Carbohydrate Polymers 87 (2012) 1020–1025

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation of cellulose films from solution of bacterial cellulose in NMMO

Gao Shanshan, Wang Jianqing*, Jin Zhengwei

Tianjin University of Science & Technology, Tianjin 300222, China

ARTICLE INFO

Article history:
Received 5 May 2011
Received in revised form 26 May 2011
Accepted 15 June 2011
Available online 11 August 2011

Keywords:
Bacterial cellulose
Dissolution
NMMO
Film

ABSTRACT

Bacterial cellulose (BC) was dissolved in *N*-methylmorpholine *N*-oxide (NMMO) to prepare regenerated BC films (RBC) with phase inversion. The solubility of BC, supermolecule on structure, morphology, thermal and physical properties of the films were investigated by Fourier transform infrared spectroscopy (FT-IR), solid-state cross polarization/magic angle spinning ^{13}C nuclear magnetic resonance (CP/MAS ^{13}C NMR), wide-angle X-ray diffraction (WAXD), scanning electron microscope (SEM), and thermogravimetric analysis (TGA). The investigation suggested BC was dissolved completely in NMMO. From the C_6 signal shifts to the amorphous area, the crystallinity of materials decreased from 79.20% to 38.17%, and the transformation from cellulose I to II occurred. It was also found that the banded structure of the native materials was replaced by homogeneous and densified sections, so RBC films had better mechanical and barrier properties, and do thermal stability was similar to that of the native BC.

1. Introduction

Promising bioenergy materials which have excellent biodegradable and environmental friendly properties could replace the existing petroleum-based plastics and chemicals (Tuula, Harry, Geoff, & Paul, 2007;Wu, Wang, Li, Li, & Wang, 2009). Cellulose as the main ingredient of biomass in nature is clean, nontoxic, renewable, and degradable, especially bacterial cellulose (BC) which is biosynthesized by Acetobacter, Rhizobium, Grobacterium, and Sarcina (Nattakan, 2009; Omer, Salman, Taous, & Joong, 2009) and cultured in pure medium containing carbon and nitrogen sources (Nakagaito, Iwamoto, & Yano, 2005). Compared with plant cellulose (PC), BC possesses an extremely fine and pure fiber network structure which is made up of random assemblies of ribbon shaped fibrils (Nakagaito et al., 2005; Brigid, Deirdre, Gidley, & Neal, 2009), and it does not contain any lignin, hemicellulose and pectin (Muenduen & Nirun, 2008). BC shows unique properties such as high mechanical strength and biocompatibility (Chen, Jeffrey, & Ali, 2009; Gao, Shen, & Lu, 2010; Yan, Chen, Wang, Wang, & Jiang, 2008; Zhou, Zhang, Cai, & Shu, 2002). After free water was completely removed from BC pellicle, the ribbon improved the Young's modulus (Chen et al., 2009) and was used as supporting framework in composites (Nattakan, 2009) and membranous materials. In addition, if the BC could be chemically modified especially when dissolved in solvents, the modification, cross-linking (Xie, Hou, & Sun, 2007) and derivatization of BC would be carried out in a homogeneous solution. However, in the solid crystalline polymer, there are many hydrogen bonds in the BC chains which give interior stability which only a spot of solvents can break. The traditional chemistry methods such as viscose and cuprammonium route (Qi, Chang, & Zhang, 2008; Cai, Wang, & Zhang, 2007) lead the degradation of cellulose, and crystallinity and degree of polymerization decreased. These factors would affect the properties of the materials, such as acid and alkali resistance and biocompatibility and also produce by-product. Therefore it is important to find a solvent of scientific and practical interest for the dissolution of BC. Considerable efforts have been devoted to find good cellulose solvents, but only a limited number of solvent systems such as PF/DMSO, N_2O_4/DMF, LiC1/DMAc, DMSO/TEAC, NMMO, pyridine or imidazole based ionic liquids, NaOH/urea and NaOH/sulfourea have been developed (Fink, Weigel, Purz, & Ganster, 2001; Zhang, Wu, Zhang, & He; Cai et al., 2007). *N*-Methylmorpholine-N-oxide monohydrate (NMMO) is an effective "green" solvent for plant cellulose in industrial fiber-making (Lyocell process). The solution is supposed to be an entirely physical process without any chemical changes being caused in the pulp or in the solvent (Thomas, Antje, Immanuel, & Andreas, 2002), and offers a better alternative being faster, easier and more reproducible (Anne & Gabrielle, 2004) than traditional technologies and transforms the system from heterogeneous to homogeneous (Ana, Carmen, Armando, & Carlos, 2007). The transparent solutions could be processed into filaments, films, fillers and nonwovens with phase inversion methods which the cellulose could precipitate from the coagulation.

In this paper, NMMO was used to dissolve the bacterial cellulose and regenerated bacterial cellulose films had been prepared, and the dissolubility of native materials, molecular and supermolecular structure, morphology, thermal stability and physical properties of the films were also assessed.

* Corresponding author. Tel.: +86 22 6027 0014; fax: +86 22 6027 3395.
E-mail address: jianqw@tust.edu.cn (W. Jianqing).

0144-8617/$ – see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2011.06.040

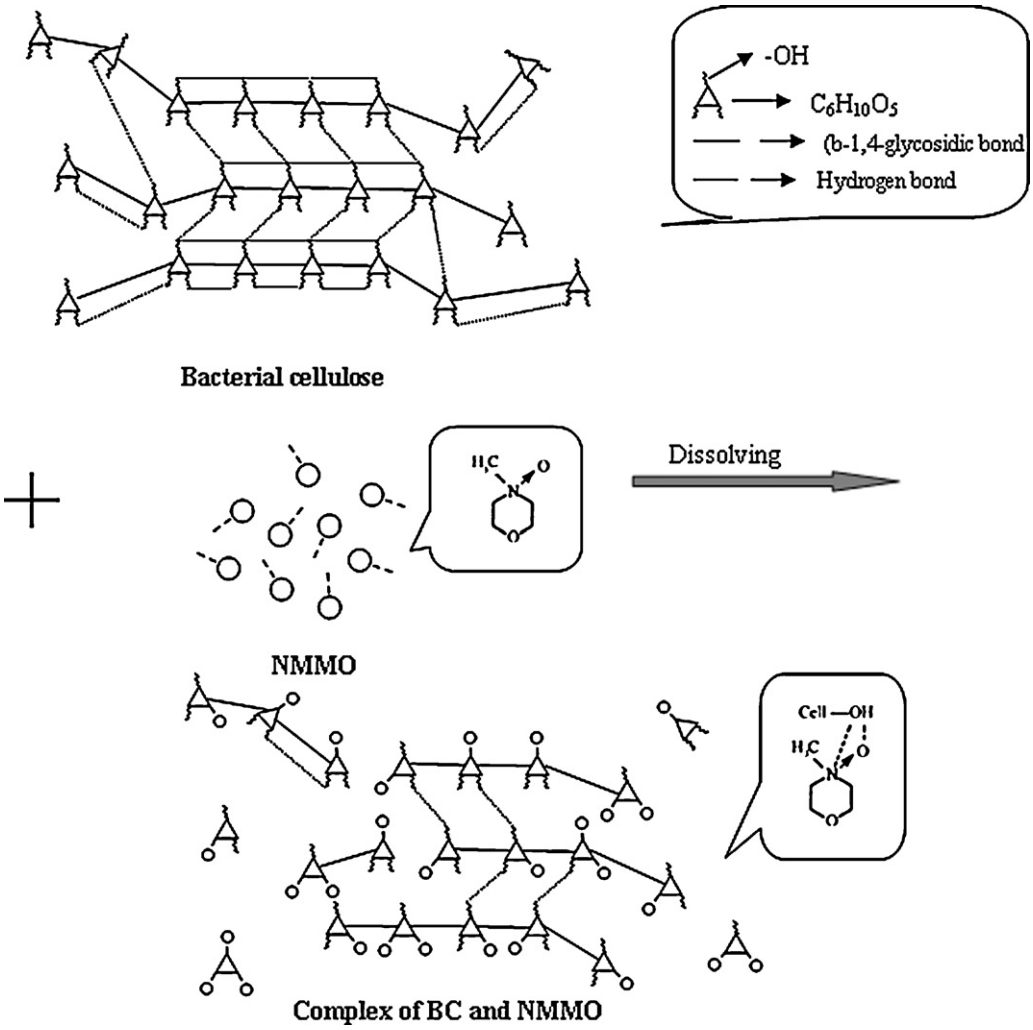

Fig. 1.

2. Materials and methods

2.1. Materials

The gel-like bacterial cellulose pellicles were provided as an experimental material by the School of Bioscience & Bioengineering, South China University of Technology (Guangzhou). NMMO used as solvent containing 60 wt.% water was purchased from Huaian Huatai Co. Ltd. Propyl gallate (chemically pure) was obtained from China National Pharmaceutical Group Corporation (Shanghai). Glycerin (analytical reagent) was obtained from Tianjin North Tian Yi Chemical Reagent Company. Deionized water was homemade.

2.2. Pretreatment of BC

BC pellicles were purified to remove bacteria cells by washing in running water firstly, then in 5% (w/v) NaOH and finally in 2.5% (v/v) NaOCl (Nattakan, Chandeep, Saharman, Takashi, & Ton, 2009). Thereafter, the pellicles were washed with deionized water twice and centrifuged. Finally, the pellicles were oven dried at 80 °C and cut into fraction.

2.3. Dissolution of BC and preparation of RBC films

Propyl gallate (0.2 wt.%) as an antioxidant was added in aqueous NMMO solution which was distilled until the surplus water decreasing to 13.3 wt.% and under ultrasonic agitation at 75 °C for 30 min, then the dried BC pieces (5.0 wt.%) were added into this beaker at 95 °C for 7 h with static. The dissolved cellulose was stirred at 100 °C for 2 h to form homogeneous and transparent bacterial cellulose/NMMO/H_2O solution, and the above mixture was poured and casted with a copper string onto the glass to obtain RBC film. The mean thickness of films was 50.0 μm measured by a high accuracy film thickness tester DRK(204) (Drick Instruments Co. Ltd., China) and five measurements were carried out for each sample. Subsequently, the films were soaked quickly into the water to remove the residual NMMO completely, followed by being plasticized in glycerin solution (30.0 wt.%) and air-dried.

2.4. Microscopic observation

The gelatinous pellicle solution was spread on an object slide and with cover glass, which was observed through a biological photomicroscope (UB100, UOP Photoelectric Technology Co. Ltd., Chongqing).

2.5. Fourier transform infrared spectroscopy (FT-IR) analysis

The molecular structures and chemical bonds of BC and RBC films were tested using a Fourier transform infrared spectrometer (VECTOR 22, Bruker Co. Ltd., Germany) from finely ground

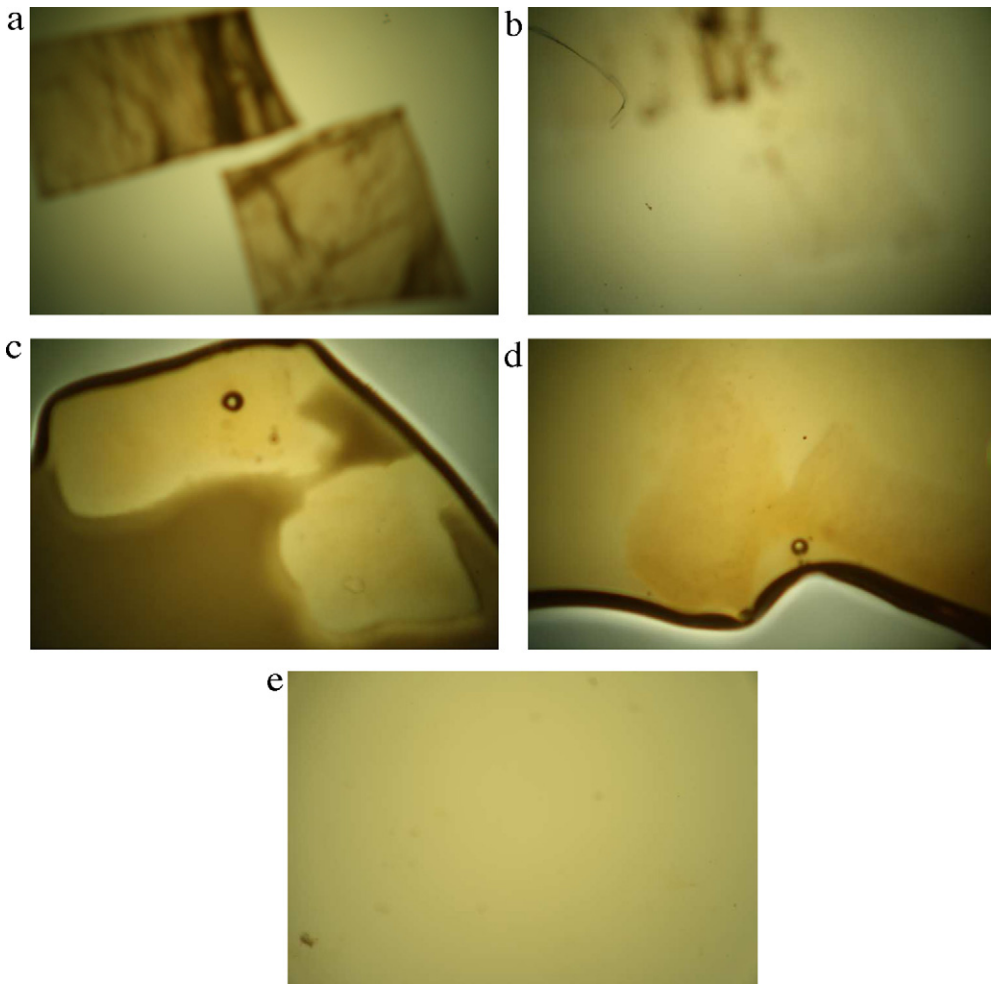

Fig. 2.

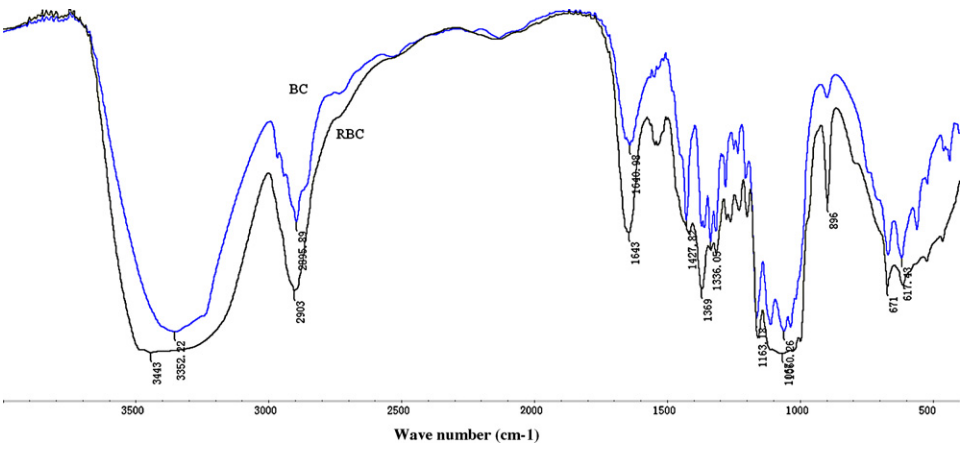

Fig. 3.

sample (1.0 wt.%) in KBr pellets in the range of 4000–700 cm^{-1} at a resolution of 4 cm^{-1}. The background spectrum and CO_2 peak were subtracted from the sample spectra.

2.6. CP/MAS ^{13}C NMR measurements

Structural of BC and RBC films through solid-state CP/MAS ^{13}C NMR measurements were conducted at room temperature on a Bruker AV300 spectrometer equipped with a CP MAS probe operating at 300 MHz.

2.7. X-ray diffraction measurement

X-ray was carried out with a Riguka D/max-C X-ray diffractometer using Cu Kα radiation (λ = 0.154 nm), the diffraction patterns being collected in the 2θ range 5–50°. The degrees of crystallinity (X_c) of BC and RBC films were calculated by the ratio of the area

in a diffractogram corresponding to crystalline (S_c) and amorphous regions (S_a) as follows:

$$X_c = \frac{S_c}{S_c + S_a}$$

2.8. Morphology analysis

Scanning electron microscopy (SEM) was used to observe the cross section morphological structures of BC and RBC films. Samples were stripping off in liquid nitrogen and sputter coated with gold and examined using a S-4700 (Hitachi Ltd.).

2.9. Thermal analysis

Thermal stability of BC and RBC films were performed using thermogravimetric analysis (TGA) with a thermal analyzer (TGA Q500 V20.6, USA). The samples weighed between 4 and 5 mg. The scans were run from room temperature to 600 °C at a rate of 10 °C per minute under nitrogen flow.

2.10. Physical properties

Compressed RBC films had been punched to prepare dog bone-shape specimens using cutter press. The tensile properties of the films were determined with an RG T-3 universal testing instrument using a 300 N load cell, and the crosshead rate used in the test was 30 mm/min, according to a Chinese National Standard of GB/T13022-1991 or a American Society for Testing Materials Standard (ASTM G 21-1996), and five measurements were carried out for each data point.

The oxygen permeability (OP) value of RBC films was determined with GDP-C shieldability testing equipment according to the Chinese National Standard of GB/T 1038-2000. The water vapor permeability (WVP) of RBC films was obtained according to the Chinese National Standard of GB 1037-1988 with sheet-cup method, and the results were reported as the average value from measurements of at least three specimens.

3. Results and discussion

Cellulose molecules are linear and have a strong tendency to form intra and intermolecular hydrogen bonds (Bochek, 2003) in the crystalline regions (Beatriz, Mohamed, & Elisabete, 2006). When in NMMO, the solvent permeates through the crystalline and amorphous phase, the lone pairs of the oxygen atom in N → O may form hydrogen bonds with the hydroxyl groups of BC. Fig. 1 shows the dissolving mechanism of BC in NMMO. The hydrogen bond networks of the BC are broken and form complex of BC and NMMO with new hydrogen bonds. However, the crystalline regions are diminished and the amorphous areas are increased.

When BC dissolves in NMMO contains two steps with swelling and dissolving. Fig. 2 shows the microphotographs of bacterial cellulose dissolved in NMMO at different times. When the BC was immersed in the solvent at the beginning, the bands and textures were very clear, after 0.5 h, flaxen bacterial cellulose got pale and the edges were blurred. In the swelling time between 4 h and 7 h, from Fig. 2(c and d), BC became transparent and the textures were opaque. After stirring for 9 h, BC was completely dissolved in the solvent where a transparent and homogeneous solution was formed in Fig. 2(e).

To investigate the effects of NMMO on the structure of cellulose during the process, the molecular structures of the RBC films and BC were tested by FT-IR spectra copy as displayed in Fig. 3. Both BC and RBC films had peaks at frequencies in 3352 cm^{-1} of ν_{OH}, ν_{C-H} in 2895 cm^{-1}, and at 1640 cm^{-1} was the hemiacetal group of cellulose 4′ side, the δ_{CH_2-OH} was in the 1427 cm^{-1}, while the intensity of absorption was weakened in the solution, and the ν_{C-O} in 1060 cm^{-1} was the characteristic peak of cellulose, the characteristic peak of β-D-glucose glycosidic bond existed in the 896 cm^{-1}, and it was acute in the dissolved BC. FT-IR showed new groups were not generated when BC was dissolved directly in NMMO.

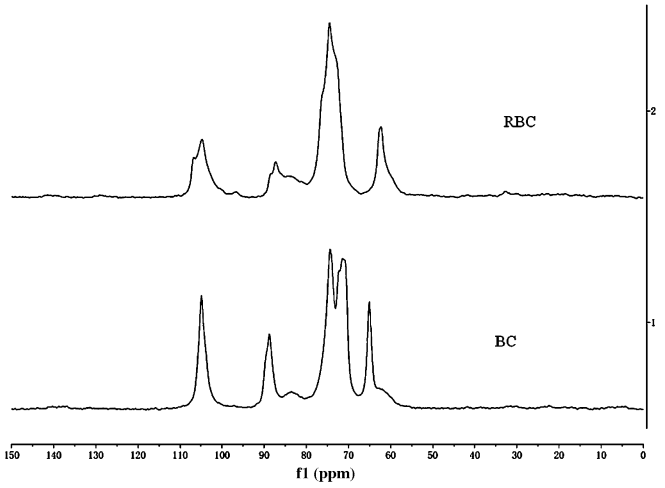

Fig. 4.

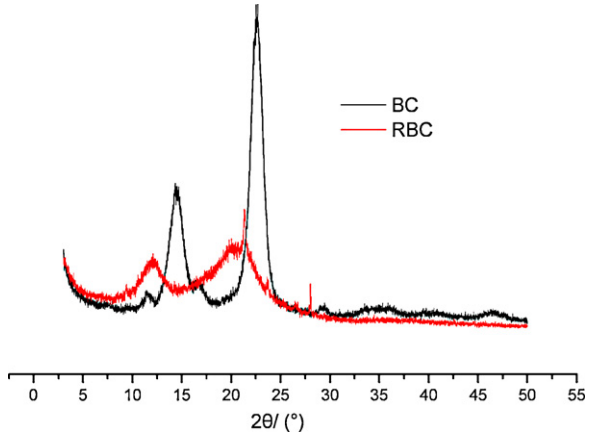

Fig. 5.

The evidence for the molecular structures of BC and RBC films was provided by the CP/MAS ^{13}C NMR spectrums and shown in Fig. 4. The strong peaks in 60–110 ppm were all the carbon signals. In BC spectrum, 104.90 ppm was the C_1 signal, 88.78 ppm was the C_4 signal which belonged to the crystalline region, and C_2, C_3, C_5 signals in 74.36 ppm, 65.06 ppm came from the C_6 signal of the crystalline region. However in RBC spectrum, the 65.06 ppm of C_6 signal shifted to 62.3 ppm which was in the amorphous region. This meant that most of the cellulose crystal structure was broken, and turned into amorphous cellulose after dissolving.

Combining with PC, the macromolecules of BC are biphasic ranges which are crystalline and amorphous phase. Fig. 5 shows a comparison of the wide angle X-ray diffraction (WAXD) profiles of BC and RBC films. Table 1 illustrated the supermolecular structure of BC transformed crystal from I to II after BC dissolved in NMMO, and the degree of crystallinity was found to be significantly reduced from 79.20% to 38.17%, so the longer the immersion times, the more cellulose was dissolved and hence smaller cellulose crystallites remained and the macromolecules of BC separated and turned into grains.

Table 1

Crystalline transformation	Position of characteristic peak 28/(?)			Crystallinity (%)
	101	$10\bar{1}$	002	
BC I	14.33	16.65	22.68	79.20
BC II	11.53	20.63	21.32	38.17

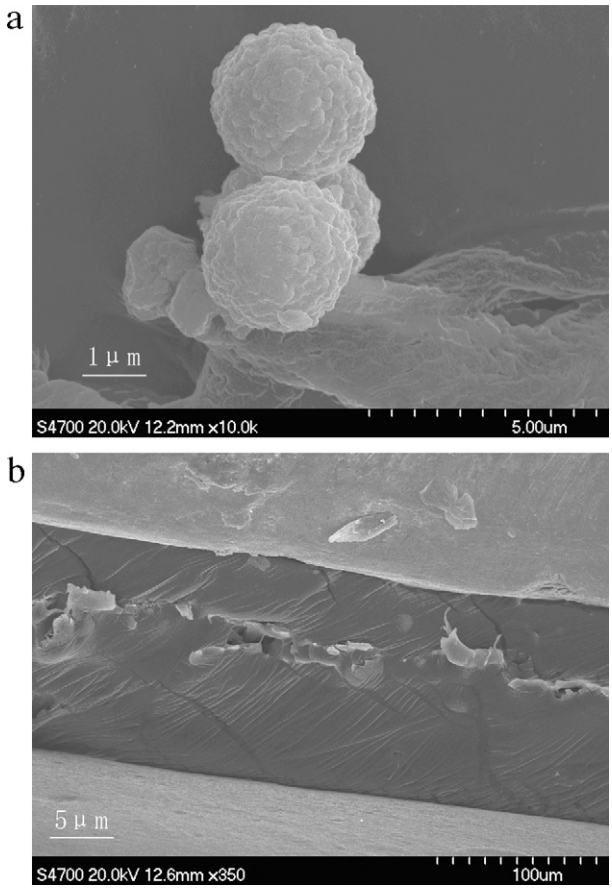

Fig. 6.

Fig. 6 shows the SEM photographs of the fracture surface of BC and RBC films. Picture (a) was the morphology of BC which comprised slender fibrils, lying in various directions and giving an image of network structure, while when BC was dissolved, the bonded structure was broken and formed homogeneous solution. The structure of RBC film was dense, therefore uniform structure of the films would have excellent physical properties.

The thermal properties of BC and RBC films were studied by TGA measurements in nitrogen. Fig. 7 shows the TGA and DTG thermograms of BC (a) and RBC (b). As can be seen, the cellulose displayed two steps of weight loss, the first step occurred around 80 °C with about 5.7 wt.% of weight loss corresponding to the moisture evaporation in cellulose. And there was a very slight mass loss until a temperature of 272 °C for the two samples. On further heating there was a sharp weight loss, the decomposition temperature occurred at 309 °C for BC and 312 °C for RBC, and with about 25 wt.% and 20 wt.% of weight loss, respectively. This indicates that the thermal stability of RBC film is better than BC.

Table 2 gives informations about the properties of RBC films. Because of higher crystallinity, the films had better mechanical properties and up to 14.31 MPa and the elongation at break was 54.70%. Based on the high tensile strength and dense and uniform structure, the lower oxygen permeability (OP) and water vapor

Table 2

Samples	σ_b (MPa)	ε (%)	OP (cm^3 mm/m^2 d kPa)	WVP (g mm/m^2 h Pa)
RBC films	14.31	54.70	0.0435	0.0386

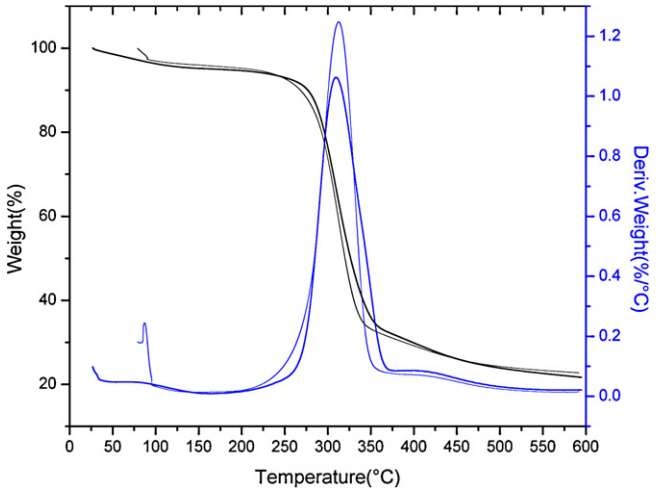

Fig. 7.

permeability (WVP) obtained, so the films had better barrier properties, and these properties would be of great relevance for its applications.

4. Conclusion

In summary, the bacterial cellulose was completely dissolved in NMMO and regenerated bacterial cellulose films were successfully prepared. The supermolecuar structure, the morphology and the thermal and physical properties of bacterial cellulose were investigated. In the process of dissolution, there were no new functional groups generated. The crystalline form of BC transformed from I to II and the crystallinity was reduced with the most crystal structures replaced with amorphous areas. The dense and uniform structure of the regenerated BC films improved the mechanical and barrier properties, and the thermal stability of regenerated BC film had changed very little after dissolving. According to these results, the use of BC cellulose films as green materials is promising for applications in filtering, spinning and food packaging or other fields.

Acknowledgements

The authors greatly appreciate the financial support from China Postdoctoral Science Foundation and Key Projects in the National Science & Technology Pillar Program during the Eleventh Five-Year Plan Period (Grant No.: 2006BAD30B02).

References

Ana, G. C., Carmen, S. R. F., Armando, J. D. S., Carlos, P. N., et al. (2007). Characterization and evaluation of the hydrolytic stability of trifluoroacetylated cellulose fibers. *Journal of Colloid and Interface Science*, *316*, 360–366.

Anne, L. D., & Gabrielle, H. (2004). Conformation and dn/dc determination of cellulose in N,N-dimethylacetamide containing lithium chloride. *Carbohydrate Polymers*, *58*, 233–243.

Beatriz, A. P. A., Mohamed, N. B., & Elisabete, F. (2006). Mercerized linters cellulose: characterization and acetylation in N,N-dimethylacetamide/lithium chloride. *Carbohydrate Polymers*, *63*, 19–29.

Bochek, A. M. (2003). Effect of hydrogen bonding on cellulose solubility in aqueous and nonaqueous solvents. *Russian Journal of Applied Chemistry*, *76*, 1711–1719.

Brigid, A. M. K., Deirdre, M., Wehr, J. B., Gidley, M. J., & Neal, W. M. (2009). Mechanical and structural properties of native and alkalitreated bacterial cellulose produced

by Gluconacetobacter xylinus strain ATCC 53524. *Cellulose*, *16*, 1047–1055. doi:10.1007/s10570-009-9340-y

Cai, J., Wang, L. X., & Zhang, L. N. (2007). Influence of coagulation temperature on pore size and properties of cellulose membranes prepared form NaOH–urea aqueous solution. *Cellulose*, *14*, 205–215. doi:10.1007/s10570-007-9106-3

Chen, K. C., Jeffrey, M. C., & Ali, D. (2009). Effect of different additives on bacterial cellulose production by Acetobacter xylinum and analysis of material property. *Cellulose*, *16*, 1033–1045. doi:10.1007/s10570-009-9346-5

Fink, H., Weigel, P., Purz, H. J., & Ganster, J. (2001). Structure formation of regenerated cellulose materials from NMMO-solutions. *Progress in Polymer Science*, *26*, 1473–1524.

Gao, Q. Y., Shen, X. Y., & Lu, X. K. (2010). Regenerated bacterial cellulose fibers prepared by the NMMO·H_2O process. *Carbohydrate Polymers*, doi:10.1016/j.carbpol.2010.09.029

Muenduen, P., & Nirun, J. (2008). Biosynthesis and characterization of bacterial cellulose–chitosan film. *Carbohydrate Polymers*, *74*, 482–488.

Nakagaito, A. N., Iwamoto, S., & Yano, H. (2005). Bacterial cellulose: the ultimate nano-scalar cellulose morphology for the production of high-strength composites. *Applied Physics A*, *80*, 93–97. doi:10.1007/s00339-004-2923-3

Nattakan, S., Chandeep, S., Saharman, G., Takashi, N., & Ton, P. (2009). All-cellulose nanocomposites by surface selective dissolution of bacterial cellulose. *Cellulose*, *16*, 435–444. doi:10.1007/s10570-009-9285-1

Omer, S., Salman, K., Taous, K., & Joong, K. P. (2009). Production of bacterial cellulose in static conditions by a simple fed-batch cultivation strategy. *Korean Journal of Chemical Engineering*, *26*(6), 1689–1692. doi:10.1007/s11814-009-0232-5

Qi, H. S., Chang, C. Y., & Zhang, L. N. (2008). Effects of temperature and molecular weight on dissolution of cellulose in NaOH/urea aqueous solution. *Cellulose*, *15*, 779–787. doi:10.1007/s10570-008-9230-8

Thomas, R., Antje, P., Immanuel, A., Andreas, H., et al. (2002). Cellulose solutions in N-methylmorpholine-N-oxide (NMMO) degradation processes and stabilizers. *Cellulose*, *9*, 283–291.

Tuula, T. T., Harry, B., III, Geoff, D., & Paul, G. (2007). Biomimetic engineering of cellulose-based materials. *Trends in Biotechnology*, *25*, 299–306.

Wu, R. L., Wang, X. L., Li, F., Li, H. Z., & Wang, Y. Z. (2009). Green composite films prepared from cellulose, starch and lignin in room-temperature ionic liquid. *Bioresource Technology*, *100*, 2569–2574.

Xie, K. L., Hou, A. Q., & Sun, Y. (2007). Chemical and morphological structures of modified novel cellulose with triazine derivatives containing cationic and anionic groups. *Carbohydrate Polymers*, *70*, 285–290.

Yan, Z. Y., Chen, S. Y., Wang, H. P., Wang, B., & Jiang, J. M. (2008). Biosynthesis of bacterial cellulose/multi-walled carbon nanotubes in agitated culture. *Carbohydrate Polymers*, *74*, 659–665.

Zhang, H., Wu, J., Zhang, J., & He, J. S. (2005). 1-Allyl-3-methylimidazolium chloride room temperature ionic liquid: a new and powerful nonderivatizing solvent for cellulose. *Macromolecules*, *38*, 8272–8277.

Zhou, J. P., Zhang, L. N., Cai, J., & Shu, H. (2002). Cellulose microporous membranes prepared from NaOH/urea aqueous solution. *Journal of Membrane Science*, *210*, 77–90.