Acid-free extraction of cellulose type I nanocrystals using Brønsted acid-type ionic liquids

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Abstract Extraction of cellulose type I nanocrystals from cotton fibers was straightforwardly carried out using exclusively Brønsted acid-type ionic liquids (ILs) via a two-step swelling/hydrolysis route, the switch between these two stages being induced by water addition. Since the whole process was achieved in a single reaction medium predominantly based on 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) and 1-(4-sulfobutyl)-3-methylimidazolium hydrogen sulfate ([SBMIM]HSO4), the process parameters were investigated in order to ensure a perfect compatibility and sequencing towards the two-step route proposed here. The impacts on nanoparticle morphology, crystallinity evolution, and cellulose type I to type II denaturation were observed under field-emission gun scanning electron microscopy and corroborated by X-ray diffraction characterizations. ILs recovery and reuse were also demonstrated, opening up new prospects of conception of multi-cycle, environmentally and economically sustainable processes.

Keywords Cellulose nanocrystals, Ionic liquids, Hydrolysis, Swelling, Recycling

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Introduction

The great potential of cellulose nanocrystals (CNCs) as nanofillers in polymeric matrices has been already underlined in numerous studies.1–7 However, there is still no example of a successful CNC-based application at a large industrial scale, the poor CNC dispersability within the polymer matrix being among the main obstacles. Development of cost-efficient and environmentally friendly CNC extraction processes also stands out as one of the major challenges to be met. To date, only two commercial entities producing CNCs for market development with capacities superior to 100 kg/day were identified: American Process Inc., Atlanta, USA (www.americanprocess.com) and CelluForce, Montréal, Canada (www.celluforce.com).

Traditional CNC extraction route, based on sulfuric acid, is applied to a large variety of cellulosic raw materials, e.g. pulps, fibers, microcrystalline cellulose (MCC). However, the use of highly concentrated sulfuric acid (64 wt%) requires further water, energy, and time-consuming purification steps, such as neutralization and dialysis. Another major consequence is the presence of residual sulfates on CNC surface, negatively impacting CNC thermal stability.8–10

In this context, alternative CNC extraction routes using, for example, subcritical water hydrolysis11 or those based on ionic liquids (ILs), have gained an increasing interest. Their chemical stability, low vapor pressure, and relatively low

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Nanocomposites 2016 VOL. 2 NO. 2
melting temperatures represent the great advantages of ILs, which allow envisaging safe- and low-energy-consuming processes. Nevertheless, considering their embodied energy and cost, the recyclability and the reuse of ILs undoubtedly seem to be essential for the conception of any environmentally and economically viable CNC extraction process.

The exceptional abilities of ILs, and particularly those of imidazolium-based ILs, to swell, to dissolve, or to hydrolyze cellulose, can be successfully used for the conversion of various lignocellulosic substrates to valuable chemicals and products. Major structural modifications of cellulose such as complete solubilization were obtained at 90–120 °C after several hours of treatments in 1-butyl-3-methylimidazolium chloride [BMIM]Cl, within a few minutes in 1-ethyl-3-methylimidazolium diethylphosphonate [EMIM]DEP, and even at room temperature after 1–2 h using 1-butyl-3-methylimidazolium acetate [BMIM]OAc. On the other hand, advanced hydrolysis of cellulose to reducing sugars was achieved in different acidic imidazolium-based ILs media, among which SO₃H-functionalized ILs greatly improved reaction kinetics and yields.

As far as CNC extraction is concerned, IL-based systems can be used for more selective and managed destructuring of native cellulose intrinsic morphology, and can eventually lead to nanoscale particles and well-defined cellulose type I nanocrystals. Cellulose nanocrystals were obtained from cotton fibers using 1-butyl-3-methylimidazolium chloride [BMIM]Cl, 1-(4-sulfobutyl)-3-methylimidazolium hydrogen sulfate ([SBMIM]HSO₄), and 1-(4-sulfobutyl)-3-methylimidazolium hydrogen dihydrogen phosphate ([SBMIM]H₂PO₄) as the reaction medium. The potential of these novel ILs for the extraction of CNCs from various lignocellulosic biomass was consolidating by the work of the group of Lemoine (Boulogne-Billancourt, France). ILs (Figure 1), cellulose fibers (pure cotton) were obtained from VWR (Louvain, Belgium) and Merck (Darmstadt, Germany), respectively.

In this regard and to propose a fully recyclable approach, we must highlight our recent work about a two-stage CNC extraction method involving IL swelling [BMIM]Cl-based treatment followed by hydrolysis under mild acid conditions. Partial unfolding of cellulose fibers could be selectively achieved in [BMIM]Cl, considerably enhancing the accessibility of the substrate towards its hydrolysis after adding only 1 wt% sulfuric acid. Interestingly, the swelling properties of [BMIM]Cl in the presence of protic derivatives such as water and H₂SO₄ could be readily tuned upon the water content. These features prompt us to conceive a recyclable acid-free extraction route where we combine [BMIM] Cl for its capability of dissolving cellulose in function of water content and 1-(4-sulfobutyl)-3-methylimidazolium hydrogen sulfate ([SBMIM]HSO₄) for its high capability of promoting hydrolytic processes. In contrast to [BMIM]HSO₄, [SBMIM]HSO₄ has shown to be an efficient Brønsted acid catalyst toward the condensation synthesis of polyesters. Combining both ILs as a single reaction medium here ensures the possibility to reuse the reaction medium for any subsequent extraction process of type I CNCs.

In the present study, cellulose nanocrystal extraction from cotton fibers was carried out with [BMIM]Cl and [SBMIM] HSO₄, without the use of sulfuric acid or any other mineral acid. Both swelling and hydrolysis of the cellulotic substrate were successively achieved in a single [BMIM]Cl/[SBMIM]HSO₄ reaction medium; the switch between the two swelling and hydrolysis steps being simply induced by water content variation. The influence of key process parameters on extraction kinetics and particles morphology was investigated, with CNCs being characterized by FEG-SEM, X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS). The focus was also made on the potential for ILs recovery and reuse involving simple liquid–liquid extractions as confirmed after Fourier transform infrared (FTIR) and NMR analyses.

Materials & methods

Materials

Cellulose fibers (pure cotton) were obtained from Lemoine (Boulogne-Billancourt, France). ILs (Figure 1), 1-butyl-3-methylimidazolium chloride [BMIM]Cl with a purity >98%, 1-butyl-3-methylimidazolium hydrogen sulfate [BMIM]HSO₄, and 1-(4-sulfobutyl)-3-methylimidazolium hydrogen sulfate [SBMIM]H₂PO₄ were purchased from Solvionic (Toulouse, France). Ethyl acetate and sulfuric acid were obtained from VWR (Louvain, Belgium) and Merck (Darmstadt, Germany), respectively.

Nanocrystal extraction

Cellulose fibers and ILs were initially dried at 105 °C. Typical CNC extraction was achieved via two-step swelling/hydrolysis route. Swelling was carried out in [BMIM]Cl (cellulose/[BMIM]Cl 2 wt%) for different quantities from 1 to 8 wt% of [BMIM]Cl.
Lazko et al. Acid-free extraction of cellulose type I nanocrystals using Brønsted acid-type ionic liquids

Nanocomposites 2016 VOL. 2 NO. 2 67

[SBMIM]HSO4 at 80 °C under constant stirring for 80 min. The hydrolysis step was initiated by water addition from 0.5 to 4 wt%. The reaction medium was kept under constant stirring at 80 °C for 8 to 24 h, depending on [SBMIM]HSO4 concentration. Additional tests were also performed at 90 °C. Finally, the reaction was quenched by adding cold water and after ice cooling for 10 min. CNCs were then purified by five consecutive washing/centrifugation cycles at 13000 rpm at 4 °C for 20 min with 10 equivalent volumes of deionized water, in order to remove ILs and hydrosoluble hydrolytic residues. Final pellet was filtered at 20 μm and cleaned CNCs were resuspended in water or freeze-dried.

Microscopy

The evolution of cellulose fiber morphology, during the swelling and hydrolysis steps, was directly observed using a Leica DM LM/P light microscope (Wetzlar, Germany) connected to a digital camera. The nanoparticle morphology was evaluated by XL 20 Phillips field emission gun scanning electron microscope (FEG-SEM) (Eindhoven, The Netherlands), in the transmission mode. Image analyses were performed manually, with particle size repartitions based on at least 100 measurements for each sample.

X-ray diffraction

The crystallinity evolution was measured by XRD using a PANalytical (Almelo, the Netherlands) diffractometer equipped with Cu anode. For each sample, diffractograms were obtained at room temperature within a 2θ range from 5° to 40°, with a step size of 0.0530°, at 40 mA and 45 kV. The crystallinity index (CI) was calculated by the peak height method using the ratio between \( I_{002} \) and \( I_{200} \) intensities, and by the peak deconvolution method using integrated crystalline peak intensities of the (1 0 1), (1 0 0), (0 0 2), and (0 4 0) diffraction planes.40–42

UV spectroscopy

Coloration changes in the reaction medium, indicating the advancement of the hydrolysis reaction, were observed by UV spectroscopy. Samples were taken from the aqueous phase recovered just after the first centrifugation. Absorbance was measured between 350 and 700 nm using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan).

X-ray photoelectron spectroscopy

The surface of CNCs was analyzed by XPS using PHI 5000 Versa Probe (Chanhasen, USA). Spectra were obtained with aluminum K-alpha radiation with a spot of 200 μm, at 47.8 W and 46° inclination. A survey scan pass energy of 187 eV was used for the general spectrum (1400–0 eV) while 23.5 eV was used for the quantification of specific elements.

FTIR spectroscopy

FTIR spectroscopy was performed using Bruker TENSOR 27 spectrometer (Ettlingen, Germany). Spectra were recorded in absorbance mode within the range of 3800–600 cm\(^{-1}\) at a nominal resolution of 1 cm\(^{-1}\).

Nuclear magnetic resonance

Composition of the recovered ILs was confirmed by proton and carbon nuclear magnetic resonance (\(^{1}\)H and \(^{13}\)C NMR). Samples were dissolved in CDCl3 (20 mg/600 μl) and analyzed with a Bruker Avance 500 Spectrometer (Billerica, USA) at a frequency of 500 and 126 MHz, respectively.

Results and discussion

Before discussing the recovery of CNCs using our approach, initial trials with [SBMIM]HSO4 were carried out in order to investigate whether this IL could effectively act as Brønsted acid catalyst instead of the currently used sulfuric acid for the production of type I CNCs, following already established two-step swelling/hydrolysis route recently described by some of us.38 In these experiments, cellulose fibers were first swollen in anhydrous [BMIM]Cl (Figure 2) and then hydrolyzed by subsequent addition of [SBMIM]HSO4 (2 to 6 wt%) and water (1 to 3 wt%). Important viscosity decrease was noticed immediately after the addition of [SBMIM]HSO4. Progressive hydrolysis and fiber size reduction were observed by optical microscopy, as well as by the yellowish color of the reaction medium. After 8 to 24 h, depending on [SBMIM]HSO4 concentration, the reaction medium was finally washed and centrifuged, and STEM pictures of the resuspended pellet clearly confirmed the efficiency of the hydrolysis reaction leading to the formation of nanoscale particles. These observations confirmed that [SBMIM]HSO4 could be successfully used as Brønsted acid catalyst for CNC extraction, with kinetics comparable to those of \( H_2SO_4 \).

Exploration tests were also carried out with non-functionalized 1-butyl-3-methylimidazolium hydrogen sulfate [BMIM]HSO4 instead of [SBMIM]HSO4 in order to investigate its applicability for cellulose hydrolysis still via two-step swelling–hydrolysis route.38 In fact, several studies already demonstrated...
In this work, swelling tests were therefore carried out at 80 °C in anhydrous mixtures of [BMIM]Cl/[SBMIM]HSO₄ with different [SBMIM]HSO₄ quantities from 1 to 8 wt% (Table 1). Swelling of cellulose fibers was successfully and quickly achieved at 1 and 2 wt% [SBMIM]HSO₄ (Figure 2). Swelling was still possible at 4 and 6 wt% but less rapid and homogeneous than previous values. For the mixture containing 8 wt% [SBMIM]HSO₄, the swelling was not achieved at all, even after several hours. The quantity of protic solvent seemed to be too high, completely inhibiting [BMIM]Cl solvating properties in agreement with the literature.18

After 80-min swelling in anhydrous [BMIM]Cl/[SBMIM]HSO₄ 4 wt% mixture, the hydrolysis step was initiated by addition of a limited quantity of water, i.e. 2 wt%. Corresponding CNCs extracted by this two-step process after 8 h of hydrolytic treatment at 80 °C are presented in Figure 4. They clearly exhibited the potential of this IL which could be efficiently used for CNC extraction, both in one and two-stage processes.25–26 However, in these studies, [BMIM]HSO₄ was systematically used as the major component, if not the only component of the reaction medium. Therefore, it was essential to explore its hydrolytic activity at lower concentrations. Indeed, the hydrolysis step induced by water addition resulted in only limited morphological modifications. These modifications would be preferentially due to the mechanical destructuration of the fibers into fibrils, and not due to any hydrolytic cleavage. Thus, even after an extended treatment over 150 h, cellulose particles longer than 500 μm and fibrils could be seen in the reaction medium (Figure 2(d)). Low concentration of [BMIM]HSO₄ was obviously not suitable for an efficient hydrolytic step leading to nanoscale cellulose particles. The impact of –SO₃H group on ILs hydrolytic activity proved significant. Functionalized IL [SBMIM]HSO₄ containing sulfonate group showed much better reactivity than [BMIM]HSO₄, and could be successfully used even at low concentrations below 4%.

Since the recyclability of ILs represents one of the major bottlenecks for the development of economically and environmentally sustainable processes, it is obviously a substantial asset to use a single mixture of two types of ILs at all times during and after CNC extraction, without any addition or separation step. Such process, in which water plays a key role, is schematically represented in Figure 3. During the first step, the swelling is achieved thanks to the presence of [BMIM]Cl in anhydrous [BMIM]Cl/[SBMIM]HSO₄ mixture. The addition of water immediately stops cellulose swelling and promotes, on the other hand, [SBMIM]HSO₄ activity enabling the hydrolysis reaction from taking place. Resulting CNCs can be recovered via washing/centrifugation cycles. Water and hydrosoluble impurities are finally removed during the last step, and both ILs are dried and recycled together.

In this work, swelling tests were therefore carried out at 80 °C in anhydrous mixtures of [BMIM]Cl/[SBMIM]HSO₄ with different [SBMIM]HSO₄ quantities from 1 to 8 wt% (Table 1). Swelling of cellulose fibers was successfully and quickly achieved at 1 and 2 wt% [SBMIM]HSO₄ (Figure 2). Swelling was still possible at 4 and 6 wt% but less rapid and homogeneous than previous values. For the mixture containing 8 wt% [SBMIM]HSO₄, the swelling was not achieved at all, even after several hours. The quantity of protic solvent seemed to be too high, completely inhibiting [BMIM]Cl solvating properties in agreement with the literature.18

After 80-min swelling in anhydrous [BMIM]Cl/[SBMIM]HSO₄ 4 wt% mixture, the hydrolysis step was initiated by addition of a limited quantity of water, i.e. 2 wt%. Corresponding CNCs extracted by this two-step process after 8 h of hydrolytic treatment at 80 °C are presented in Figure 4. They clearly exhibited
Table 1  Main experimental conditions and dimension of CNCs extracted via two-step swelling–hydrolysis route in [BMIM]Cl/[SBMIM]HSO₄ media

| [SBMIM]HSO₄/ | Swelling | Hydrolysis | CNCs length |
| [BMIM]Cl (wt%) | (h)      | (h)       | (nm)        |
| 1            | 1h:20    | 24        | 310 ± 100   |
| 2            | 1h:20    | 8         | 290 ± 95    |
| 4            | 1h:20    | 8         | 305 ± 95    |
| 6            | 1h:20    | 8         | 360 ± 140   |
| 8            | /a       | 24        | 285 ± 90    |
| 4            | /b       | 51        | 265 ± 100   |
| 6            | 1h:20    | 24        | 275 ± 90    |

*a* Poor swelling even after several hours, involving a prolonged hydrolysis step.

*b* Hydrolysis without initial swelling, involving a prolonged hydrolysis step.

*c* Dark brown CNCs suspensions and presence of contaminants on CNCs surface.

well-defined rod-like morphology and quite homogeneous size repartition of average lengths around 300 ± 100 nm and mean diameter around 20 ± 10 nm (Figure 5). In comparison, these dimensions are within the high range of sizes obtained for CNCs extracted from various cotton sources by traditional concentrated sulfuric acid hydrolysis, length and width usually between 70–300 nm and 5–20 nm, respectively.3,7,43 Finally, milky white aqueous suspensions exhibited typical flow birefringence phenomena when observed under polarized light and showed to be homogeneous and stable over 1 year at 4 °C. The overall extraction yield was 30 ± 10%. CNC purity was also confirmed by XPS, Cl and S atomic fractions related to possible traces of [BMIM]Cl and [SBMIM]HSO₄, respectively, being below theoretical detection limits (0.1% At). This represents a major advantage over CNCs extracted by traditional acid

Figure 4  Aspect of the final aqueous suspension (a) and corresponding STEM picture of CNCs (b) extracted via two-step swelling/hydrolysis route in [BMIM]Cl/[SBMIM]HSO₄ 4% reaction medium

Figure 5  Particle size repartition in number of CNCs extracted in [BMIM]Cl/[SBMIM]HSO₄ 4% reaction media via swelling/hydrolysis route
Lazko et al. Acid-free extraction of cellulose type I nanocrystals using Brønsted acid-type ionic liquids

Nanocomposites 2016  VOL. 2  NO. 2

hydrolysis which may typically contain 0.3–0.6% sulfur, taking into account the negative impact of residual sulfates on CNCs thermal stability.8,10,36,44

It is worth noting that the same [BMIM]Cl reaction medium, containing 4 wt% [SBMIM]HSO4 and 2 wt% H2O, was applied but in the presence of water added at the very beginning of the process. Under these experimental conditions, the swelling of cellulose fibers was not achieved even after a 3-h treatment. This is explained by the fact that a protic solvent such as water inhibits [BMIM]Cl solvating properties.16,38 Although it was still possible to successfully extract CNCs with quite similar morphology, the hydrolysis step was considerably extended more than 6 times (from 8 to 51 h). The same behavior was observed for the aforementioned 8% [SBMIM]HSO4 trial where, in the absence of suitable swelling, the hydrolysis step had to be extended up to 24 h in order to extract nanoscale particles. This again demonstrates the key role of the controlled swelling step, which definitely has a positive impact on hydrolysis kinetics by improving substrate accessibility.

On the other hand, if the destructuring step of cellulose is extended beyond the swelling time in [BMIM]Cl, substantial morphological defects can be observed on the final extraction product. Experiments were performed varying the [SBMIM]HSO4 content from 1 to 4%, either with a fixed H2O/[SBMIM]HSO4/1/2 g/g ratio, or with a fixed H2O/[BMIM]Cl 1/50 g/g ratio (corresponding to H2O-2% content). A homogeneous dispersion of clean and well-defined rod-like CNCs was only obtained for [SBMIM]HSO4 4% (Figure 4). On the other hand, at low [SBMIM]HSO4 contents equal to 1 and 2%, rod-like nanoparticles were systematically surrounded by residual amorphous phase which could not be clearly defined by STEM (Figure 6). Such morphological heterogeneities undoubtedly have a negative impact on overall yield and process conception with further efforts and additional purification steps being needed to obtain clear final suspensions of individual rod-like CNCs with homogeneous size repartition.

Since the quantity of acidic IL is low, a first assumption could be made that the surrounding phase results from the amorphous, not yet hydrolyzed native cellulose intrinsically present in the fiber structure. This phase could also consist of denaturized cellulose, just in the case if [SBMIM]HSO4 and water do not completely inhibit [BMIM]Cl solubilization ability. Thus, the hydrolysis step would be affected by a side reaction, characterized by an extensive cellulose solubilization and, eventually, regeneration in water medium during the final washing/purification step. The closer to the aprotic conditions, the more intense cellulose solubilization phenomena may be, so the impact of modifications induced by [BMIM]Cl could even prevail those induced by the hydrolytic action of [SBMIM]HSO4.

XRD analyses have been studied to confirm the last assumptions (Figure 7). Cellulose modifications induced by [BMIM]Cl treatment (without [SBMIM]HSO4) led to exclusively regenerated type II structure, characterized by three intense peaks at 2θ = 12.3 (1 0 1), 20.1 (1 0 ī), and 21.9 (0 0 2).32 Moreover, this cellulose I to cellulose II conversion resulted in a significant increase in the amorphous fraction, and crystallinity index was determined to be at an average of 13 % lower than the reference cellulose fiber sample.42 For extractions with 1 and 2% [SBMIM]HSO4, cellulose particles are still mainly characterized by type II conformation. However, the intensity of 12.3° peak progressively decreases with the concentration, becoming for 2% [SBMIM]HSO4 sample even less intense than some intrinsic peaks of the native cellulose. Finally, only cellulose type I peaks at 2θ = 15.0° (1 0 1), 16.7° (1 0 ī), and 22.9° (0 0 2) clearly characterize all CNCs extracted in the protic medium at higher 4% [SBMIM]HSO4 content. In comparison with the untreated reference cellulose fibers, crystallinity indices of the extracted

Figure 6 STEM pictures of heterogeneous nanoparticles resulting from combined unsequenced solubilization/hydrolysis treatments. [SBMIM]HSO4 varying from 1 to 2% and H2O varying from 0.5 to 2%
to 6% (Table 1) were carried out under the same operational conditions, 1-h 20-min swelling and 8-h hydrolysis time (except extended hydrolysis from 8 to 24 h in the specific case of 1% [SBMIM]HSO4). A very slight yellowish coloration could be noticed for 1% and 2% [SBMIM]HSO4 concentrations. At 4%, the coloration is much more pronounced, already indicating an advanced stage of hydrolysis, which likely corresponds to the advanced hydrolysis of a non-negligible part of the cellulosic substrate down to hydrosoluble residues beyond nanoscale particles. Finally, for 6% [SBMIM]HSO4 trial, an intense browning of the reaction medium and particularly high absorption values were recorded after 8 h. Such upstream information may already indicate a possible...
over-hydrolysis of the cellulosic substrate, in complementarity with yield calculation and other further analytical methods and characterizations.

An over-extended hydrolysis, observed for example after 24 h in 4 % [SBMIM]HSO₄ or even after 8 h in 6 % [SBMIM]HSO₄ media, could also affect the aspect of the final suspensions and the purity of the extracted CNCs. Usually, the coloration caused by the presence of hydrosoluble hydrolytic residues is efficiently removed after five consecutive washing/centrifugation cycles. As a result, stable milky white suspensions are obtained, containing only clean and well-defined CNCs (Figure 4). However, for the extensive hydrolysis, the dark brown coloration persists in the final suspension despite usual separation techniques and five washing/centrifugation cycles (Figure 10). Obviously, this is not caused by hydrosoluble fraction any more, but rather this is the consequence of the extracted fraction including CNCs and all other non-soluble particles.

According to STEM observation, carried out without prior sonication, the well-defined rod-like morphology of extracted CNCs is still preserved and there seems to be no specific alteration in their shape or decrease in size (Figure 10). Most likely, the coloration was caused by grape-like aggregates or numerous small irregular particles usually directly deposited on the CNC surface and remaining in suspension together with CNCs. Efforts had not been undertaken to elucidate the exact chemical structure of these insoluble hydrolytic residues, but it seems clear that the presence of such contaminants has a negative impact on CNC processibility and should be avoided. An intense browning of the reaction medium and contamination of CNC suspensions with individual small particles or large grape-like aggregates were equally noticed for prolonged hydrolyses in mild acid conditions (H₂SO₄ 4%) for extractions carried in [BMIM]Cl as previously reported. 38

It is important to note that the processing temperature could also have a significant impact on the hydrolysis kinetics. Even a slight increase in temperature from 80 to 90 °C allowed reducing the swelling step time from 80 to 40 min by 50%. Similar time reduction from 8 to 4 hours was observed for hydrolysis step at 90 °C instead of 80 °C. Therefore, temperature, as well as [SBMIM]HSO₄ and water concentration, are to be considered as key parameters for further optimization of CNCs extraction processes.

Finally, the CNC extraction route described in this work, based on the use of a single [BMMIM]Cl/[SBMIM]HSO₄ reaction medium, makes it possible to envisage efficient ways for ILs recycling. At the end of the hydrolysis step and after the first washing/centrifugation cycle, the whole medium thus consists of two main phases: the pellet containing CNCs...
and the supernatant aqueous phase containing ILs as well as hydrosoluble hydrolytic residues. According to mass balance results, more than 90% of the initially introduced ILs is systematically found in this first aqueous phase (Table 2). Finally, five washing/centrifugations cycles are essential in order to remove any eventual trace of ILs and to get highly purified CNCs. Interestingly, by combining only the first two aqueous phases, the quantity of potentially recyclable ILs approaches 100% of the theoretical value.

Further purifications, such as an additional centrifugation followed by a filtration at 0.2 μm, allowed removing traces of all insoluble particles still remaining in suspension. The removal of the hydrosoluble hydrolytic residues, carbohydrates resulting from over-hydrolyzed cellulose fiber beyond the scale of CNCs, could be carried out by liquid–liquid extraction using ethyl acetate/water 1/4 g/g biphasic system (Figure 11). In fact, even if they represent only a minor fraction (<1%), the presence of hydrosoluble hydrolytic contaminants within ILs medium might have a negative impact on ILs activity, especially on [BMIM]Cl cellulose swelling ability. After a vigorous agitation and decantation, hydrosoluble residues migrate from the aqueous phase to the superior organic phase, resulting in intense browning of the initially colorless ethyl acetate. On the other hand, ILs fraction remains solubilized in the aqueous phase, which was also confirmed by mass balance results after two phases were separated and evaporated (Table 3).

To further extend the purification of ILs, the aqueous phase was washed two more times with the ethyl acetate. Successive decoloration of the aqueous phase already demonstrates, in a qualitative way, the extraction efficiency. FTIR, as well as 1H and 13C NMR analyses, confirmed the recovery of the ILs after the third extraction, which spectra systematically corresponded to those of native compounds (Figures 12 and 13). In the 1H spectra (CDCl3, 500 MHz) peaks at δ 7.39, 7.54, and 10.51 ppm correspond to the protons of the imidazolium ring, peak at δ 4.07 ppm to the methyl, and those at δ 0.90, 1.32, 1.83, and 4.27 ppm to the butyl moieties, respectively. Triplet at δ 2.89 ppm corresponds to the [SBMIM]HSO4 aliphatic protons in α of the sulfonic acid function. In 13C spectra (CDCl3, 126 MHz) three peaks at δ 123.14, 124.84, and 139.39 ppm correspond to the chemical shifts of the imidazolium ring, while five peaks at δ 14.71, 20.75, 33.45, 37.86, and 51.08 ppm correspond to the aliphatic moieties. According to these analytical results, the process parameters established in this study seem to be suitable for both CNC extraction and recovery of the initial reaction medium.

New CNC extraction tests were subsequently carried out to verify performances of the recovered ILs media. Swelling ability was successfully confirmed for three consecutive cycles, all cellulose fibers being homogeneously swollen after only 40 min at 90 °C (Figure 2(c)). Then, concerning the hydrolysis step, it was deliberately decided to systematically complete the recovered reaction medium with some additional [SBMIM]HSO4 in order to maintain high hydrolytic activity, achieving...
CNCs extraction after only 4 h at 90 °C, as with the fresh ILs medium. In fact, [SBMIM]HSO₄ fraction might be partially consumed during the hydrolysis step, but also removed or altered during ILs purification and drying at 105 °C. Further quantitative analyses of the reaction medium during the whole process should be considered in order to confirm these assumptions and eventually readjust recycling parameters.

**Conclusion**

Well-defined rod-like cellulose type I nanocrystals were extracted from cotton fibers by two-step swelling/hydrolysis method, in acid-free reaction medium exclusively based on a single mixture of two ILs without addition of any mineral acid. The reaction medium was predominantly composed of 1-butyl-3-methylimidazolium chloride [BMIM]Cl (up to 96 wt%), inducing controlled swelling of the cellulosic fibers in anhydrous conditions. The activity of the 1-(4-sulfobutyl)-3-methylimidazolium hydrogen sulfate [SBMIM]HSO₄, generally representing 4 wt%, was then exploited during the second process step, triggering, after addition of 2 wt% water, a fast hydrolysis of unfolded fibers, eventually leading to rod-like CNCs with average diameter and length around 20 and 300 nm, respectively.

As two ILs were always together in the reaction medium, from the beginning and during the whole extraction process, it was essential to ensure a good efficiency of each step, but also a perfect compatibility and sequencing between them. More particularly, the water content is to be increased to a sufficient level during the hydrolysis step in order to avoid any intensive solubilization. Otherwise, the extraction does not lead to rod-like cellulose type I nanocrystals but to morphologically heterogeneous nanoparticles containing both cellulosics type I and type II. The impact of process parameters such as duration of each step, temperature, and [SBMIM]HSO₄ content, was also examined in order to find a suitable compromise between fiber unfolding, solubilization, hydrolysis advancement, and the final product properties. An attempt to slightly increase temperature from 80 to 90 °C allowed reducing the reaction time by half, from 9h20 to 4h40 with the overall extraction yield always being in the range of 30%. Thus, it still might be possible to consider numerous opportunities for further extraction process optimization.

Finally, the recovery of the reaction medium components, with yields reaching 95%, can be achieved by centrifugation, filtration, and liquid–liquid extraction with ethyl acetate/water biphasic system. At this stage of the study, the efficiency of the recycled reaction medium was assessed for three consecutive CNC extractions, validating perspectives for conception of multi-cycle, environmentally and economically viable processes.

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**Disclosure statement**

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