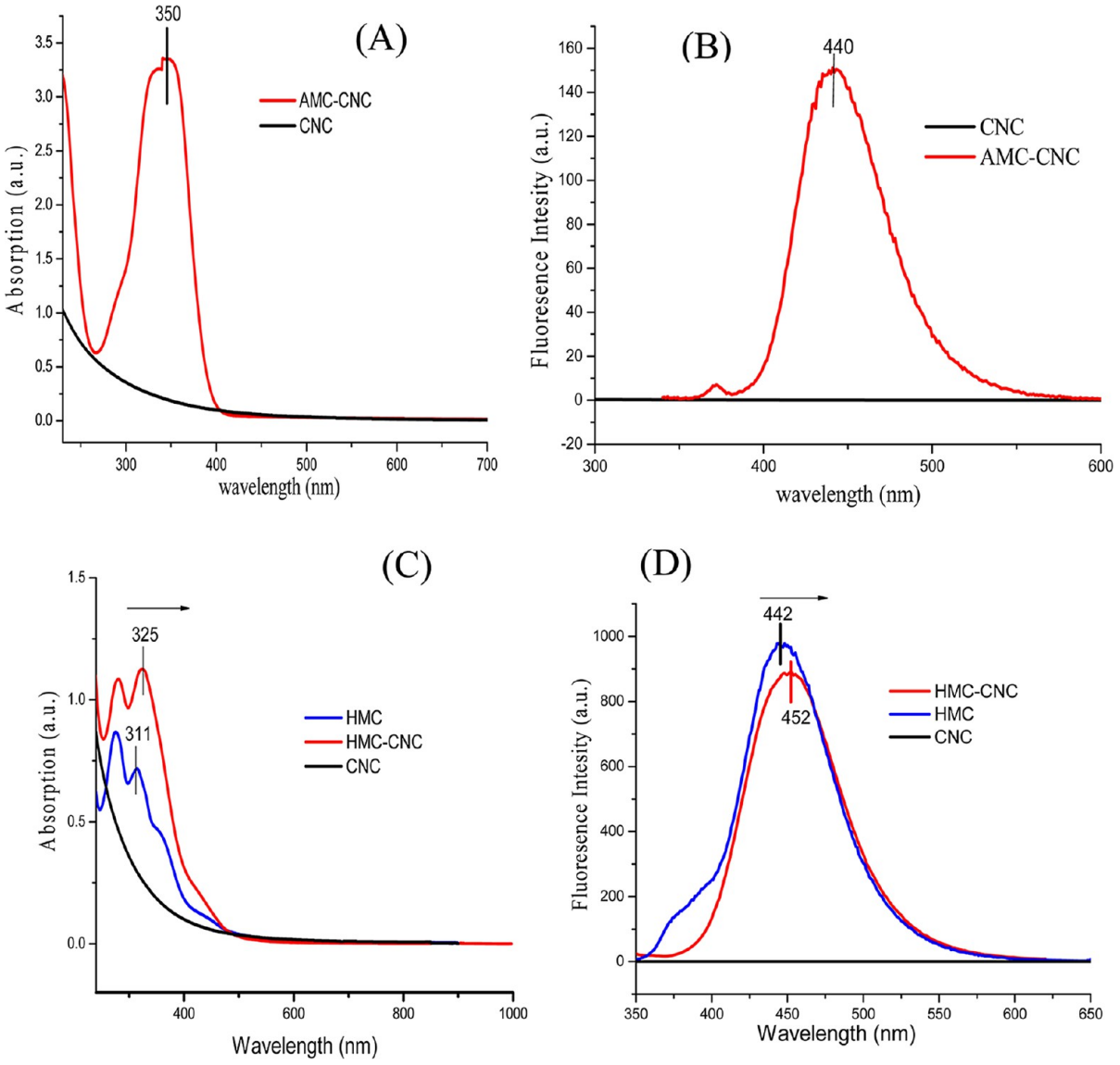

Figure 2. UV−vis (A, C) and fluorescence emission spectroscopy (B, D) of the cellulose nanocrystalline before and after modification. (Excitation wavelength = 350 nm).

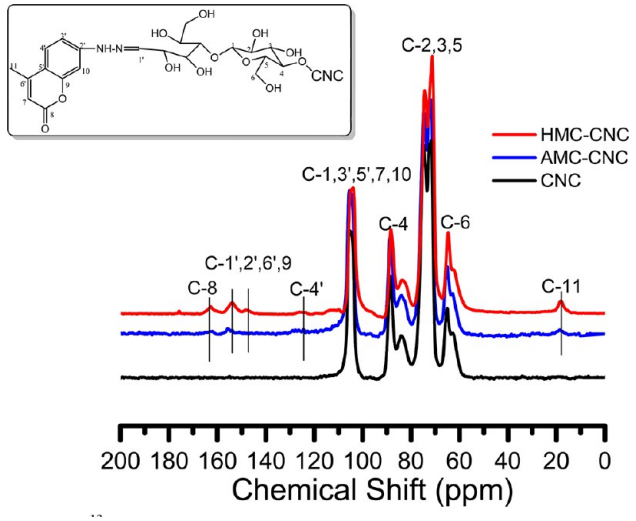

Figure 3. Solid ^{13}C NMR spectra of the cellulose nanocrystalline before and after modification.

where *N*% is the weight percentage of nitrogen in the nanocrystal sample, DS is degree of substitution per anhydroglucose group in the nanocrystal, *n* is number of nitrogen atoms in the substituent group, and *M* is the molecular weight of the substituent group. Normally, the maximum DS is 3 for reaction at each of the hydroxyl groups of the anhydroglucose units in the cellulose chain. Taking the bulk *N*% from Table 1 gives low values of DS = 0.026 for HMC−CNC and 0.027 for AMC−CNC. This assumes that *every* anhydroglucose unit (AGU) in the sample is available for reaction. As these values are based on the total number of anhydroglucose units in the nanocrystal, we write the degree of substitution thus defined as DS_{total}. However, only those on the surface of the nanocrystal are accessible, and of these, only those at the reducing end of the nanocrystal are in the hemiacetal (aldehyde) form that reacts readily with amine or hydrazo groups.

To estimate the number of available sites for reaction, consider a crude model of a nanocrystal, 20 nm wide (square cross-section) and 200 nm long. The glucan chain is approximately 0.57 nm wide, so there are 20/0.57 or ∼35 chains in one side or

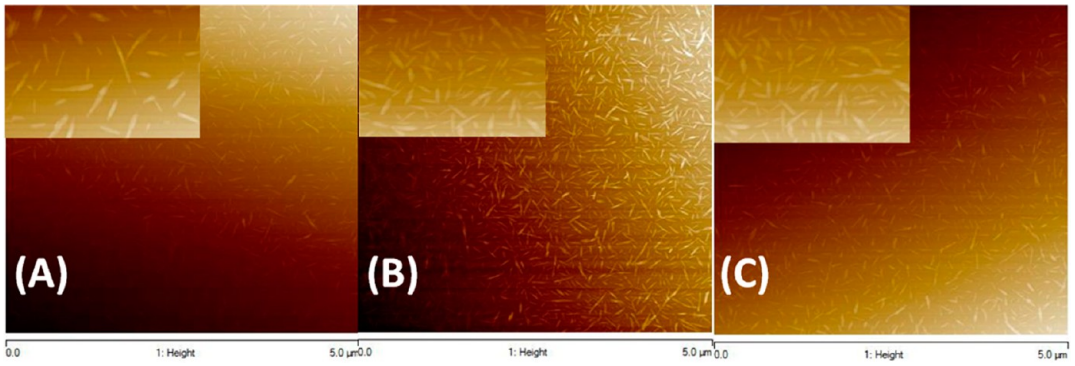

Figure 4. AFM images of the nanocrystalline cellulose (A), HMC−CNC (B), and AMC−CNC (C). (Inset: size width 2.5 μm).

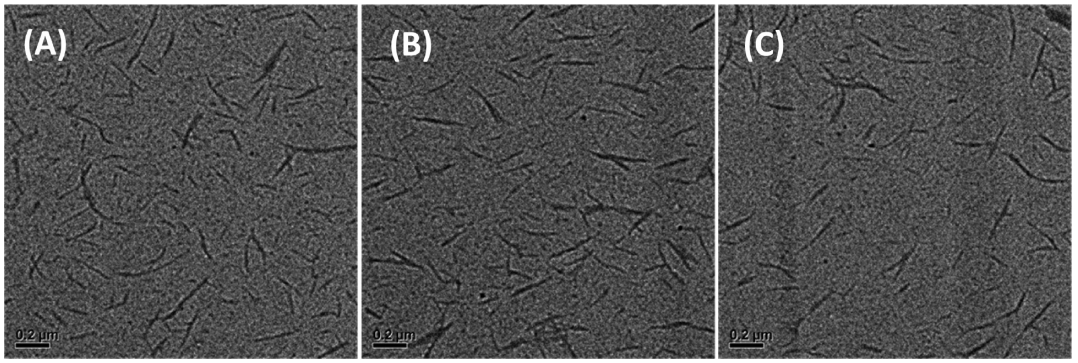

Figure 5. TEM pictures of the nanocrystalline cellulose (A), HMC−CNC (B), and AMC−CNC (C).

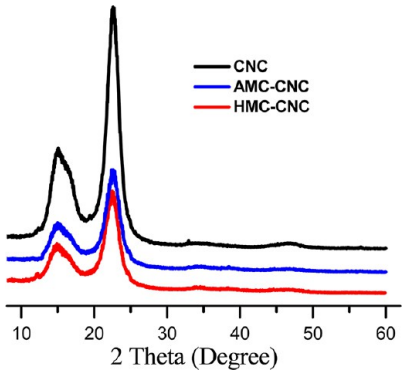

Figure 6. Wide-angle XRD diffraction patterns of the samples.

Table 1. Elemental Analysis and Surface XPS Results

sample	C (wt %)	O (wt %)	H (wt %)	N (wt %)	S (wt %)
pure cellulose (calculated)	44.44	49.38	6.18	0	0
CNC (bulk)	41.9	51.9[a]	6.5	0	0.78
AMC−CNC (bulk)	41.6	52.0[a]	6.2	0.23	0.83
HMC−CNC (bulk)	43.4	50.2[a]	5.9	0.44	0.64
CNC (surface, XPS)	40.0	53.3	–	0	–
HMC−CNC (surface, XPS)	56.2	43.2	–	0.61	–

[a]O not measured by elemental analysis; estimated by mass difference.

about 1200 chains in a nanocrystal cross-section. Lengthwise, a 200 nm long nanocrystal would have 200/0.57 = 350 AGU lengthwise and contain 1200 such chains, making a total of 420,000 AGU per nanocrystal. The number of AGU at the reducing end of a nanocrystal is 1200, and the number of surface AGU = 4 × 35 × 350 = 49,000. If we define the DS of just those AGU at the end of the nanocrystal as DS_{end} and the *DS* of those on the surface of the nanocrystal as $DS_{surface}$, then

$$DS_{end} = DS_{total} \times (420{,}000/1200) = DS_{total} \times 350 = 9.1$$

and

$$DS_{surface} = DS_{total} \times (420{,}000/49{,}000) = DS_{total} \times 8.6 = 0.22$$

Clearly, the amount of dye attached to the cellulose nanocrystal is at least an order of magnitude too large to be accommodated at the reducing end of the nanocrystal. (If despite the relatively unfavored open-ring aldehyde form of the terminal acetal groups and the effect of steric hindrance every chain end were able to react with one dye molecule, the DS_{end} would be 1.0. The actual value may be much smaller.) Even if all the surface AGU were available for reaction, the value of $DS_{surface}$ = 0.22 would mean that one in five of the surface AGU has a dye substituent. While it is possible that acid hydrolysis caused chain scission on the surface to generate sufficient reducing chain ends for the dye attachment to occur, it may also be that despite extensive extraction of the nanocrystals some dye remained physically adsorbed on the nanocrystal surface. If the original CNC sample contained aldehyde groups from glucosidic contaminants,[17] these would react with the fluorophores, but the adducts should also have been removed by the subsequent extraction process.

The covalent linkage of the dyes with D-glucose as a model for the AGU chain ends was shown by NMR for the hydrazino methylcoumarin dye. Yield of HMC-glucose was 64% (Supporting Information).

CONCLUSION

The preparation of fluorescent cellulose nanoparticles under mild aqueous conditions has been demonstrated. The general strategy of using the reducing groups at one end of the cellulose nanocrystals as linkage sites for fluorescent and other tags is attractive, but it is very difficult to confirm that this is the only mechanism involved. Detection and quantification of the small number of adduct groups relative to the mass of the nanoparticles requires sensitive analytical methods.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and physical and spectral data for the 7-hydrazino-4-methylcoumarin (HMC) and model glucose dye adducts. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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