A Facile Approach for Fabricating Fluorescent Cellulose

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ABSTRACT: Fluorescent cellulose nanocrystals (CNCs) were prepared through a two-step approach. Reactive amino groups were first introduced onto the CNCs through a silanization reaction with 3-aminopropyltrimethoxysilane. The fluorescent moieties were then attached onto the cellulose by covalent grafting between the amino groups and 1-pyrenebutyric acid *N*-hydroxy succinimide ester or fluorescein isothiocyanate. The synthesized fluorescent CNCs were investigated and characterized with attenuated total reflectance Fourier transform infrared spectroscopy, ultraviolet–visible absorbance and fluorescence spectroscopy, confocal microscopy, and dynamic light scattering. The same fluorescent functionalization strategy could also be applied to other cellulose materials, such as microcrystalline cellulose and bulky paper sheets. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 3639–3644, 2010

Key words: biomaterials; fluorescence; fluoropolymers

INTRODUCTION

Polysaccharide crystals originating from amylose, cellulose, and mannan have shown promising applications in biosystems and biodegradable composites because of their sustainability, renewability, biocompatibility, and biodegradability.[1] Of the polysaccharide crystals, cellulose crystals are the most important and extensively investigated ones. For example, microcrystalline cellulose (MCC), produced from purified cellulose after the removal of amorphous regions through selective acid hydrolysis, exhibits great potential applications in pharmaceuticals, foods, papers, and structural composites.[2] Recently, cellulose nanocrystals (CNCs), prepared by the controlled acid hydrolysis of cellulose fibers, have attracted tremendous attention and have been used in drug carriers, the targeted delivery of therapeutics, the measurement of residual dipolar couplings and bioimaging agents, and thermoplastics as reinforcing additive.[3–6]

Fluorescent dyes have been widely used in the construction of fluorescent sensors because of their spectral properties in response to environmental changes.[7] For example, fluorescein isothiocyanate (FITC) and its derivatives were used in pH sensors because their fluorescence intensities depend on the pH value.[8] Pyrene and its derivatives were used in oxygen sensors because their luminescences were readily quenched by oxygen molecules[9] and in dicarboxylic acids sensors because these acids were able to change the fluorescence spectra by conformational reorganizations.[10] In addition, fluorescent dyes (e.g., quantum dot) are generally used in biochemistry and life science research for bio-imaging and for tracking or analyzing biological molecules by means of the fluorescent emission at a specific frequency.[11,12]

Fluorescent CNCs are expected to have potential applications in the fabrication of sensors for biological systems because of their good biocompatibility and biodegradability. However, the direct fluorescent functionalization of cellulose is difficult because of the poor reactivity of the surface hydroxyl groups of the cellulose. Generally, the preactivation of the cellulose surface is required before its functionalization. It was reported that cellulose could be activated with oxidants, such as sodium hypochlorite[13] and sodium periodate,[14] functional epoxy derivatives,[4] xyloglucan and its derivatives,[15] and silane coupling agents.[16] To introduce fluorescent groups (moieties) into cellulose, varied fluorescent dyes and photoluminescent polymers have been investigated, including fluorescein-5′-isothiocyanate,[4] rhodamine B isothiocyanate (chloride) and rhodamine B (perchlorate),[17] 5-(4,6-dichlorotriazinyl) aminofluorescein,[18] poly(fluorene-*co*-fluorenone),[13] and hydrolyzed poly[2-(3-thienyl) ethanol butoxy carbonyl methyl urethane].[19]

This article reports a unique and simple method for fluorescently functionalizing CNCs. The method consisted of two steps: the introduction of reactive amino groups onto cellulose by a silanization

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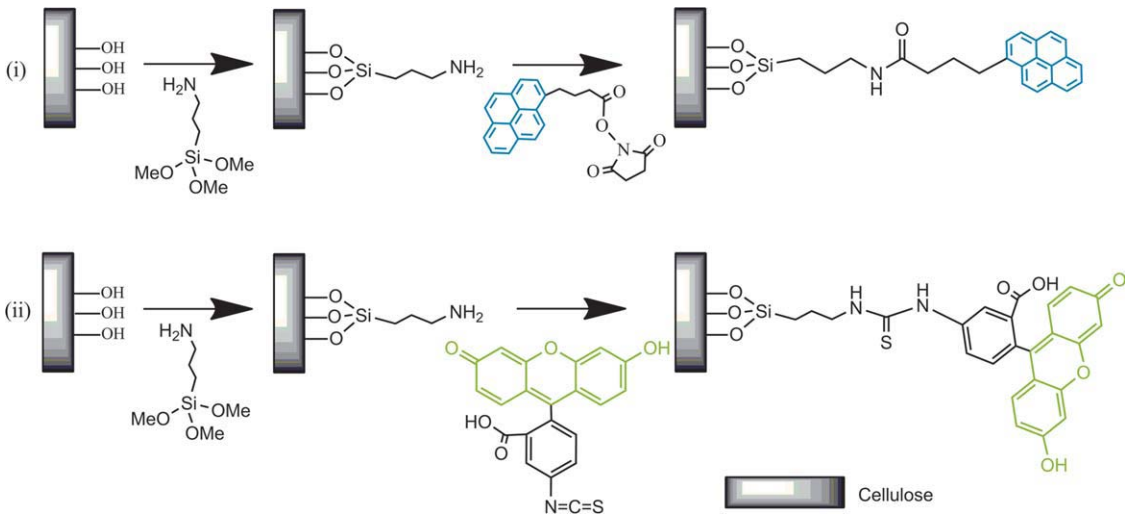

Scheme 1 Schematic illustration of the fluorescent functionalization of cellulose. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

reaction followed by fluorescent functionalization with 1-pyrenebutyric acid *N*-hydroxy succinimide ester (PSE) or FITC. The method was also successfully applied to other cellulose materials (MCC and bulky paper sheets).

EXPERIMENTAL

Materials

MCC (20 μm), 3-aminopropyltrimethoxysilane (APS), FITC, and PSE were obtained from Sigma-Aldrich (St. Louis, MO). Sulfuric acid, Whatman no. 1 filter paper (FP), anhydrous tetrahydrofuran (THF), acetone, and anhydrous *N*,*N*-dimethylformamide (DMF; dried with 4-Å molecular sieves before use) were purchased from Fisher Scientific (Pittsburgh, PA).

Preparation of the CNCs

The CNCs were prepared by the controlled acid hydrolysis of MCC. Typically, MCC (5 g) was mixed with 64 wt % sulfuric acid (45 mL) into a 100-mL beaker and was hydrolyzed at 45°C for 2 h. After the hydrolysis, the CNCs were collected by centrifugation, thoroughly rinsed with deionized water, washed with acetone, and dried *in vacuo.* The average particle diameter of the resuspended CNCs in water was 470 nm, as measured with dynamic light scattering (DLS).

Activation of cellulose

Reactive amino groups were introduced onto the surface of the cellulose materials by reaction with APS. In brief, dried CNCs (or cellulose microcrystals or Whatman no. 1 FP; 0.2 g) were mixed with APS (2.86 mmol, 0.5 mL) and anhydrous DMF (20 mL). The reaction lasted for 2 h at room temperature. The activated cellulose substrates were separated by centrifugation, rinsed with anhydrous DMF three times, and dried *in vacuo.*

Preparation of the fluorescent cellulose

The fluorescent cellulose was prepared by the functionalization of the activated cellulose materials described previously with PSE or FITC. Typically, the previously activated CNCs (or cellulose microcrystals or FP; 0.15 g), DMF (15 mL), and PSE (0.052 mmol, 20 mg) or FITC (0.051 mmol, 20 mg) were placed into a 50-mL, three-necked flask. The mixture reacted under stirring at room temperature for 19 h. The resulting functionalized cellulose was separated by centrifugation, washed with anhydrous DMF until there was no PSE or FITC detected in the supernatant by ultraviolet–visible (UV–vis) spectroscopy, washed with acetone, and dried *in vacuo.* In the control experiments, all of the conditions were the same, except that original (inactivated) cellulose materials were used.

Characterizations

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 viable-temperature UV–vis spectrophotometer (Cary 50 Bio, Varian, Palo Alto, CA). Fluorescence spectra were recorded on an MOS-250 fluorescence spectrometer (Biologic, Claix, France). The average particle diameters were determined by DLS with a Zetasizer Nano ZS instrument (Malvern Instruments,

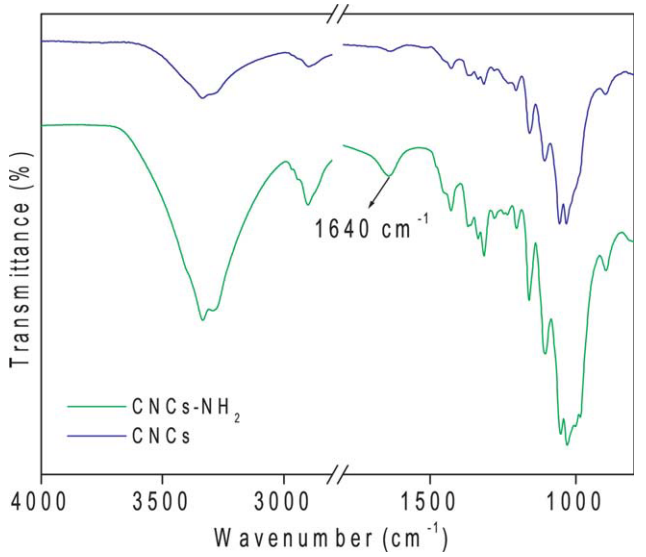

Figure 1 ATR FTIR spectra of the CNCs and CNC–NH_2. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

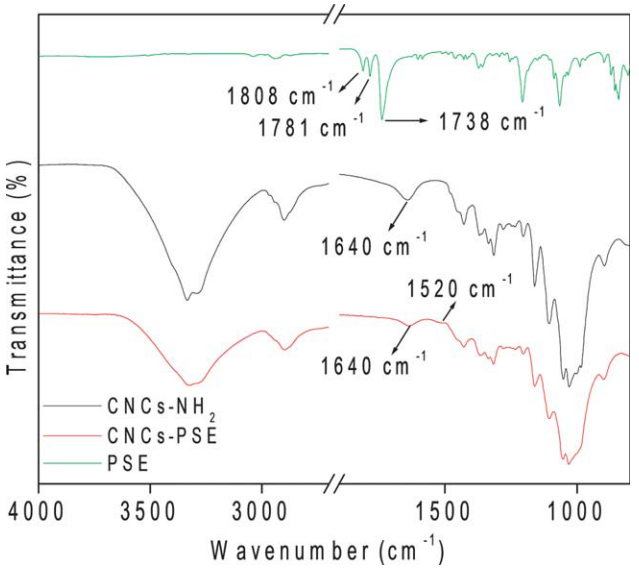

Figure 2 ATR FTIR spectra of the PSE, CNC–NH_2, and CNC–PSE. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Worcestershire, England) equipped with a 633-nm laser. Confocal images were taken on a Zeiss Axiovert 200M motorized inverted microscope (Thornwood, NY) equipped with 10× conventional and 20× Differential Interference Contrast (DIC) objectives and 40, 63, and 100× DIC oil-immersion objectives.

RESULTS AND DISCUSSION

Activation of the CNCs

To enhance the reactivity of the CNC surface, highly reactive amino groups (—NH_2) were introduced through a silanization reaction with APS, as shown in the first step of the reaction shown in Scheme 1 (i,ii). The introduction of amino groups was verified by ATR FTIR spectroscopy. As shown in Figure 1, absorption bands at 3638–2992 cm^{-1} (—OH stretching), 2897 cm^{-1} (C—H stretching), 1427 cm^{-1} (—OCH— in-plane bending), 1369 cm^{-1} (C—H deformation), 891 cm^{-1} (—COC— deformation), and 657 cm^{-1} (—OH out-of-plane bending) were observed in the FTIR spectrum of pristine CNCs,[20] whereas a new peak at 1640 cm^{-1} appeared in the spectrum of the activated CNCs, which was attributed to the stretching vibration of the amino group (—NH_2).[21] Other cellulose materials (MCC and FP) were surface-activated through the same reaction.

Preparation and characterization of the fluorescent cellulose

PSE has a highly reactive *N*-hydroxyl succinimide group, which could readily react with the primary amine on the silanized CNCs in DMF.[22] The reaction resulted in fluorescently functionalized CNCs (CNC–PSE), as shown in the second step of the

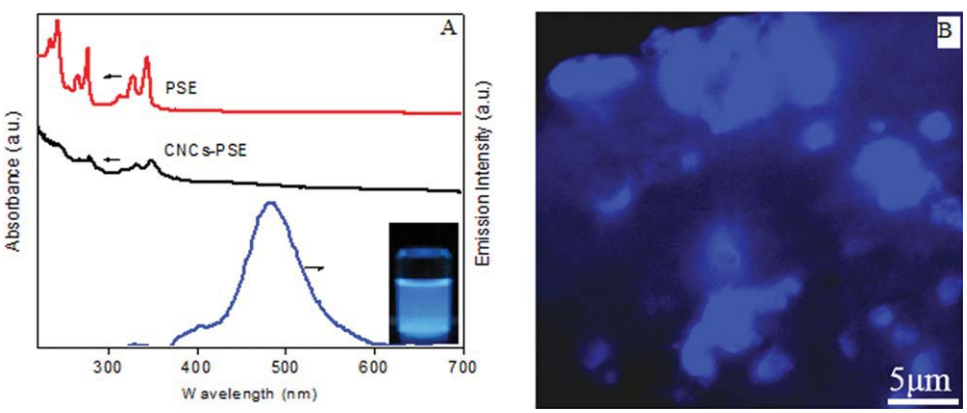

Figure 3 (A) UV–vis absorbance and emission spectra of the CNC–PSE in THF (Excitation wavelength, $\lambda_{excitation}$ = 325 nm; the inset is the CNC–PSE suspension illuminated by a UV lamp). (B) Confocal fluorescence image of the CNC–PSE ($\lambda_{excitation}$ = 338 nm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

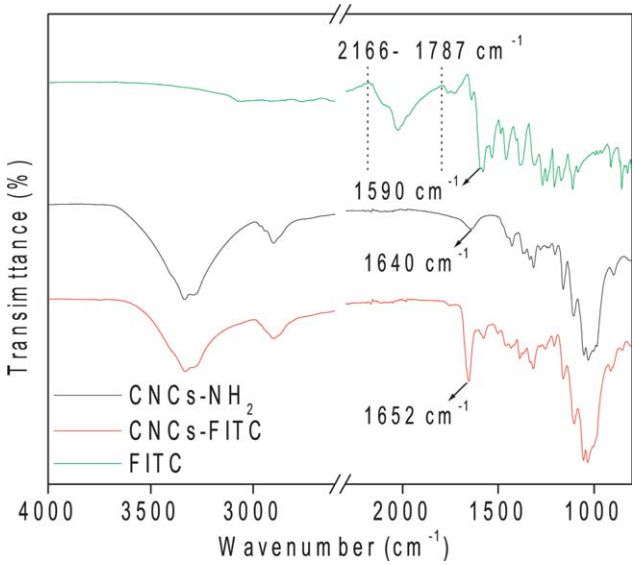

Figure 4 ATR FTIR spectra of the FITC, CNC–NH_2, and CNC–FITC. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

reactions in Scheme 1(i). The resulting CNC–PSE was characterized as follows.

Three peaks at 1808 cm^{-1} (C=O symmetric stretching of succinimidyl ester), 1781 cm^{-1} (C=O antisymmetric stretching of succinimidyl ester), and 1738 cm^{-1} (C=O stretching), were observed in the ATR FTIR spectrum of PSE (Fig. 2).[21] On the other hand, these peaks disappeared in the spectrum of the CNC–PSE (Fig. 2); this suggested that the PSE completely reacted with the activated cellulose. In addition, amide bands at 1640 (amide I) and 1520 cm^{-1} (amide II) in the FTIR spectrum of CNC–PSE further verified the successful preparation of the CNC–PSE.

The fluorescent properties of the CNC–PSE were studied with UV–vis absorbance and emission spectrometry; the spectra are shown in Figure 3(A). Pure PSE had seven characteristic absorption peaks of the pyrene moieties at 235, 244, 266, 277, 313, 328, and 344 nm,[22] whereas CNC–PSE only showed five characteristic absorbance peaks at 245, 268, 315, 330, and 349 nm. Redshifts were observed in the spectrum of CNC–PSE. When illuminated by a UV light, the CNC–PSE sample appeared blue, as shown in the inset of Figure 3(A). The emission spectrum of the CNC–PSE showed two weak monomer emissions at 383 and 401 nm and a strong excimer emission at 483 nm. The blue fluorescence characteristic of the CNC–PSE was further confirmed by a confocal fluorescence microscope, as shown in Figure 3(B). We also found that the CNCs formed a stable aqueous suspension, whereas the CNC–PSE was not well dispersible in aqueous medium because of the introduction of the hydrophobic PSE moieties.

Likewise, FITC with an isothiocyanate reactive group (—N=C=S) was successfully attached to the surface of the APS-activated CNCs by an addition reaction between the isothiocyanate and amino groups,[23,24] as shown in the second step of the reactions in Scheme 1(ii). The resulting CNC–FITC was green when illuminated by a UV lamp. In contrast, the inactivated cellulose crystals in the control experiment did not react with FITC under the same experimental conditions; thereby, there was no FITC detected by UV light on the surface of the inactivated CNCs after the reaction.

In the ATR FTIR spectrum, as shown in Figure 4, two characteristic absorbance bands at 1787–2166 cm^{-1} (N=C=S stretching) and at 1590 cm^{-1} (C=O stretching) were observed in the FITC. After FITC was reacted with the activated CNCs, the characteristic absorbance bands of N=C=S disappeared. In addition, a significant carboxyl stretching vibration peak appeared at 1652 cm^{-1} (C=O stretching) in CNC–FITC; this indicated that the reaction between the activated CNCs and FITC occurred. The UV–vis

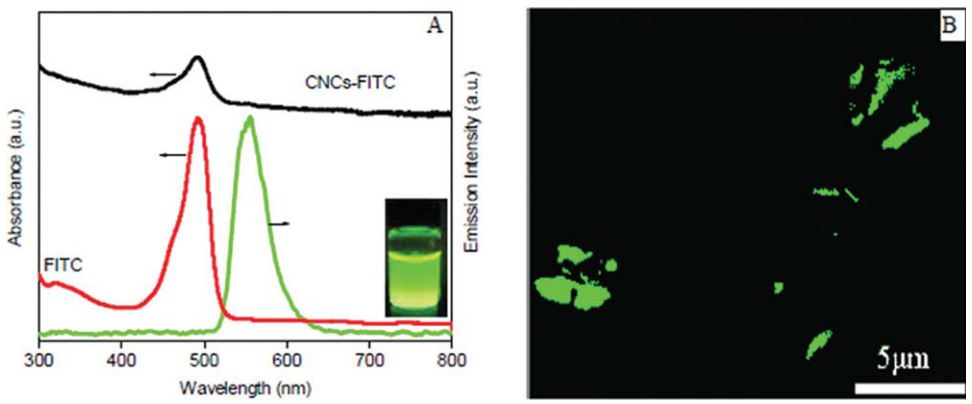

Figure 5 (A) UV–vis absorbance and emission ($\lambda_{excitation}$ = 490 nm) spectra of the CNC–FITC in aqueous sodium carbonate (pH = 11; the inset is the CNC–FITC suspension illuminated by a UV lamp). (B) Confocal fluorescence image of CNC–FITC ($\lambda_{excitation}$ = 492 nm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

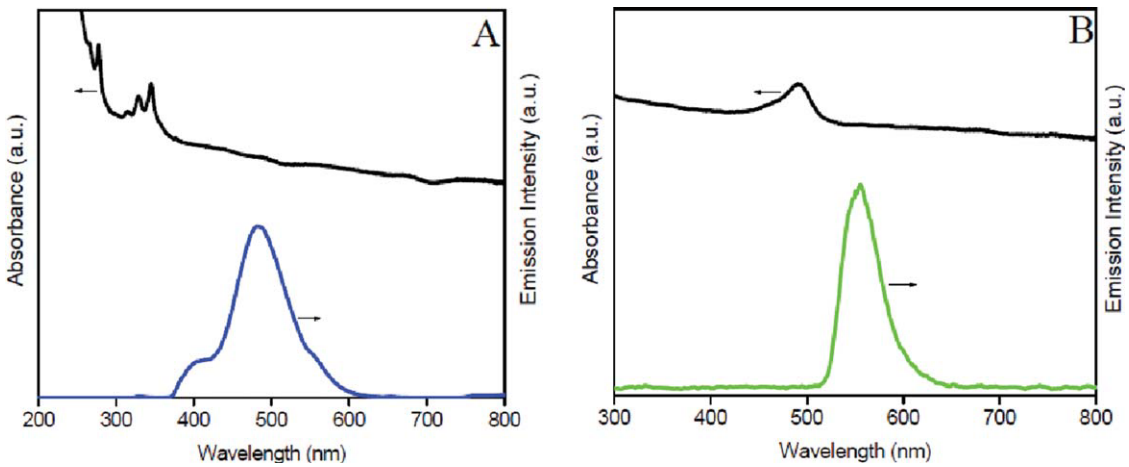

Figure 6 (A) UV–vis absorbance and emission spectra of the (A) MCC–PSE in THF ($\lambda_{excitation}$ = 325 nm) and (B) MCC–FITC in aqueous sodium carbonate (pH = 11, $\lambda_{excitation}$ = 490 nm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

absorbance and emission spectra of the CNC–FITC suspension in aqueous sodium carbonate (pH = 11) are shown in Figure 5(A). CNC–FITC had a characteristic absorption peak at 493 nm and appeared green when illuminated by a UV lamp (see the inset).[9] The green fluorescence feature of CNC–FITC was further verified by a confocal fluorescence microscope, as shown in Figure 5(B). There was no significant redshift observed in the spectra of CNC–FITC, compared to that of the pure FITC. CNC–FITC showed an emission peak at 555 nm. The UV–vis and emission spectra verified the fluorescent properties of the CNC–FITC. The CNC–FITC had an average particle size of 363 nm, as measured by DLS. The CNC–FITC was dispersible and stable in aqueous media because of electrostatic and hydration forces, hydrophobic interactions, and hydrogen bonding.

With the same strategy, PSE and FITC were covalently grafted onto the surface of MCC as well. The fluorescent properties of MCC–PSE and MCC–FITC were verified by UV–vis absorbance and emission spectra, as shown in Figure 6. The MCC–PSE exhibited absorbance peaks at 269, 279, 314, 329, and 346 nm. A slight redshift was observed in the MCC–PSE as well. The emission of MCC–PSE was similar to that of CNC–PSE. MCC–FITC had also an absorbance peak at 493 nm and an emission peak at 555 nm in sodium carbonate solution (pH = 11), as did CNC–FITC.

The fluorescent functionalization discussed previously could be also applied to bulky cellulose material. For example, FP activated by APS was successfully labeled with PSE and FITC. As shown in Figure 7, the PSE-labeled FP was blue under a UV lamp, whereas the FITC-labeled FP was green under a UV lamp. In contrast, PSE or FITC could not be immobilized onto the inactivated FP under the same experimental conditions. As a result, the inactivated FP did not undergo a color change when illuminated by a UV lamp (picture not shown here). This verified the necessity of the surface activation for introducing the fluorescent moieties on cellulose materials.

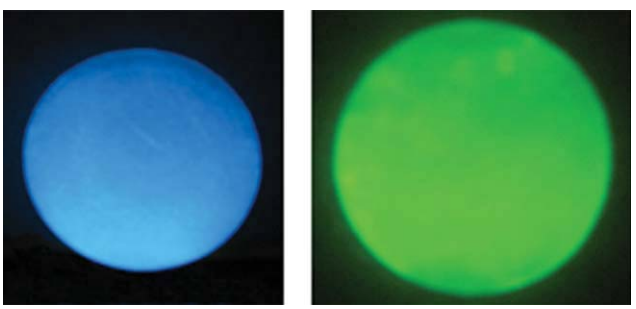

Figure 7 Photos of the FP–PSE (left) and FP–FITC (right) in aqueous sodium carbonate (pH = 11) illuminated by a UV lamp. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CONCLUSIONS

A new approach for the preparation of fluorescent cellulose was developed, which consisted of two simple reactions, the activation of the cellulose surface by the introduction of reactive amino groups through a silanization reaction followed by the fluorescent functionalization through the reaction with PSE or FITC. The method could be applied to different cellulose substrates, including CNCs, MCC, and bulky paper sheets. The fluorescent cellulose may have potential applications in functional papers, sensors, and bioimaging.

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