Carbohydrate Polymers 74 (2008) 942–945

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Homogenous reactions of cellulose from different natural sources

Sherif M.A.S. Keshk *

AinShams University, Institute of Environmental Studies and Research, Basic Science Department, Abbasia 11566, Cairo, Egypt

ARTICLE INFO

Article history:
Received 3 March 2008
Received in revised form 7 May 2008
Accepted 27 May 2008
Available online 7 June 2008

Keywords:
Bacterial cellulose
Kenaf
Microcrystalline cellulose
Triester hypoiodous cellulose

ABSTRACT

Two different homogenous reactions on bacterial cellulose (BC), kenaf fiber (KF) and microcrystalline cellulose (MC) were performed to monitor their chemical reactivity. The first reaction was selective oxidation of the primary hydroxyl group with sodium chlorite in the presence of catalytic amount of sodium chloride. While, the second was the formation of triester hypoiodous cellulose using potassium iodate and potassium iodide. The chemical structures of these derivatives were investigated using FT-IR and solid state ^{13}C NMR spectroscopies. The BC fibrils required the shortest time among these cellulose samples for both reactions, whereas the viscosity values of BC after iodination and oxidation have the best values compared to KF and MC. FT-IR results show the absence of the hydroxy group of BC and a weak absorption band in both KF and MC. On the other hand, the crystallinity index (CI) of BC is higher than those of both KF and MC. FT-IR spectra of the oxidized different cellulose samples, confirmed the presence of a strong absorption band at around 1590 cm^{-1} that attributed to vibration band of carbonyl group of carboxylic moiety. Moreover, in the ^{13}C NMR spectrum of oxidized cellulose, the lack of signal at 62 ppm and the appearance of signal at 171 ppm indicated that the primary alcohol group is completely oxidized to carboxylic acid. These results showed that BC had a higher reactivity than other samples due to its great purity and low degree of polymerization.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Acetobacter xylinum produces highly crystalline cellulose extracellularly using glucose as a carbon source. Bacterial cellulose (BC) differs from plant cellulose with respect to its high crystallinity and purity (free from lignin and other biogenic products), separable in a simple way, high water-absorption capacity and better mechanical strength (Keshk, 2002). There are many researches on BC production control from various carbon sources and the influence of several additives on the nascent structure of it (Keshk & Sameshima, 2005, 2006; Masaoka, Ohe, & Sakota, 1993; Yoshinaga, Tonouchi, & Watanabe, 1997). Few researches have been discussed the chemical reactivity and heterogeneous reactions of BC in wet and dry state (Kim, Nishiyama, & Kuga, 2002). The carboxmethylation and cyanoethylation reactions were performed on BC (wet state), viscose grade pulp and cotton linter to study the reactivity of these cellulose samples (Keshk & Nada, 2003). BC showed the highest reactivity and selectivity with the lowest degradation; instead of BC have the highest crystallinity index and the lowest degree of polymerization (Keshk & Nada, 2003). Moreover, the homogenous reaction of dissolved BC with acetic anhydride and phenyl isocyanate showed products with high degree of substitution value up to 3.0 compared to plant cellulose without explaining the reason of this reactivity (Schlufer, Schmauder, Dorn, & Heinze, 2006). Since cellulose dissolves in 85% phosphoric acid with limited hydrolysis, its homogenous oxidation of the primary hydroxyl groups with halogen oxides have been achieved (Green, 1980; Pagliaro, 1998). The primary hydroxyl groups could be completely (>90%) oxidized to carboxylic acids. 6-Carboxycellulose is an important derivative as a wound-healing agent (to prevent post-surgical adhesions), a bone regenerating and periodontal therapy (Finn, Schow, & Schneiderman, 1992; Pollack & Bouwsma, 1992). Interestingly, iodination of cellulose in 85% phosphoric acid using mixture of KIO_3 and KI gave triester of hypoiodous acid (Pagliaro, 1999). In this study, homogenous derivatizations (selective oxidation and iodination) using phosphoric acid will be performed on different cellulose samples (BC, KF and MC) to reveal their chemical reactivates. Furthermore, the physicochemical characterizations of cellulose derivatives are studied.

2. Materials and methods

2.1. Materials

Bacterial cellulose was produced from *Acetobacter xylinum* ATCC 10245 using molass as carbon source (Keshk & Sameshima 2006) and dried as reported in our previous work (Kai & Keshk, 1998; Kai & Keshk, 1999). Kenaf fiber was prepared from kenaf bast using combination of ammonium oxalate and sodium hydroxide by refluxing and followed by acidic chlorite (Keshk, Suwinarti, & Sameshima, 2006). α-Cellulose was obtained from kenaf fiber by treating with 17.5% NaOH (TAPPI Test Method T 203 om-93.,

---

* Tel.: +202 120339402; fax: +202 24053215.
E-mail address: keshksherif@gmail.com

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.05.022

1994). Microcrystalline cellulose (Merck, Darmstadt, Germany) was used without any purification treatments. The other chemicals and solvents were of laboratory grade (Nacalai Tesque, INC, Kyoto, Japan), and used without further purification.

2.2. Iodination procedure

Iodination of cellulose samples was performed according to Pagliaro (1999). Cellulose sample (1.0 g) was dissolved in H_3PO_4 (30 ml, 85% w/w) until clear and viscous solution was obtained (3 h). Then mixture of finely powdered KI (1.1 mol/3 Glc residue) and KIO_3 (2.1 mol/3 Glc residue) was rapidly added in fume hood. After 24 h the cellulose hypoiodite was precipitated by adding cold EtOH (96%). Iodination was carried out with iodine in presence of HIO_3 (Scheme 1).

2.3. Oxidation procedure

The selective oxidation of cellulose samples with sodium chlorite was performed according to Pagliaro method (1998). In which, the cellulose samples (1.0 g) was dissolved in H_3PO_4 (30 ml, 85% w/w) until clear and viscous solution was obtained (3 h) then NaCl (0.10 g) was added, and after 30 min $NaClO_2$ (1.2 mol/mol primary hydroxyl) was added slowly to the cellulose solution at 4 °C. After 24 h at 4 °C, the oxidized cellulose was precipitate in a fume hood with cold ethanol under vigorous stirring. The precipitate was filtered and washed several times with aq. EtOH (80% v/v).

2.4. Carboxyl content analysis

The same procedure as described in the United States Pharmacopeia (USP, 1990) was used. Briefly, about 0.5 g of the sample was accurately weighted and dispersed in 50 ml of a 2% (w/w) solution of calcium acetate for 30min. The suspension was the titrated with standardized 0.1 N NaOH solution using phenolphthalein as an indicator. The volume of NaOH solution consumed was corrected for the blank. The carboxyl content in the sample was calculated from the following relationship:

$$\text{COOH content} = \frac{(\text{N} \cdot \text{V} \cdot \text{MW})_{\text{COOH}}}{\text{Weight of the Sample (mg)}} \times 100$$

where N and V are the normality and volume of NaOH, respectively.

2.5. Viscosity measurement

The viscosity of different samples of cellulose samples was measured by TAPPI test method (TAPPI Test Method T 230 om-89, 1994) using cupriethylendiamine (CED) as a solvent and a capillary viscometer, to give an indication of the average degree of polymerization of the cellulose samples and their derivatives. The viscosities determined as centipoises (cP) were converted to degree of polymerization (DP) according to the following formula:

$$\text{DP}^{0.905} = 0.75[954 \log(X) - 325],$$

where X = TAPPI viscosity in cP.

2.6. FT-IR spectroscopy

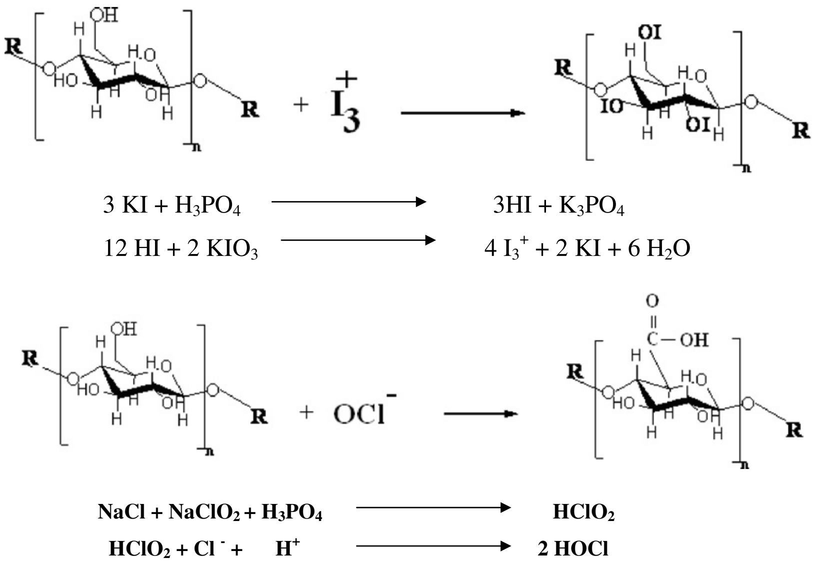
To study the physical structure of the cellulose samples and their derivatives, vibration spectroscopy was applied according to Abbott, Palmer, Gordon, and Bagby (1988) that obtained with a Bruker IFS 66 FT-IR spectrometer. The reproducibility of the spectra was verified on two-sample preparation; from 64 to 100 scans were taken with a resolution of 4 cm^{-1}. The crystallinity index (CI) was calculated as the ratio of the absorbance of band at 1430 cm^{-1} to the band at 900 cm^{-1} according to Nada, Shabaka, Yousef, and Nour (1990).

2.7. ^{13}C NMR analyses

The CP/MAS ^{13}C NMR spectra were recorded (at 292 ± 1 K) on the Joel CMX-300 instrument operating at 7.0 T. A double air-bearing probe and a zirconium oxide rotor were used. The MAS rate was in the 4–5 kHz range. Acquisition was performed with a standard CP pulse sequence using a 5 μs proton 90 pulse, a 1200 μs contact pulse and 3 s delays between repetitions.

3. Results and discussion

FT-IR spectra of the different samples of cellulose were recorded in the range of 4000–400 cm^{-1}. A slight difference is observed in the region of the intermolecular hydrogen bonding (3200–3400 cm^{-1}) of BC (Fig. 1). Each spectrum has two peaks at 1430 cm^{-1} and 900 cm^{-1} that assigned to amorphous and crystal-



$$3\,KI + H_3PO_4 \longrightarrow 3HI + K_3PO_4$$

$$12\,HI + 2\,KIO_3 \longrightarrow 4\,I_3^{+} + 2\,KI + 6\,H_2O$$

$$R\text{-cellulose} + I_3^{+} \longrightarrow$$

$$+\ OCl^{-} \longrightarrow$$

$$NaCl + NaClO_2 + H_3PO_4 \longrightarrow HClO_2$$

$$HClO_2 + Cl^{-} + H^{+} \longrightarrow 2\,HOCl$$

Scheme 1. Reaction mechanism of cellulose.

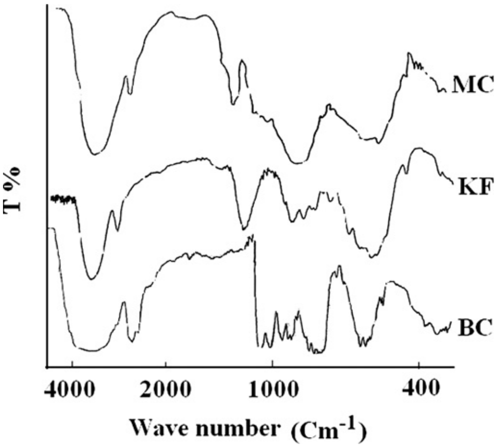


Fig. 1. FT-IR spectra of different cellulose samples.

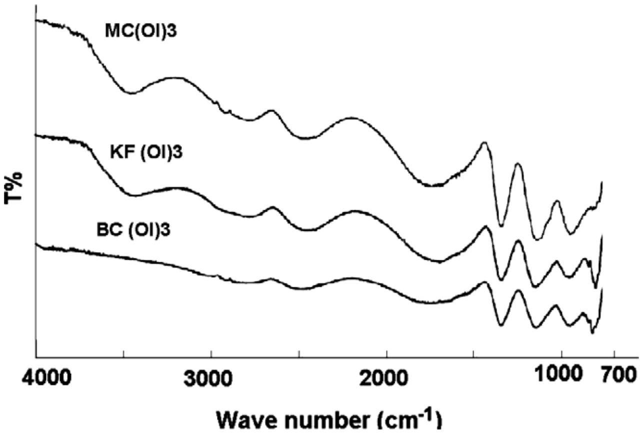


Fig. 2. FT-IR spectra of different cellulose hypoiodous samples.

line regions, respectively (Nada et al., 1990). The crystallinity index (CI) was calculated as the ratio of the absorbance of band at 1430 cm^{-1} to band at 900 cm^{-1}, which showed increase in crystallinity index of BC compared to KF and MC (Table 1). Also the shift of the maximum absorption band of stretching vibration of OH group of BC to lower wave number (3280 cm^{-1}) is more than the other two samples of cellulose (3479 cm^{-1} and 3387 cm^{-1} for KF and MC, respectively. This shift proves that BC is more crystalline than both samples. The reactivity of these samples of cellulose towards addition of a finely powdered mixture of potassium iodate and potassium iodide to a solution of cellulose in 85% phosphoric acid were studied. Among halogens, molecular iodine is the least powerful halogenating agent and organic iodinations are generally conducted in the presence of a strong oxidizing acid. Interestingly, iodinations carried out with iodine in the presence of HIO_3 have been ascribed to the species I_3 (Scheme 1). FT-IR spectra (Fig. 2) showed the absence of the OH group peak of BC and a weak absorbance in both KF and MC. This indicates that BC in phosphoric acid has the highest reactivity toward iodination reaction. Furthermore, all the spectra showed a strong absorption band at 530 cm^{-1} assigned to the stretching mode of I—O bond. From elemental analysis (Table 1), the triester hypoiodous BC has the highest iodine content compared to those of the other two samples; even BC has the highest degree of crystallinity. This may be attributed to the use of phosphoric acid that degrades the crystal structure of cellulose. So the crystallinity does not play an important factor as in the case of heterogeneous reactions (Keshk & Nada, 2003). Moreover the viscosity of BC is lower than that of KF, which reflects the lowest degree of polymerization of BC (Table 1). These results showed that the reactivity of BC can be attributed to the increased number of the end groups in BC chains due to its lower degree of polymerization than those of KF and MC. The selective oxidation of different cellulose samples yield a polymer with a degree of oxidation at C-6 of about 90% for BC, 84% for KF and 80% for MC (Scheme 1 and Table 1). The three native celluloses had various crystallinities index (Table 1), These values of each native cellulose sample were unchanged sharply after the acidic chloride oxidation; neither carboxylate nor aldehyde groups are probably formed inside cellulose I crystallites by the oxidation, and thus significant amounts of carboxylate and aldehyde groups are present only on the crystal surfaces and in disordered regions. FT-IR spectra of oxidized forms of different cellulose samples showed a broad absorption band at 3460 cm^{-1}, that confirms the stretching frequency of the —OH group (Fig. 3). In addition to the band at 2940 cm^{-1} that confirms C–H stretching vibration. The presence of a strong absorption band at 1590 cm^{-1} confirms the presence of COO— group. The bands around 1423 and 1325 cm^{-1} are assigned to —CH2 scissoring and —OH bending vibration, respectively. The band at 1061 cm^{-1} is due to $^{\cdot}CH_2$—O—CH_2 stretching. The ^{13}C NMR spectra of the oxidized and non-oxidized KF at different times (24 and 36 h) are shown in Fig. 4. In the spectrum of non-oxidized KF, the peak at 105 ppm is assigned to C-1 and the strong peak at 74 ppm to C-2, C-3 and C-5. Moreover, the two doublet peaks appeared at 84 ppm and at 65 ppm are corresponding to C-4 and C-6, respectively (Horri, Yamamoto, Kitamaru, Tanahashi, & Higuchi, 1987; Ek, Wormald, Ostelius, Iversen, & Nystrom, 1995). In the spectrum of oxidized KF, the lack of signal at 63 ppm and the appearance of signal at 171 ppm indicate that primary alcohol group is oxidized to yield carboxylic acid. Moreover, the cellulose

Table 1
Physicochemical characterization of different samples of cellulose

Samples	Viscosity	CI	COOH content	*I* (%)
BC	20.56	2.90	–	–
$BC(OI)_3$	2.76	1.09	–	90
BC—COOH	1.74	1.25	30.56	–
KF	109	1.30	–	–
$KF(OI)_3$	1.23	0.62	–	77
KF—COOH	1.24	1.23	25.45	–
MC	2.08	1.20	–	–
$MC(OI)_3$	1.27	0.65		72
MC—COOH	1.25	1.19	23.52	–

Crystallinity index have been calculated from FT-IR spectra.

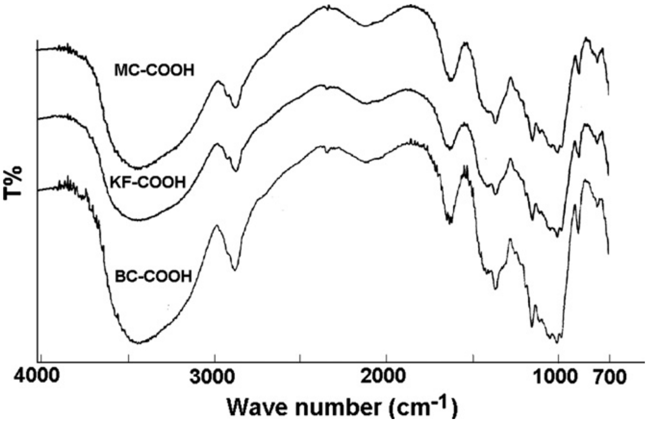


Fig. 3. FT-IR spectra of different oxidized cellulose samples.

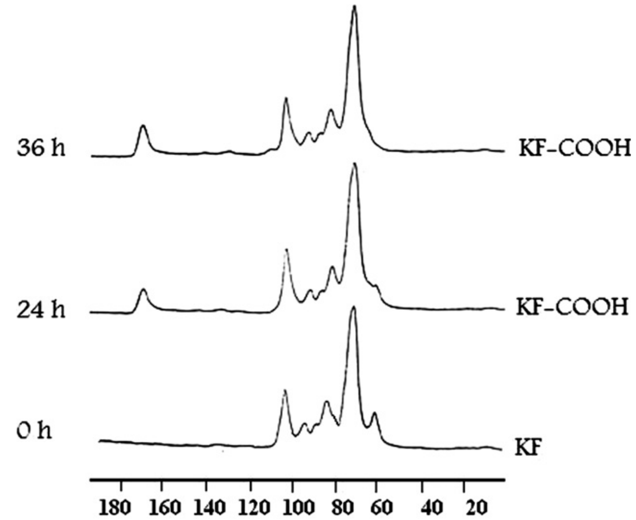


Fig. 4. Solid state ^{13}C NMR spectra of crystalline components in the cellulose and oxidized cellulose from kenaf.

structure was retained and practically no ring cleavage between C-2 and C-3 had occurred. From this result, $NaClO_2$/NaCl mixture is a good oxidizing regent for primary hydroxyl group of cellulose rather than other regents because of the need to reduction step after the oxidation. Moreover, it has been reported that chlorine bubbled into a cellulose solution in 85% phosphoric acid at ambient temperature do oxidize and degrade the polysaccharide to a very limited extent even after 40 h (Pagliaro, 1998). From all these results, BC is considered to be good for both heterogeneous and homogenous reactions than those of plant and wood cellulose.

4. Conclusion

The triester hypoiodous BC has the highest iodine content as well as the oxidation form has the highest carboxyl content compared to the other two samples. These results clearly demonstrated that the crystallinity of BC does not play an important factor in both heterogeneous and homogeneous reactions of cellulose. This can be attributed to the increased number of the end groups in BC chains compared to other samples.

Acknowledgements

I am grateful to Dr. Eji Togawa (Tsukuba University, Japan) for solid state ^{13}C NMR measurements and his generous support. I would like also to express my deep thanks to Prof. Dr. A. S. Hamad Elgazwy, Department of Chemistry, and Faculty of Science Ain Shams University for his valuable discussion.

References

Abbott, T., Palmer, D., Gordon, S., & Bagby, M. (1988). Solid state of plant polymers by FTIR. *Journal of Wood Chemistry Technology, 8*, 1–28.

Ek, R., Wormald, P., Ostelius, J., Iversen, T., & Nystrom, C. (1995). Crystallinity index of microcrystalline cellulose particles compressed into tablets. *International Journal of Pharmaceutics, 125*, 257–264.

Finn, M. D., Schow, S. R., & Schneiderman, E. D. (1992). Osseous regeneration in the presence of four common hemostatic agents. *Journal of Oral and Maxillofacial Surgery, 50*.

Green, J. (1980). In W. Pigman & D. Norton (Eds.), *The carbohydrates* (pp. 1106–1120). New York: Academic Press.

Horri, F., Yamamoto, H., Kitamaru, R., Tanahashi, M., & Higuchi, T. (1987). Transformation of native cellulose crystal induced by saturated steam high temperatures. *Macromolecules, 20*, 2946–2949.

Kai, A., & Keshk, S. A. M. S. (1998). Structure of nascent microbial cellulose I. *Polymer Journal, 30*, 996–1002.

Kai, A., & Keshk, S. (1999). Structure of nascent microbial cellulose II. *Polymer Journal, 31*, 61–65.

Keshk, S. (2002). *Gluconacetobacter xylinus*: A new resource for cellulose. *Egypt Journal of Biotechnology, 11*, 305–310.

Keshk, S., & Nada, A. (2003). Heterogeneous derivatization of bacterial and plant celluloses. *Biotechnology Bioscience Asia, 1*, 39–41.

Keshk, S., & Sameshima, K. (2005). Evaluation of different carbon sources for bacterial cellulose production. *African Journal of Biotechnology, 4*, 478–482.

Keshk, S., & Sameshima, K. (2006). The utilization of sugar cane molasses with/without the presence of lignosulfonate for the production of bacterial cellulose. *Applied Microbiology and Biotechnology, 71*, 291–296.

Keshk, S., Suwinarti, W., & Sameshima, K. (2006). Physicochemical characterization of different treatment sequences on kenaf bast fiber. *Carbohydrate Polymer, 65*(2), 202–205.

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

Masaoka, S., Ohe, T., & Sakota, N. (1993). Production of cellulose from glucose by *Acetobacter xylinum*. *Journal of Fermentation and Bioengineering, 75*, 18–22.

Nada, A., Shabaka, A., Yousef, A., & Nour, A. (1990). Infrared spectroscopic and dielectric studies of Swollen cellulose. *Journal of Applied Polymer Science, 40*, 731–739.

Pagliaro, M. (1998). Autocatalytic oxidations of primary hydroxyl groups of cellulose in phosphoric acid with halogen oxides. *Carbohydrate Research, 308*, 311–317.

Pagliaro, M. (1999). New iodination of cellulose in phosphoric acid. *Carbohydrate Research, 315*, 350–353.

Pollack, R. P., & Bouwsma, O. (1992). Application of oxidized regenerated cellulose in periodontal therapy. *Compendium, 13*, 888–892.

Schlufer, K., Schmauder, H., Dorn, S., & Heinze, T. (2006). Efficient homogeneous chemical modification of bacterial cellulose in the ionic liquid 1-*N*-butyl-3-methylimidazolium chloride. *Macromolecule Rapid Communication, 27*, 1670–1676.

TAPPI Test Method T 203 om-93 (1994). Alpha-, beta- and gamma-cellulose in pulp. TAPPI PRESS, Atlanta.

TAPPI Test Method T 230 om-89 (1994). Viscosity of pulp (capillary viscometer method). TAPPI PRESS, Atlanta.

Yoshinaga, F., Tonouchi, N., & Watanabe, K. (1997). Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material. *Bioscience Biotechnology Biochemistry, 61*, 219–222.