Biomacromolecules **2007,** *8,* 1973−1978

Surface Modification of Bacterial Cellulose Nanofibers for Property Enhancement of Optically Transparent Composites: Dependence on Acetyl-Group DS

Shinsuke Ifuku,*,† Masaya Nogi,‡ Kentaro Abe,‡ Keishin Handa,§ Fumiaki Nakatsubo,‖ and Hiroyuki Yano†

Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Japan, International Innovation Center, Kyoto University, Kyoto, Japan, Mitsubishi Chemical Group Science and Technology Research Center, Mitsubishi Chemical Corporation, Yokohama, Japan, and Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Received January 29, 2007; Revised Manuscript Received March 16, 2007

Bacterial cellulose (BC) nanofibers were acetylated to enhance the properties of optically transparent composites of acrylic resin reinforced with the nanofibers. A series of BC nanofibers acetylated from degree-of-substitution (DS) 0 to 1.76 were obtained. X-ray diffraction profiles indicated that acetylation proceeded from the surface to the core of BC nanofibers, and scanning electron microscopy images showed that the volume of nanofibers increases by the bulky acetyl group. Since acetylation decreased the refractive index of cellulose, regular transmittance of composites comprised of 63% BC nanofiber was improved, and deterioration at 580 nm because of fiber reinforcement was suppressed to only 3.4%. Acetylation of nanofibers changed their surface properties and reduced the moisture content of the composite to about one-third that of untreated composite, although excessive acetylation increased hygroscopicity. Furthermore, acetylation was found to reduce the coefficient of thermal expansion of a BC sheet from 3×10^{-6} to below 1×10^{-6} 1/K.

Introduction

Bacterial cellulose (BC), an indigenous dessert food known as a “nata-de-coco”, is produced by *Acetobacter xylinum* and consists of ribbon-shaped nanofibers structured in a weblike network.[1,2] Because the nanofibers are made up of a bundle of cellulose microfibrils which are aggregates of semicrystalline extended cellulose chains, bacterial cellulose nanofibers have excellent physical properties; their Young’s modulus is 138 GPa,[3] their tensile strength is estimated to be at least 2 GPa,[4] and remarkably, their thermal-expansion coefficient in the axial direction is as small as 0.1×10^{-6} 1/K.[5]

Recently, Yano et al. discovered that bacterial cellulose nanofibers had very promising characteristics as a reinforcing material for optically transparent plastics.[6] Because of the size effect, the composite in a variety of resin was optically transparent even at high fiber content such as 70%. Moreover, the nanocomposites exhibited remarkable characteristics, such as low thermal expansion and high strength because of the effect of BC nanofiber reinforcement, while maintaining the ease of bending of pure polymers. BC nanocomposites have been investigated in detail in terms of, for instance, the relationship between the transparency of the BC nanocomposite and the refractive index of the resin[7] and the fiber content dependency of the transparency and thermal expansion of the composites for future material application.[8] Although these results indicated that BC has extraordinary potential as a reinforcement material in optoelectronic devices, for instance, as a substrate for bendable displays, the composite is hygroscopic owing to several hydroxyl groups on the cellulose nanofiber, and moisture absorption causes deformation of the composite, spoiling the advantage of dimensional stability against temperature. Hence, in a previous paper, Nogi et al. prepared a nanocomposite reinforced with acetylated BC nanofibers with a low-degree-of-substitution (DS) sample of 0.17.[9] Even at low DS, acetylation was found to effectively reduce the hygroscopicity of BC nanocomposites without sacrificing transparency, because hydrophilic hydroxyl groups on the nanofiber surface were replaced by less hydrophilic acetyl groups. Further improvement of hygroscopicity is expected by further acetylation, although the relationships between DS value and material properties are still unclear. It is desirable to investigate acetyl DS dependency for various practical applications such as those related to optoelectronics devices. In this paper, we prepared BC acetates with different degrees of substitution and their composites and then characterized hygroscopicity and other properties in detail.

Experimental Section

Acetylation of Bacterial Cellulose Nanofiber. BC pellicles were a kind gift from Dr. Y. Kuwana (Fujicco Co., Ltd.). The bacterial strain *Acetobacter xylinum* FF-88 was incubated for 10 days in a static culture containing 5% (v/v) coconut milk (nitrogen content: 0.8%, lipid: 30%) and 8% (w/v) sucrose, adjusted to pH 3.0 by acetic acid. The BC fiber content in the pellicles was approximately 1% (v/v). BC pellicles, cut into about 10 cm × 10 cm × 1 cm thick pieces, were boiled in 2% NaOH for 2 h to remove the bacterial cell debris. The residue was thoroughly washed under running tap water for 2 days and then was compressed to 1 mm thickness by pressing at 2 MPa and 20 °C for 2

---

* To whom correspondence should be addressed. Tel: +81-774-38-3658. Fax: +81-774-38-3658. E-mail: sifuku@rish.kyoto-u.ac.jp.

† Research Institute for Sustainable Humanosphere, Kyoto University.

‡ International Innovation Center, Kyoto University.

§ Mitsubishi Chemical Group Science and Technology Research Center, Mitsubishi Chemical Corporation.

‖ Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University.

10.1021/bm070113b CCC: $37.00

min to roughly remove the water bulk. The compressed BC pellicles were soaked in acetone. The water in the sample was replaced repeatedly by acetone. The sample was then placed in a Petri dish containing a mixture of 40 mL of acetic acid, 50 mL of toluene, and 0.2 mL of 60% perchloric acid.[10] Then, the desired amount of acetic anhydride was added with stirring. The mixture was allowed to stand for 1 h at room temperature. After acetylation, the BC sample piece was washed thoroughly with methanol and then was hot-pressed at 2 MPa and 80 °C for 4 min to obtain a dried sheet.

Fabrication of BC Nanocomposites. Acetylated BC sheets cut into 4 cm × 3 cm were impregnated with neat acrylic resin (tricyclodecane dimethanol dimethacrylate (TCDDMA), Mitsubishi Chemical Corp. refractive index: 1.532) under a reduced pressure of −0.09 MPa for 12 h. The resin-impregnated BC sheets were cured using UV curing equipment (20 J/cm^2, a F300S UV lamp system and a LC6 benchtop conveyer, Fusion UV systems Japan). The BC nanocomposite sheets thus obtained were about 100 μm thick, and the fiber content of the original BC nanocomposite was about 40%.

Fourier Transform Infrared (FT-IR)−Attenuated Total Reflection (ATR) Spectroscopy. Infrared spectra of the BC samples were recorded with an FT-IR spectrometer (Spectrum One, Perkin-Elmer Japan Co., Ltd) equipped with an ATR attachment (Universal ATR, Perkin-Elmer Japan Co., Ltd). All the spectra were obtained by accumulation of 16 scans, with resolution of 4 cm^{-1}, at 400−4000 cm^{-1}. In our measurement condition, the depth of the FTIR-ATR method is about 1.5−15 μm at 400−4000 cm^{-1}.

Scanning Electron Microscopy (SEM). The acetylated and untreated BC sheets were coated with platinum by an ion sputter coater and were observed with a field-emission scanning electron microscope (JSM-6700F, JEOL Ltd.).

X-ray Diffraction. Equatorial diffraction profiles were obtained with Ni-filtered Cu Kα ($\lambda = 0.15418$ nm) from an X-ray generator (UltraX 18HF, Rigaku Corp.) operating at 30 kV and 100 mA.

Moisture Content. Moisture content was evaluated by exposing samples under a constant relative humidity. After measuring the sample weight equilibrated in 55% RH at 20 °C with a saturated aqueous solution of $Mg(NO_3)_2$, the sample was oven-dried at 105 °C for 24 h, and then the moisture content was determined on the basis of the oven-dried weight.

Regular Light Transmittances. Regular light transmittances were measured at wavelengths from 200 to 1000 nm using a UV−vis spectrometer with an integrating sphere 60 mm in diameter (U-4100, Hitachi High-Tech. Corp.). Regular transmittance was measured without the reference by placing the specimens 25 cm from the entrance port of the integrating sphere.

Coefficient of Thermal Expansion (CTE). The CTEs were measured by a thermomechanical analyzer (TMA/SS6100, SII Nanotechnology Inc.). Specimens were 25 mm long and 3 mm wide with a 20 mm span. The measurements were carried out three times from 20 °C to 165 °C by elevating the temperature at a rate of 5 °C/min in a nitrogen atmosphere in tensile mode under a load of 3 g. The CTE values were determined in the second run. For this measurement, the cellulose samples were heated at 180 °C for 4 h in vacuo to postcure the acrylic resin.

Results and Discussion

Characterization of Acetylated BC Nanofibers. BC samples were acetylated according to the procedure in ref 10. Figure 1 shows the degree of substitution (DS) of acetylated BC pellicles plotted against the amount of acetic anhydride (Ac_2O) added to the cellulose specimen containing about 500 mg of dry cellulose.

The DS values of the cellulose derivatives were calculated from the C content in the elemental analysis data. BCs acetylated from 0 to 1.76 DS were obtained by adjusting the amount of acetic anhydride added under acidic conditions. This reaction is applicable for several esterifications of BC nanofibers using corresponding carboxylic acid and acid anhydride. The initial steep rise in DS gradually changed to a gentle one as the amount of reagent increased. The observed nonlinearity indicates that cellulose nanofibers were acetylated inhomogeneously; that is, after initial rapid surface acetylation, the inside of the cellulose nanofiber was acetylated slowly with the collapsing crystal structure of cellulose I.

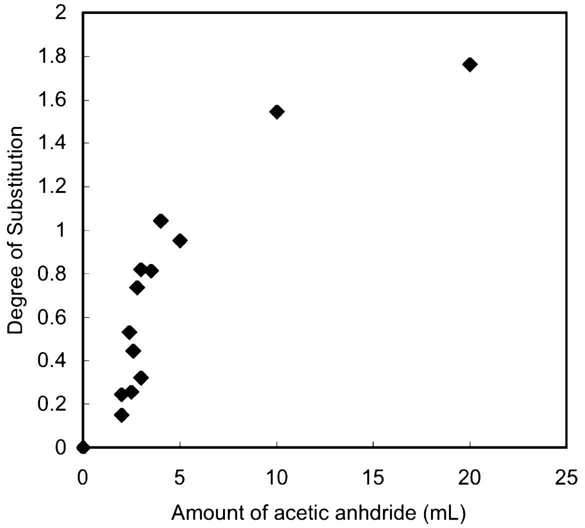

Figure 1. DS of acetylated BC plotted against the amount of acetic anhydride.

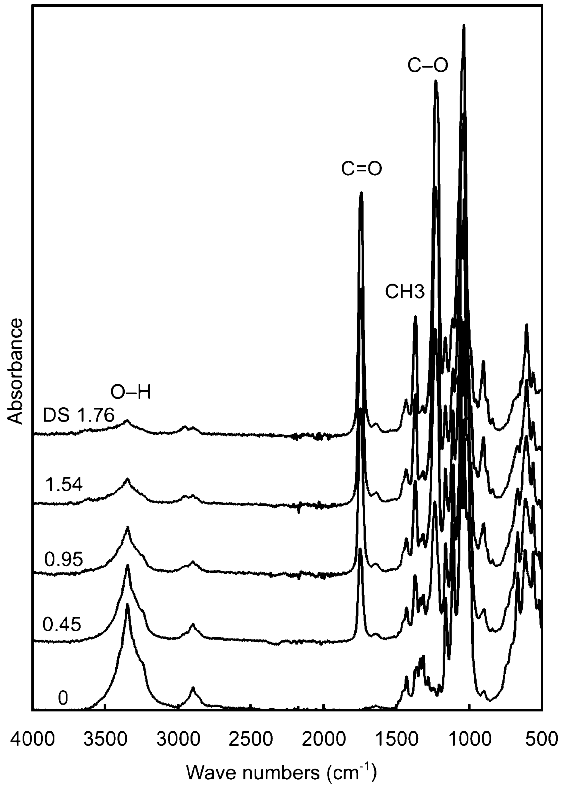

Figure 2. ATR-IR spectra of a series of acetylated BC.

Figure 2 shows the FT-IR−ATR spectra of a series of acetylated BC. These spectra are normalized by an absorption band at 1428 cm^{-1} derived from the cellulose main chain.

Corresponding to the increase in DS values from 0 to 1.76, the O−H band at 3348 cm^{-1} decreased monotonously as a result of esterification, and three bands at 1742 cm^{-1}, 1369 cm^{-1}, and

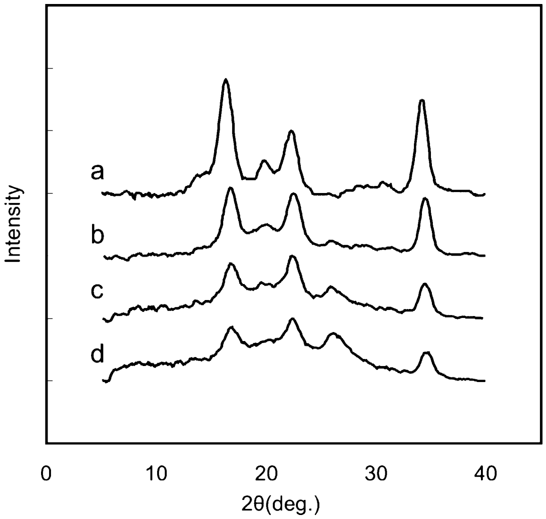

Figure 3. X-ray diffraction profiles of (a) untreated, (b) DS 0.45, (c) DS 0.95, and (d) DS 1.55.

1229 cm^{-1}, assigned, respectively, to the C=O stretching vibration mode, the CH_3 asymmetric bending vibration mode, and the C−O stretching vibration mode of the acetyl group, increased.[10,11]

The X-ray diffraction profiles of a series of acetylated BC samples are shown in Figure 3.

The diffraction pattern of the DS 0.45 sample (Figure 3b) seems to almost agree with that of the untreated sample (Figure 3a). On the other hand, the DS 0.95 and 1.55 samples (Figure 3c and 3d) had smaller diffraction peaks from cellulose I than those in the untreated and DS 0.45 samples. In addition, these samples gave a well-defined pattern of cellulose triacetate at $2\theta = 26°$ and an amorphous halo. These features also clearly indicate that acetyl groups were introduced from the surface to the core of BC nanofibers as described in ref 10.

Acetylation of bacterial cellulose nanofiber is considered to change its shape with the change of crystal structure of cellulose I as shown in Figure 3. Scanning electron microscope (SEM) images of untreated and esterified BC nanofibers are shown in Figure 4 a−c.

Owing to the addition of toluene as a poor solvent into the acetylation reaction system, fiber shapes were kept even in the high-DS sample, although cellulose acetate fibers were generally dissolved in acetic acid from the surface. All nanofibers of dried BC sheets existed separately without coagulation, since the surface tension was reduced by the replacement of water with organic solvent acetone or MeOH. The untreated BC sample consisted of thin nanofiber ribbons (Figure 4a). The appearance of the nanofibers in the DS 0.45 (Figure 4b) seems to be almost the same as that of the untreated sample. On the other hand, the volume of nanofibers in DS 1.55 sample obviously increases as shown in Figure 4c. This change in volume also indicates that bulky acetyl groups were heterogeneously introduced to the inside of nanofibers after the surface reaction.

As a result, acetylated BC with a variety of DS was obtained by adjusting the amount of acetylation reagent. The reaction proceeds from the surface to the core of semicrystalline nanofibers, and the dimensions and crystal structure of the nanofibers were changed with the increase of acetyl DS. These changes affected various physical properties of the BC nanocomposites.

Characterization of Acetylated BC Nanocomposites. Figure 5 shows the regular transmittance of transparent BC nanocomposites plotted against acetyl DS at the 580 nm wavelength, which is the center of the visible light spectrum from 380 to 780 nm.

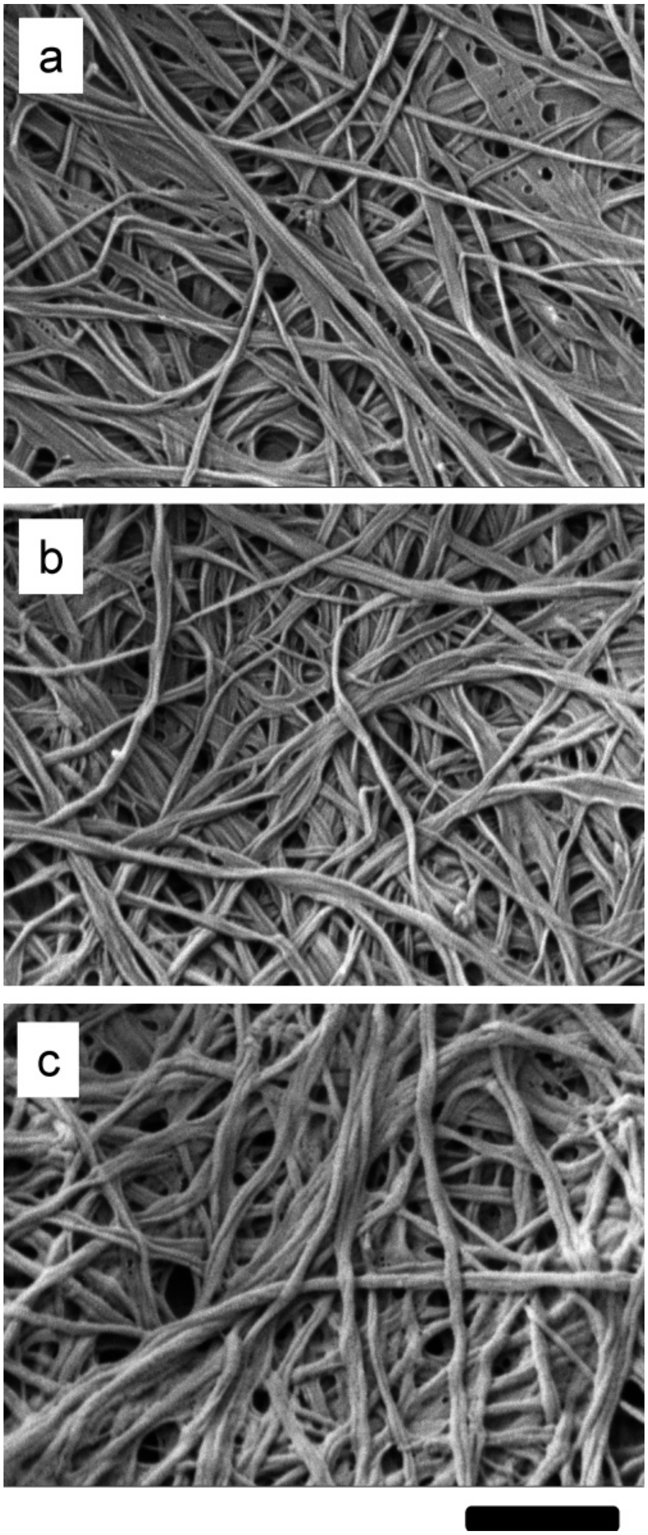

Figure 4. SEM images of (a) untreated, (b) DS 0.45, and (c) DS 1.55 BC samples. For all photographs, the length of the scale bar is 500 nm.

The regular transmittance of the untreated BC nanocomposite was 78.2%. However, the transparency was improved with increasing DS until DS 0.74 and reached 87.8%. Surprisingly, since the regular transmittance of TCDDMA film with the same thickness was 91.2% including surface reflection (Fresnel's reflection), the optical loss of the composite because of fiber

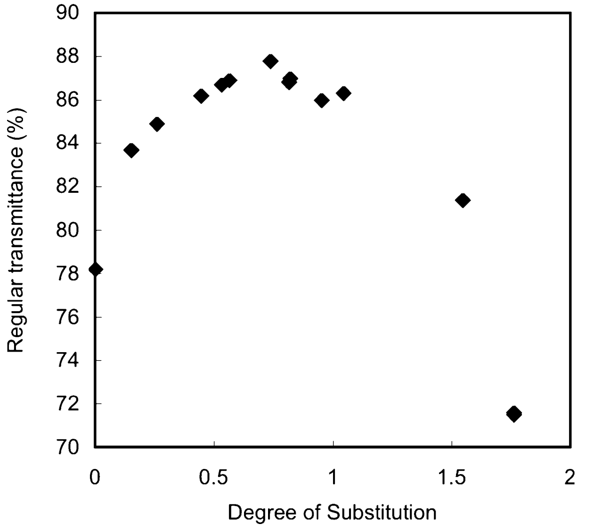

Figure 5. Regular transmittance of a series of acetylated BC nanocomposite at 580 nm.

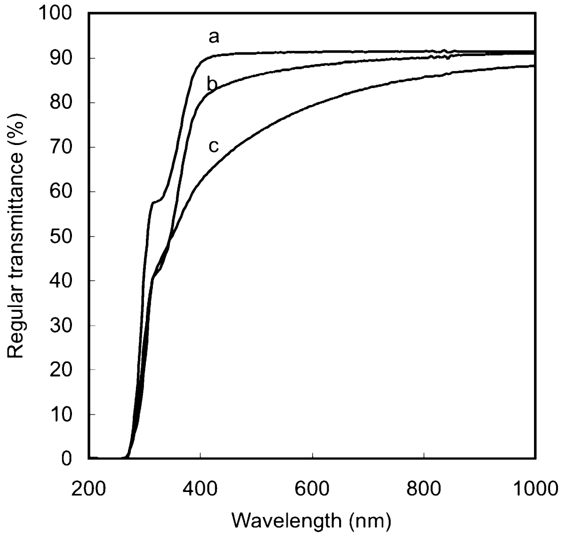

Figure 6. Regular transmittance spectra of (a) TCDDMA, (b) acetylated BC composites, and (c) untreated BC composites.

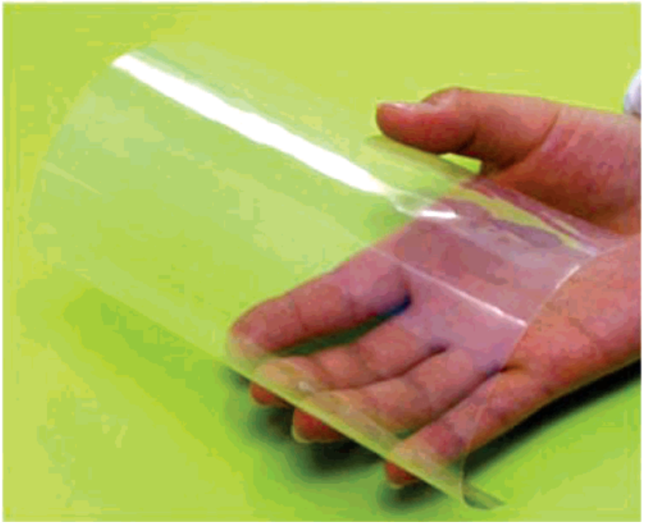

Figure 7. Appearance of optically transparent acetyl BC nanocomposite.

reinforcement was reduced from 13.0% to 3.4% by acetylation, even with a high fiber content of 63% (Figure 6), as shown in Figure 7.

Nogi et al. reported that the optimum refractive index of resin to obtain the highest transparency nanocomposite with untreated BC is 1.56−1.60, which is higher than the refractive index of TCDDMA resin (1.532).[7] However, it is known that acetylation decreases the refractive index of cellulose, and the refractive index of cellulose triacetate was reduced to about 1.470−1.475, which is considerably lower than that of TCDDMA.[12,13] Consequently, it is assumed that the refractive index of cellulose nanofiber decreased by acetylation and came close to the refractive index of the TCDDMA resin at about DS 0.74.[9] This result clearly indicates that refractive index of cellulose can be adjusted by acetylation to agree with that of a variety of resins.

Affinity between fiber and resin should also relate to the high transparency. Since the hydrophilic nanofiber surface became hydrophobic by acetylation, the affinity of the nanofiber surface and hydrophobic TCDDMA was increased, allowing rapid penetration of the resin into the cavities of a BC sheet. As a result, scattering of visible light at the interface of cellulose nanofibers decreased. In fact, impregnation of untreated BC sheet with neat resin took about 12 h under reduced pressure, while in case of the acetyl sample, the composite turned mostly transparent within 1 h at atmospheric pressure.

However, introduction of further acetyl groups steeply decreased transparency, and regular transmittance at 580 nm fell to 71.5% in the DS 1.76 sample. Considering that the refractive index of cellulose acetate decreases with the increase of acetyl DS, it can be speculated that refractive index of the BC might become less than that of TCDDMA by excess acetylation. In addition, since nanofibers became thicker by the inner introduction of bulky acetyl groups as shown in Figure 4c, these thickened fibers would interfere with transmission of visible light.

Although the BC nanocomposite has very promising characteristics as a reinforcement material for optically functional composites, it is considerably hygroscopic compared to the general acrylic resin. High moisture absorption causes deformation of the composite, which is a serious disadvantage for its application as an optoelectronics device. Nogi et al. demonstrated that even at slight acetylation, the moisture content of the nanocomposite was reduced effectively,[9] and additional acetylation seemed to further reduce moisture content. Thus, we investigated the relationship between the acetyl DS and the moisture absorption of the composite to minimize hygroscopicity. Figure 8 shows the moisture contents of a series of acetylated BC nanocomposites at 20 °C and 55% RH.

The moisture content of the original sample was 1.5%, which is considerably higher than that of TCDDMA acrylic resin such as 0.35%. The moisture absorption steeply decreased as the DS value increased and became less than 0.50% at DS 0.45−0.56. This is obviously because the nanofiber surface became hydrophobic by the introduction of acetyl groups, and this change in surface properties resulted in reducing the absorption of water.[14] However, by further acetylation, the moisture contents increased linearly from 0.44% to 1.92% as the DS values increased from 0.56 to 1.76. This result indicates that by excess chemical modification, bulky acetyl groups were introduced into the core of cellulose crystalline, and the water molecules were allowed to penetrate into the internal spaces of the cellulose acetate fiber. Consequently, the optimum DS value to obtain a low-hygroscopic nanocomposite is about 0.5; hygroscopicity of the sample is less than one-third that of the original composite.

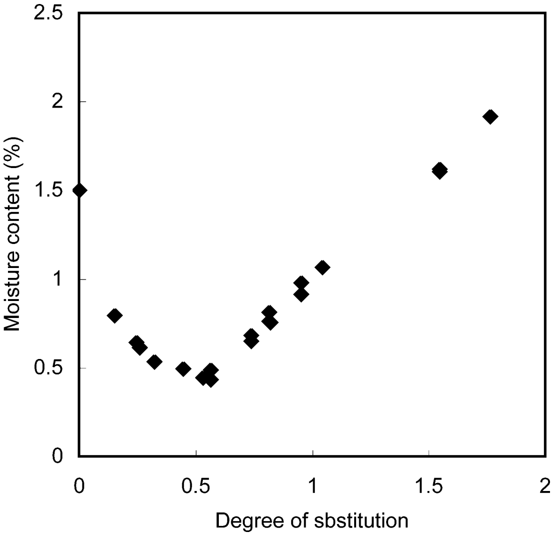

Figure 8. Moisture content vs degree of substitution of acetylated BC/resin nanocomposites.

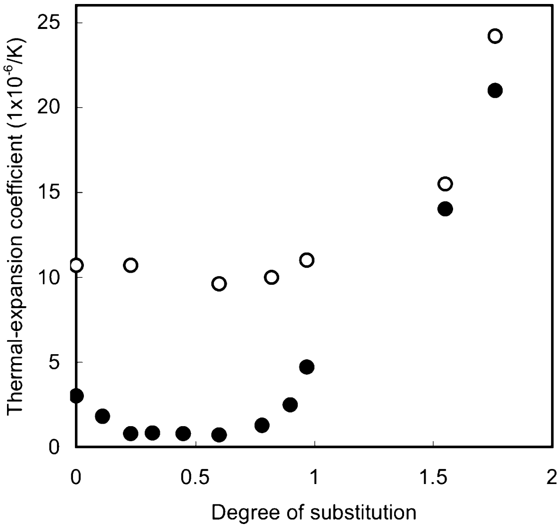

Figure 9. The CTE of a series of acetylated BC samples (filled circles) and their nanocomposites (open circles).

The nanofiber reinforced composite showed incredibly low thermal expansion.[6] For a wider use of optically transparent polymers in optoelectronic devices such as substrates for flexible displays and components for precision optical devices, their low thermal expansion must be maintained. Acetylation of BC may influence the coefficient of thermal expansion (CTE) of the composites with the change of crystal structure. Hence, we clarified the changes of the CTE of the composites against degree of acetylation. The CTE of BC samples and their nanocomposites from 20 °C to 150 °C plotted against the acetyl DS is shown in Figure 9.

Surprisingly, the CTE of BC sheets decreased with increasing acetyl content until DS 0.6, and the CTE from DS 0.23 to 0.6 was lower than 1×10^{-6} 1/K. One of the plausible reasons for the decrease of CTE is explained as follows. In low acetyl DS samples (0.23−0.6), the crystallinity of nanofibers changed little as shown in Figure 3. However, the surface and amorphous region was preferentially acetylated as described before and became much less mobile by the introduction of bulky acetyl groups, after which thermal expansion was restricted. After resin impregnation, the CTEs of the acetyl composites were not improved and stayed around 10×10^{-6} 1/K in DS 0−0.82 composites. This is presumably because the interaction among nanofibers becomes weak because of decreased interfibrillar hydrogen bonding by acetylation. Consequently, the acetylated BC sheet will be deformed easily by the thermal expansion of the resin.[9] For improvement of the CTE of the acetyl composite, it is important to make the nanofiber network interaction strong to restrict the thermal expansion of the matrix. We have already confirmed that strengthening of this interaction effectively reduces the CTE, and these findings will be reported and discussed elsewhere. On the other hand, for samples above DS 0.78, the CTE of acetylated BC samples and their composites increased with increase of DS. This is obviously because extensive acetylation reduced the degree of crystallinity of the cellulose nanofiber.

Conclusion

Bacterial cellulose could be acetylated with a variety of DS values. We found that acetylation improved various properties of BCs and their composites. The composite had an optimum DS for property enhancement, and excessive acetylation reduced these properties. Acetylation decreased the refractive index of cellulose, and the loss in regular transmittance of transparent nanocomposite at 580 nm from neat acrylic resin was reduced from 13% to 3.4% at a fiber content of 63%. Acetylation effectively reduced the hygroscopicity of BC nanocomposites, and water absorption at 20 °C became less than 0.5%. Furthermore, the CTE of a BC nanofiber sheet decreased to below 1×10^{-6} 1/K by acetylation. Consequently, surface acetylation of BC nanofibers without collapsing crystal structure is desirable for the enhancement of various properties of optically transparent composites.

Acknowledgment. This paper was prepared through helpful discussion with Dr. A. N. Nakagaito, RISH, Kyoto University. We would like to acknowledge Prof. Dr. L. Berglund, Biofibre Materials Centre, Royal Institute of Technology, for valuable suggestions and revision of the manuscript. This work was supported by a grant-in-aid from the International Innovation center, Kyoto University, Special Coordination Funds for Promotion Science and Technology from Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by fellowship from Japan Society for the Promotion of Science.

References and Notes

(1) Ross, P.; Mayer, R.; Benziman, M. Cellulose biosynthesis and function in bacteria. *Microbiol. Rev.* **1991**, *55*, 35−58.
(2) Iguchi, M.; Yamanaka, S.; Budhiono, A. Bacterial cellulose—a masterpiece of nature's arts. *J. Mater. Sci.* **2000**, *35*, 261−270.
(3) Sakurada, I.; Nukushina, Y.; Ito, T. Experimental determination of elastic modulus of crystalline regions in oriented polymers. *J. Polym. Sci.* **1962**, *57*, 651−660.
(4) Page, D. H.; Elhosseiny, F. The mechanical-properties of single wood pulp fibers. 6. Fibril angle and the shape of the stress-strain curve. *J. Pulp Pap.-Can.* **1983**, *84*, 99−100.
(5) Nishino, T.; Matsuda, I.; Hirao, K. All cellulose composite. *Macrolecules* **2004**, *37*, 7683−7683.
(6) Yano, H.; Sugiyama, J.; Nakagaito, A. N.; Nogi, M.; Matsuura, T.; Hikita, M.; Handa, K. Optically transparent composites reinforced with networks of bacterial nanofibers. *Adv. Mater.* **2005**, *17*, 153−155.
(7) Nogi, M.; Handa, K.; Nakagaito, A. N.; Yano, H. Optically transparent bionanofiber composites with low sensitivity to refractive index of the polymer matrix. *Appl. Phys. Lett.* **2005**, *87*, 243110.
(8) Nogi, M.; Ifuku, S.; Abe, K.; Handa, K.; Nakagaito, A. N.; Yano, H. fiber-content dependency of the optical transparency and thermal expansion of bacterial nanofiber reinforced composites. *Appl. Phys. Lett.* **2006**, *88*, 133124.
(9) Nogi, M.; Abe, K.; Handa, K.; Nakatsubo, F.; Ifuku, S.; Yano, H. Property enhancement of optically transparent bionanofiber composites by acetylation. *Appl. Phys. Lett.* **2006**, *89*, 233123.

(10) Kim, D.; Nishiyama, Y.; Kuga, S. Surface acetylation of bacterial cellulose. *Cellulose* **2002**, *9*, 361−367.

(11) Hurtubise, F. G. The analytical and structural aspects of the infrared spectroscopy of cellulose acetate. *Tappi* **1962**, *45*, 460−465.

(12) Boerstoel, H.; Maatman, H.; Picken, S. J.; Remmers, R.; Westerink, J. B. Liquid crystalline solutions of cellulose acetate in phosphoric acid. *Polymer* **2001**, *42*, 7363−7369.

(13) Scherer, J. R.; Bailey, G. F. Water in polymer membranes. 1. Water sorption and refractive index of cellulose acetate. *J. Membr. Sci.* **1983**, *13*, 29−41.

(14) Bledzki, A. K.; Gassan, J. Composites reinforced with cellulose based fibres. *Prog. Polym. Sci.* **1999**, *24*, 221−274.

BM070113B