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Original Article

DISSOLUTION STUDY OF BACTERIAL CELLULOSE (*NATA DE COCO*) FROM LOCAL FOOD INDUSTRY: SOLUBILITY BEHAVIOR & STRUCTURAL CHANGES

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ABSTRACT

Dissolution and regeneration of bacterial cellulose (BC) extracted from *nata de coco* using different ratio of sodium hydroxide (NaOH) and urea as a solvent was investigated. The dissolution and structural properties of regenerated BC were determined by Fourier transform infrared spectrometric analyzer and X-ray diffraction. The solubility of BC increased significantly with the increasing concentration up to 8%. In contrast, urea alone had no effect on solubility of BC but an increase in concentration of urea (up to 4% w/v) combined with 6% or 8% of NaOH resulted in an increase of BC solubility. X-ray diffraction and FTIR results indicate that NaOH is the primary agent in cleaving of the inter- and intra-hydrogen bonds in cellulose. An increase in urea concentration was found to be responsible for the transformation of cellulose I to II in the presence of NaOH.

Keywords: Nata de coco, Bacterial cellulose, NaOH/urea, Solubility, X-ray diffraction, FTIR.

INTRODUCTION

Cellulose is not only plentiful source of biopolymer but also hydrophilic, biodegradable and chemically modifiable [1,2]. However, its insolubility in common solvents and non-thermoplastic nature limit its pharmaceutical application. In fact, development of special non-derivatizing cellulose dissolving system has been a long standing goal in the field of cellulose research because of the rigid long chains and strong intra and inters hydrogen bonds [3, 4].

In earlier period, regenerated cellulose film was mainly prepared by using cuprammonium. However, it was later shown to be hazardous to the environment [5]. Hence, there is a need arises to identify a new environmental-friendly solvent system for cellulose dissolution. Some non-derivatizing solvent systems like inorganic complexes (cuoxam, cuen), lithium chloride/N,N-dimethylacetamide (LiCl/DMAc) [6], ammonia/ ammonium salt (NH_3/NH_4SCN) [7], N-methylmorpholine-N-oxide monohydrate (NMMO) [2] and ionic liquids came in flux. Nevertheless, they are restricted to laboratory scale because of the concerns like viscosity, toxicity and high cost variably. Recently, cheaper, environmental-friendly and less toxic NaOH complex solvent system were developed by Zhang's group [1, 8, 9, 10]. They proposed that cellulose with low degree of polymerization (DP<300) can be easily dissolved in a narrow range of NaOH solution (7 to 10 wt %) at temperature below 0°C. Other researchers reported that treatment with alkali solution at higher concentration cause polymorphic transformation from cellulose I to cellulose II within the crystalline domains [11]. It has also been shown that the transformation occurred in the concentrations up to 12% [12]. However, cellulose of high degree of polymerization has been found to be difficult to be dissolved in an alkali solution. In another studies, a combination of an alkaline solution with urea and thiourea were found to be useful in dissolving the material. It has been shown that the combine solutions have more dissolution capacity than NaOH solution alone [4, 9, 10]. Zhang et al. also reported that NaOH/urea solvent system was also suitable for homogenous reaction mixture for etherification of cellulose and synthesis of cellulose polyelectrolyte, containing acylamino and carboxyl groups with acrylamide [10, 13, 14].

Bacterial cellulose (BC) synthesized by *Acetobacter xylinum* is a promising biopolymer and has been used in a number of biomedical applications [15, 16, 17, 18]. Unlike the plant cellulose, BC is pure and possesses higher degree of polymerization [19, 20]. In this work, we investigate the dissolution behavior of bacterial cellulose extracted from nata de coco in different NaOH/urea combinations and study the influence of solvent system on structure, solubility and crystallinity of BC specifically.

MATERIALS AND METHODS

Materials

Nata de coco, was further purified, lyophilized, characterized and identified as described in British Pharmacopoeia (2010) to obtain bacterial cellulose. Sodium hydroxide, urea, hydrochloric acid (HCl) (Sigma Aldrich) and other reagents were of analytical grade quality and used without further purification.

Dissolution of Bacterial Cellulose (BC)

The 2% w/v BC solution was prepared by dispersing microfibrilated BC at various NaOH (0, 2, 4, 6, 8, 10% w/v) and urea concentrations (0, 2, 4, 6% w/v), and maintained at -15°C for 24 h. The frozen solution was thawed, stirred vigorously to obtain a homogenous cellulose solution as described by Chang et al. (2010) [21], and observed under an optical microscope (Olympus, Fluoview FV 1000, Japan).

Regeneration of BC

The regenerated BC fibers used for X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) studies were obtained by neutralizing NaOH with HCl, and the regenerated fibers were subsequently washed with distilled water until all alkalinity was removed from the samples. These samples were coded on the basis of NaOH/urea concentration used for BC solubilization (Table 1).

Solubility of Bacterial Cellulose

The percentage solubility of BC in varying ratios of NaOH/urea solvent system was determined by using the following equation:

$$S_a\ (\%) = [W_1/(W_1 + W_2)] \times 100$$

Where, S_a is the percent solubility of BC, W_1 is the weight of dissolved BC, and W_2 is the weight of insoluble BC after centrifugation at 7000 rpm for 10 min. The effect of solvent composition on cellulose fiber was recorded by an optical microscope (Olympus, Fluoview FV 1000, Japan).

FT-IR Analysis

The IR spectra of samples were recorded in order to analyze the structural changes by using FT-IR Spectra 2000 (Perkin Elmer, Waltham, MA, USA) at room temperature. The KBr disk method was

adopted for FTIR experiment and samples were scanned over the range of 4000–400 cm^{-1}.

X-Ray Diffraction

In order to investigate the crystalline structure of the samples, XRD of regenerated BC was performed by an X-ray diffractometer (D8 Advance; Bruker AXS, Inc., Madison, WI, USA) using CuKά radiation at 40 kV and 50 mA in differential angle range of 5°- 60° 2θ.

RESULTS AND DISCUSSION

Solubility of BC

The combination effect of NaOH (0%–10% w/v) and urea (0%–6% w/v) concentration on solubility of BC were investigated (Table 1). The results indicated that the BC solubility was increased significantly with increasing NaOH concentration, peaking at 8%. In contrast, urea alone had no effect on BC solubility. Solubility of BC (2%) increased rapidly from 3.65% to 40.65% when the NaOH concentrations were increased from 2% to 8%, but not from 8% to 10%. These findings are in accordance to those of Cai and Zang (2005 & 2006) [1, 3] who used plant cellulose, but at different NaOH concentrations.

On the other hand, solubility of BC dramatically increased by the addition of urea from 2% to 4%. An increasing urea concentration (up to 4% w/v) combined with 6% or 8% of NaOH resulted in an increase of BC solubility by 6.86% (29.22% to 36.08%) and 4.66% (40.65% to 45.31%), respectively. The increase in solubility was insignificant ($P< 0.05$) when the urea concentration was increased from 4% to 6%. From the data, it was apparent that solubility rapidly improved from 6% to 8% NaOH in combination with 2% to 4% urea.

These results are consistent with previous studies [4] reporting that the size of NaOH-water hydrates; which plays a primary role in cellulose dissolution, is concentration-dependent. Native cellulose chains are very dense with an inter-sheet distance of about 10 A° and a crystalline diameter of only 10 nm. Hence, at low NaOH concentrations, these hydrodynamic diameters of NaOH-water hydrates may be too large to penetrate the crystalline region; alternatively, the quantity of NaOH may be insufficient to dissolve the cellulose. By contrast, at higher concentrations, the NaOH molecule may position itself more closely towards the cellulose chain and form soda cellulose I. Soda cellulose I is capable of absorbing more alkali solution and converting into soda cellulose II, which is apparently water soluble [4].

Macro structural changes of BC samples in different ratio of NaOH/Urea were observed by optical microscope. Here, we analyzed and compared the swelling and dissolution behavior of BC in different solvents in terms of transparency and un-dissolved fraction of fibers. Figure 1 b to d showed urea alone does not affect the shape of BC fiber; it was similar as the untreated cellulose fiber (Figure 1a). However, combination of urea and NaOH caused remarkable change on the shape of the fibers. As clear from the image, increased amount of urea (0 to 6% w/v) with constant concentration of NaOH (2% w/v) caused more gelation and swelling of the cellulose fibers (Figure 1e to 1h). Similarly as NaOH concentration increased from 2% to 4% both swollen and unswollen fibers could be seen from Figures 1e and 1i, but as the concentration increased from 6% to 10%, the fiber structure was highly swollen, exhibited state of gelation and destruction of cellulose crystal (Figures 1j to 1l).

X-Ray Diffraction of Regenerated Cellulose

The XRD pattern with corrected intensities is displayed in Figure 2. The diffractogram of untreated and only urea-treated (0%–6% w/v) BC were similar, with four peaks typical of cellulose I (Figure 2a), located at 2θ = 14°, 16°, 23°, and 34°, corresponding to the (1 0 1), (1 0 $\bar{1}$), (0 0 2), and (0 4 0) crystallographic planes, respectively [22]. When two peaks present in the diffractogram at 2θ = 14° and 16° are more pronounced, they represent a higher crystalline cellulose content. Therefore, the urea content alone does not affect the crystallinity of BC and does not cause crystalline transformation (Figure 2a). Similar patterns of diffraction were also reported by Oudiani et al. (2011) [11] upon treating plant cellulose with urea. By contrast, NaOH-treated cellulose displayed peaks typical of cellulose II polymorph at 2θ = 11°, 20°, 22°, and 37° correspond to the (1 0 1), (1 0 $\bar{1}$), (0 0 2), and (0 4 0) crystallographic planes, respectively (Figure 2b). Two characteristic peaks at 14° and 16° of cellulose I are an indication of cellulose I-to-II conversion. The peaks present in the diffractogram at 2θ = 14° and 16° were smeared, appearing as one broad peak in regenerated BC treated with 2% and 4% NaOH, while the peaks appeared to have disappeared in 6%–10% NaOH-treated regenerated BC (Figure 2b). In the absence of NaOH, the diffractogram displayed only peaks typical of cellulose I, while the diffractogram of cellulose treated with 2% (w/v) NaOH displayed the appearance of the diffraction at 2θ = 20° suggesting the beginning of polymorphic transformation from cellulose I to II. At increasing NaOH concentrations, major peaks shifted towards cellulose II (2θ = 20°, 22°). While urea alone does not affect the crystalline structure, an increasing amount of urea in the presence of constant NaOH concentration causes increased transformation of cellulose I to II, indicated by the appearance of diffraction at 2θ = 20°, 22° (Figure 2c).

The above data indicate that NaOH is the primary agent in the cleaving of inter- and intra-hydrogen bonds in cellulose. The resulting NaOH-water hydrates penetrate into the amorphous area of cellulose and destroy the neighboring crystalline region. After breakage of inter-hydrogen bonding, active cellulose strands reform an aggregate, which is prevented by the addition of urea that blocks active strand formation of cellulose by functioning as hydrogen bonding donor and acceptor between solvents [3].

FT-IR Analysis

The spectral range of 1500–800 cm^{-1} in FTIR is known for its sensitivity in detecting polymorphic changes [23]. The FTIR spectra (Figure 3a) of regenerated BC treated with varying ratios of urea were similar to untreated cellulose; supporting the above mentioned XRD data that urea does not cause structural changes.

The spectra of BC treated with 6%-10% NaOH, were not identical to untreated BC spectra (Figure 3b). Most apparently, the bands were poorly resolved and broader in length, primarily due to a reduction in cellulose crystallinity, known to occur during mercerization, as observed in the XRD data. The more prominent alkali-induced spectral changes were observed in the band shift from 2900 cm^{-1} (corresponding to the C–H stretching vibration) to higher wavenumber values (2924 cm^{-1}) and by the strong reduction in the band intensity, corresponding to the reduction in cellulose crystallinity. Additionally, the FTIR absorption band at 1430 cm^{-1} corresponding to symmetric CH_2 bending vibrations, decreases as the NaOH concentration increases. Also known as the crystalline band, its reduced intensity indicates a reduction in degree of crystallinity. By contrast, the amorphous band at 894 cm^{-1} (assigned to C–O–C stretching at β-(1-4)-glycosidic linkages) displayed increased intensity compared to untreated cellulose. The characteristic band of cellulose I at 1111 cm^{-1} disappeared, while the band at 1162 cm^{-1} (assigned to C–O–C), shifted to a lower wave number (1157 cm^{-1}) with weak intensity. Previous studies have reported [23, 24] that the intra-molecular hydrogen bonds for 2-OH···O-6 and 3-OH···O-5, and the intermolecular hydrogen bonds for 6-OH···O-3′ in cellulose I, appear at 3455–3410, 3375–3340, and 3310–3230 cm^{-1}, respectively, along with the valence vibration of hydrogen-bonded OH groups at 3570–3450 cm^{-1}. It was apparent that the maximum absorbance of hydrogen-bonded O–H stretching is shifted to higher wave numbers from 3367 cm^{-1} to 3421, 3446, 3456, 3458 cm^{-1} as the concentration of NaOH increased due to the intra-molecular hydrogen bond of 2-OH···O-6 formed by transformation (Figure 3b).

The spectra of cellulose treated with 2% NaOH, along with increased urea concentration, showed a significant difference ($P<0.05$) in absorption of the band at 1644 cm^{-1}, corresponding to C–O stretching vibration of C–O–H group. This peak became stronger as urea concentration increased. By contrast, regenerated BC without NaOH displayed a single peak at 1645 cm^{-1} without urea, but as the

urea concentration increased, the band split into two absorption peaks at 1665 and 1635 cm^{-1}. The latter peak was allocated to the OH of the water absorbed from the cellulose.

The crystalline peak at 1111 cm^{-1} disappeared due to broadening of the band at 1060 cm^{-1}corresponding to C–O stretching (Figure 3c).

Generally, the ratio of absorption of peak at 1372 cm^{-1} to those at 2900 cm^{-1} (A_{1372}/A_{2900}) could also be used to measure the degree of crystallinity [25]. The results are in accordance with the XRD data, where crystallinity decreased from 0.93±.04 to 0.62±0.12 as alkali concentration increased from 0 to 10%. There was no change in crystallinity by urea treatment (0.93±0.04 to 0.91±0.03 in 0 to 6% urea treatment without alkali), but a substantial effect on the NaOH-treated sample (Table 2).

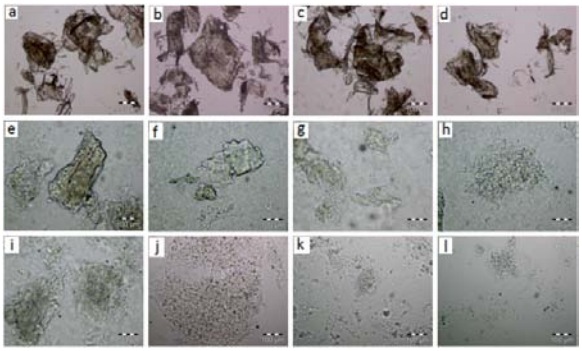

Fig. 1: Macromorphology changes of bacterial cellulose fiber in different concentration of NaOH/urea (a) 0-0, (b) 0-2, (c)0-4, (d) 0-6, (e) 2-0, (f) 2-2, (g) 2-4, (h) 2-6, (i) 4-0, (j) 6-0, (k) 8-0, (l) 10-0.

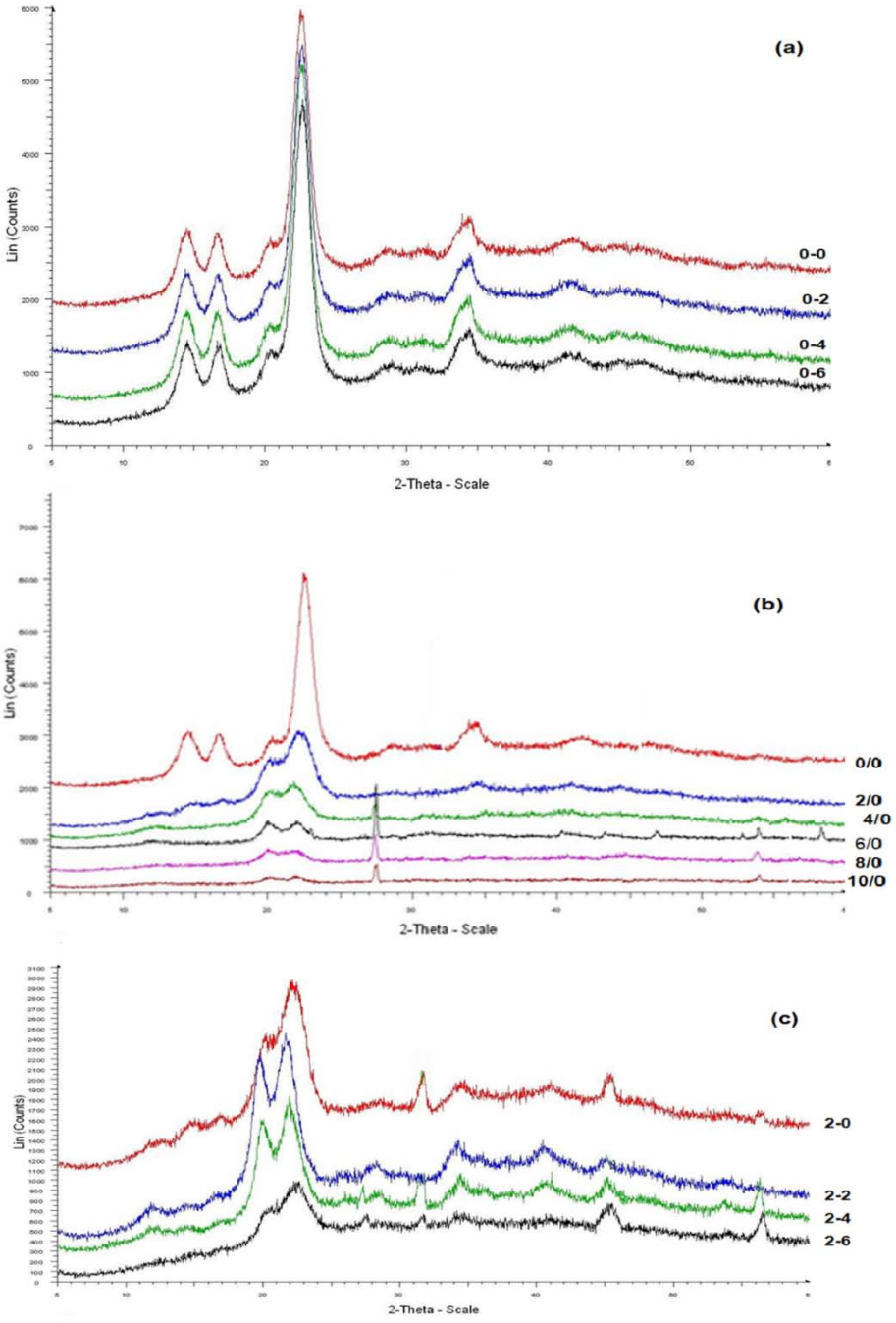

Fig. 2: X-ray diffraction data of regenerated bacterial cellulose treated with (a) different concentration of urea, (b) different concentration of NaOH, (c) different concentration of NaOH/urea.

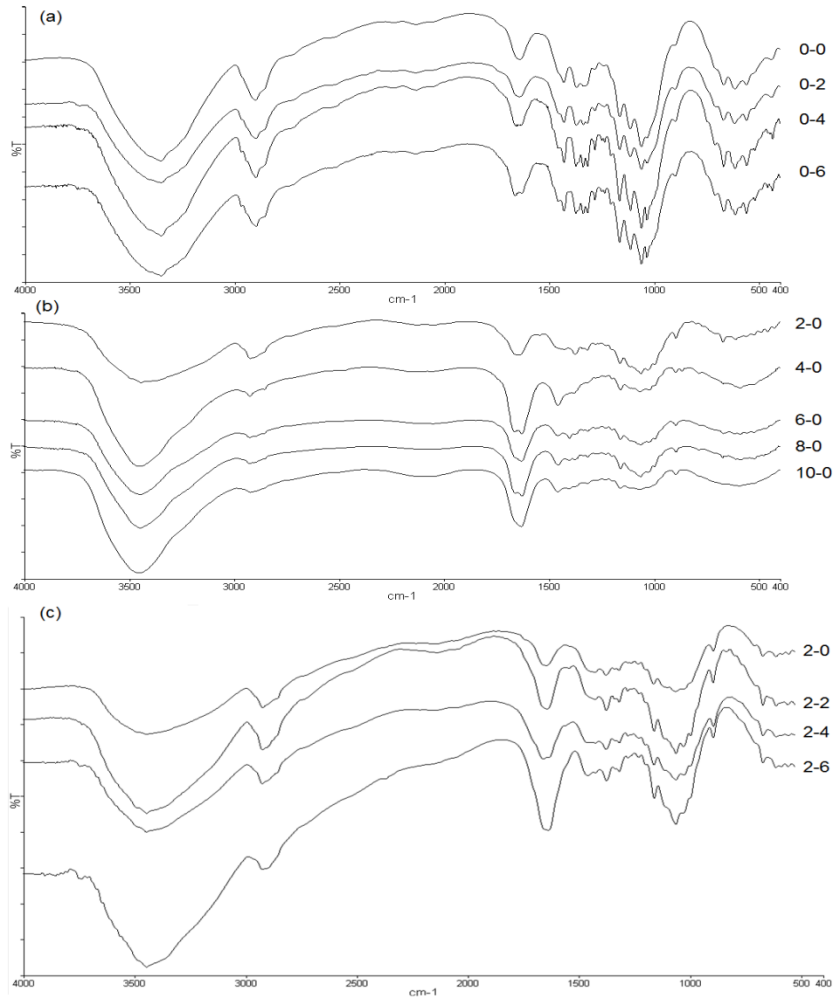

Fig. 3: Comparative FTIR spectra of regenerated bacterial cellulose treated with (a) different concentration of urea, (b) different concentration of NaOH, (c) different concentration of NaOH/urea.

Table 1: Formulation code for regenerated bacterial cellulose on the basis of solvent composition for bacterial cellulose and percentage solubility of BC

Formulation code for regenerated cellulose	Concentration of NaOH (% w/v)	Concentration of urea (% w/v)	Solubility of BC (%)
0-0	0	0	0
0-2	0	2	0
0-4	0	4	0
0-6	0	6	0
2-0	2	0	3.65±0.95
2-2	2	2	5.15±1.35
2-4	2	4	6.59±0.67
2-6	2	6	6.80±0.72
4-0	4	0	17.11±1.53
4-2	4	2	20.70±0.87
4-4	4	4	23.77±0.85
4-6	4	6	24.22±1.53
6-0	6	0	29.22±0.95
6-2	6	2	32.74±2.52
6-4	6	4	36.08±0.84
6-6	6	6	36.74±1.37
8-0	8	0	40.65±0.86
8-2	8	2	43.75±1.08
8-4	8	4	45.31±1.19
8-6	8	6	46.07±0.75
10-0	10	0	43.16±2.07
10-2	10	2	45.35±2.26
10-4	10	4	46.82±1.46
10-6	10	6	46.53±1.75

Table 2: Relative crystallinity of regenerated cellulose

% w/v Ratio of NaOH/Urea	Crystallinity
0/0	0.93
0/2	0.93
0/4	0.94
0/6	0.93
2/0	0.88
4/0	0.73
6/0	0.70
8/0	0.66
10/0	0.62

CONCLUSION

In summary, the work reports the effect of alkali treatment with urea on bacterial cellulose solubility, structure and crystallinity, along with the effect on hydrogel appearance and swelling properties. From X-ray diffraction and FTIR analysis it was found that sodium plays important role in transformation of cellulose I to II more than 2% NaOH concentration. Urea doesn't affect structure and crystallinity alone but in presence of NaOH it increased the mercerisation process of BC.

CONFLICT OF INTERESTS

Declared None

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