ELSEVIER

Polymer 41 (2000) 2033–2039

polymer

PVA containing chito-oligosaccharide side chain

Yong-Woo Cho[a], Sung-Soo Han[b], Sohk-Won Ko[a,*]

[a]*Department of Fiber and Polymer Science, Seoul National University, Seoul 151-742, South Korea*
[b]*School of Textiles, Yeungnam University, Kyongsan 712-749, South Korea*

Received 12 February 1999; received in revised form 26 April 1999; accepted 17 May 1999

Abstract

A chito-oligosaccharide (COS) with the number average degree of polymerization (DP_n) of 9 was immobilized on poly(vinyl alcohol) (PVA) by crosslinking with a bifunctional compound, *N*-methylolacrylamide (NMA). The reaction was composed of two successive steps, the reaction of COS with NMA in an acidic medium in the first step and the reaction of the COS–NMA with PVA in an alkaline medium in the second step. The reaction products were characterized by ^{1}H NMR, differential scanning calorimetry (DSC), and X-ray diffractometry. The relative crystallinity of PVA containing COS side chain (PC) was found to be lower than that of PVA due to the bulky and rigid COS backbones. The tensile modulus of PC film was lower than that of PVA film, but its tensile strength was higher. The antimicrobial activity of PC was excellent in spite of its low COS content. The PC is considered to be a good candidate for a new biomaterial having various functional properties of chitosan coupled with good mechanical properties of PVA.

Keywords: Poly(vinyl alcohol); Chito-oligosaccharide; *N*-methylolacrylamide

1. Introduction

Poly(vinyl alcohol) (PVA) is generally prepared by the saponification of poly(vinyl ester)s, such as poly(vinyl acetate) (PVAc). PVA fibers and films are known to possess high tensile and impact strength, high tensile modulus, and excellent resistance to alkali, oils and solvents [1,2]. Also, PVA has gained increasing attention in the biomedical field due to its bioinertness [3–5]. PVA hydrogels resemble organic tissue and have a high elastic modulus even though their water content is very high.

Chitosan is a β(1 → 4)-2-amino-2-deoxy-D-glucan which is manufactured by *N*-deacetylation of chitin. It shows good antimicrobial activity [6,7] and has biocompatibility for most tissues including skin and bone. It accelerates wound healing [8] and exhibits good blood compatibility and hemostatic activity [9]. It has been expected to be used in such industrial and biomedical applications as an antibacterial fiber, drug carrier, immobilizer of biological molecules, membrane, wound-healing agent, and artificial skin [10–12]. However, applications of chitosan fibers and films are limited mainly due to its poor mechanical properties such as brittleness and rigidity. Therefore, it is interesting to prepare a biomaterial having both good biocompatibility and mechanical properties.

For this reason, the blends of PVA/chitosan [13–15] (or chitin [16]) and cellulose/chitosan [17–20] (or chitin [21]) have been extensively studied. Since Miya et al. [13] reported that PVA and chitosan formed a clear homogeneous blend of which the tensile strength was greater than the sum of the component values, a PVA/chitosan blend has become a topic of great interest in the biomedical field. Nakatsuta and Andrady [14] studied the permeability and diffusion of vitamin B-12 through a PVA/chitosan blend membrane. Chandy and Sharma [15] studied immobilization of bioactive molecules on a PVA/chitosan blend membrane, which showed good permeability for small molecules and blood compatibility. Lee et al. [16] reported that PVA/β-chitin blends prepared using formic acid as a cosolvent showed improved mechanical properties in comparison with those of homopolymer. A cellulose/chitosan blend was studied by Hasegawa et al. [17–19]. A cellulose/chitosan blend film prepared by dissolution in chloral/dimethlyformamide showed improved mechanical properties and higher solute permeability. Yang and Ko [21] reported that a cellulose/chitin blend was prepared using dimethylacetamide/LiCl as a cosolvent and spun by wet spinning method. However, no study has been reported on the synthesis of biomaterials on which chitosan is linked through covalent bonding.

* Corresponding author. Tel: +82-2-880-7185; fax: +82-2-885-1748.
E-mail address: sohkwonk@gong.snu.ac.kr (S.-W. Ko)

0032-3861/00/$ - see front matter
PII: S0032-3861(99)00351-1

In this study we synthesized a new biopolymer containing chito-oligosaccharide (COS) side chain. Poly(vinyl acetate) (PVAc) was prepared by the bulk polymerization of vinyl acetate (VAc). The PVA was produced by the saponification of the PVAc. Chitosan was depolymerized by sodium nitrite. The COS was immobilized on PVA by crosslinking with the bifunctional compound, *N*-methylol acrylamide (NMA). The PVA containing the COS side chain (PC) was characterized by ^{1}H NMR, differential scanning calorimetry (DSC), and X-ray diffractometry, and its mechanical properties and antimicrobial activity were investigated.

2. Experimental

2.1. Deacetylation of chitosan [22,23]

A chitosan of an initial 83.9% degree of deacetylation (DD) was obtained from Kumho Chemicals Co., Ltd., Korea. The chitosan was treated with a 50% aqueous sodium hydroxide solution under N_2 flow for 2 h at 100°C. After cooling, the mixture was filtered and washed with water several times. The above procedure was repeated. The DD of chitosan was measured by the alkaline titration method.

2.2. Depolymerization of chitosan [24,25]

Two grams of chitosan was dissolved in 100 ml of a 2% aqueous acetic acid solution. Sodium nitrite (0.125 mol with respect to 1 mol of glucosamine), was slowly stirred into the chitosan solution for 3 h at room temperature. The reactant was poured into methanol. The precipitate was filtered, washed thoroughly with methanol, and dried under vacuum at 40°C. The molecular weight of COS was determined with a Waters liquid chromatograph (Waters Associates Milford, MA) with pullulan standard (column, Waters hydrogel linear, 30 cm × 2; eluent, 0.15 M CH_3COOH buffered with 0.5 M CH_3COONa; flow rate, 1 ml/min; temperature 30°C).

2.3. Reaction of COS with NMA

One gram of COS with DP_n of 9 was dissolved in 5 ml of a 4% aqueous acetic acid solution. NMA (0.5 mol with respect to 1 mol of glucosamine) and a small amount of polymerization inhibitor were stirred into the COS solution for 0.5 h at 70°C. The reactant was poured into acetone. The precipitate was filtered, washed with acetone, and dried under vacuum at 40°C.

2.4. Reaction of acrylamidomethyl chito-oligosaccharide (COS–NMA) with PVA

One gram of PVA was dissolved in 100 ml of water for 6 h at 95°C. The PVA solution was then slowly cooled down to room temperature. COS–NMA was dissolved in water for 2 h at room temperature. The COS–NMA solution and an aqueous sodium hydroxide solution were successively added to the PVA solution. Reaction mixtures were stirred for different reaction time at different temperatures. The reactant was neutralized with 1 M hydrochloric acid solution and poured into acetone. The precipitate was filtered, washed with water/acetone mixture and cold water. It was then dissolved in DMSO. Insoluble substances (unreacted COS–NMA) were removed by centrifuge. The solution was poured into acetone and its precipitate was filtered, washed with acetone, and dried under vacuum at 40°C.

2.5. Preparation of PVA and PC films

PVA was dissolved in water for 6 h at 95°C to prepare 3 g/dl solution. The homogeneous solution was poured into a stainless steel plate and dried in a drying oven for 1 day at 40°C. The obtained film was evacuated in a vacuum oven for 3 days at 40°C to remove the residual solvent inside the film. PC film was prepared by the same method.

2.6. Measurements

The ^{1}H and ^{13}C NMR spectra were obtained using an FT NMR spectrometer (UNITY 300, Varian).

X-ray diffraction patterns were recorded on a MXP-18 X-ray diffractometer (Mac Science) using Ni-filtered CuKα-radiation at 40 kV and 200 mA.

The thermal properties of products were measured using a differential scanning calorimeter (DSC7, Perkin–Elmer) with a heating rate of 20°C/min.

Tensile tests were carried out using a UTM (LR10K, Lloyd). The distance between the two grips was 5 cm and the crosshead speed was 5 cm/min. The tensile modulus was determined within 0.5% elongation. The tensile modulus and strength given were the average values of 10 samples.

AATCC Test Method 100-1993 was used for determining antimicrobial activity of the PC. In this method, a gram-positive bacterium, *Staphylococcus aureus* (American Type Culture Collection No. 6538), was used. One gram of sample was placed in 1 ml of a culture medium containing *Staphylococcus aureus* and then kept for 24 h at 37°C. The medium was diluted with 100 ml phosphate buffer solution (pH 7.2) and cultivated on an agar for 24 h at 37°C. The results were expressed as reduction in bacteria (%) by the following formula:

$$\text{Reduction in bacteria (\%)} = [(C - A)/C] \times 100$$

where A is the number of bacteria recovered from the test specimen and C, the number of bacteria recovered from the control (at "0" contact time).

3. Results and discussion

PVA was prepared by the saponification of PVAc. DP_n and DS of the PVA were 3300 and over 99%, respectively. The chitosan with DD 94.5% was produced by alkaline treatment. Sodium nitrite, which is able to perform

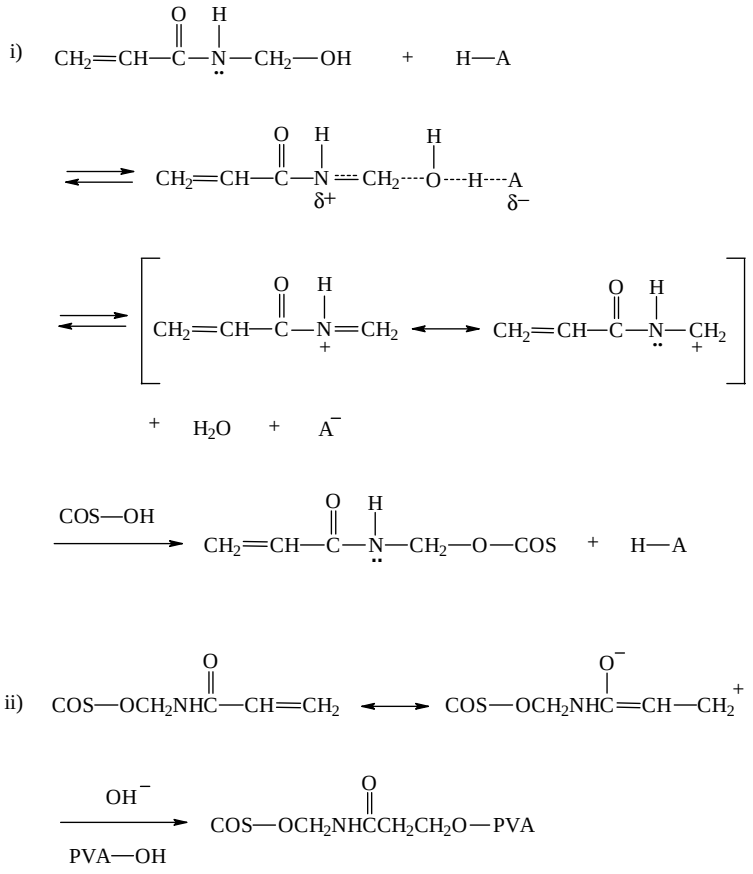

Fig. 1. Synthesis of PC.

depolymerization reaction under mild conditions, was used to depolymerize chitosan. Sodium nitrite and amino groups of chitosan react with 1:1 stoiochiometry according to the depolymerization mechanism [24,25]. The DP_n of COS was about 9.

The COS was immobilized on PVA using NMA (CH_2=CHCONHCH$_2$OH). The NMA with two different functional groups (*N*-methylol and vinyl groups) has widely used for crosslinking of cellulose. Numerous studies have been reported on the reactions of NMA with cellulose [26–30]. The reaction, as shown in Fig. 1, was composed of two successive steps. The first step is the reaction of COS with NMA in an acidic medium. *N*-methylol group of NMA reacts with hydroxyl group of COS under acidic condition. The transfer of the proton from the acid catalyst to the oxygen atom and elimination of hydroxyl group are

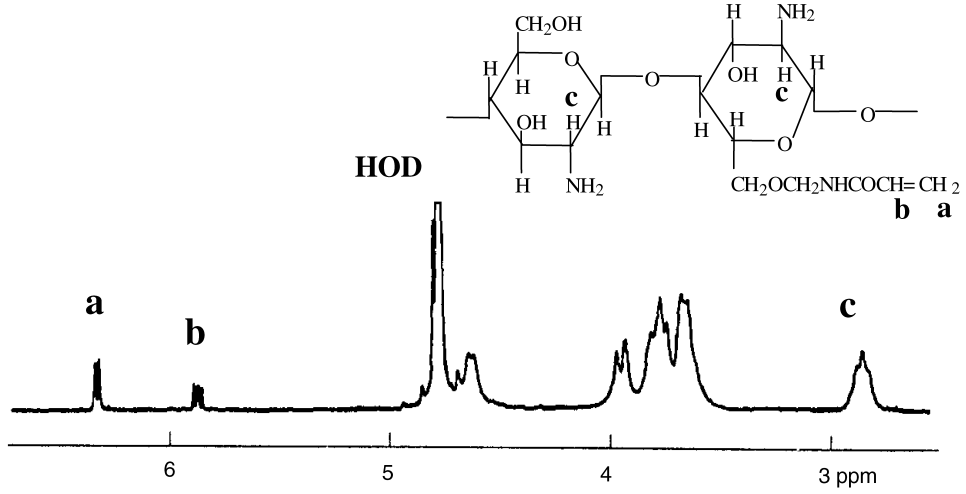

Fig. 2. ^{1}H NMR spectrum of COS–NMA.

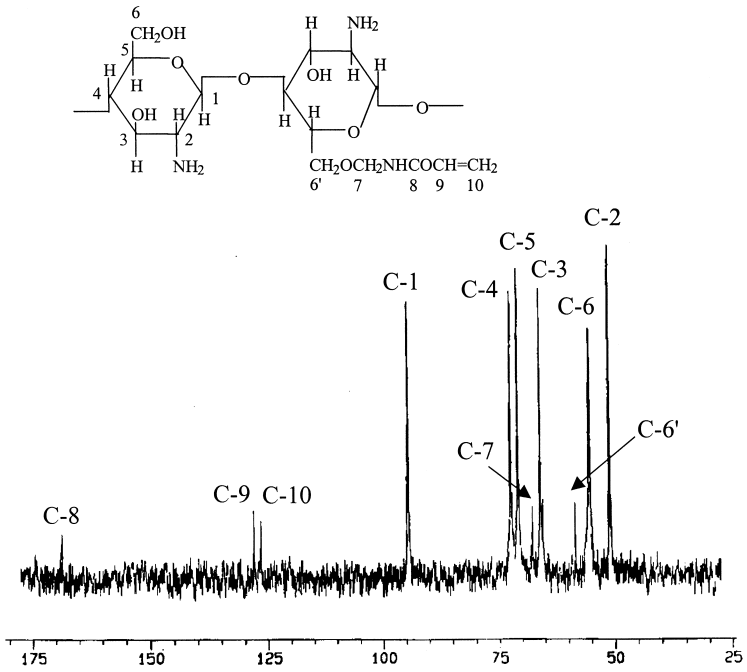

Fig. 3. ^{13}C NMR spectrum of COS–NMA.

favorable due to the mesomerically stabilized amidomethylene [carbonium–immonium] ion. Thus, the condensation reaction of *N*-methylol group of NMA with hydroxyl group is much more facile than other alcohol condensation reactions. Acetic acid is used not only as a catalyst in this reaction but also as a reagent, which transforms the amino groups of COS into ammonium salts. An ammonium salt has a positive charge and cannot act as a nucleophile. Therefore, the reaction between the NMA and hydroxyl group of COS is dominant. Fig. 2 shows the ^{1}H NMR spectrum of COS–NMA in D_2O [31]. The COS is insoluble to water, but the COS–NMA is soluble to water. The peaks of vinyl groups of COS–NMA are observed at about 5.8 and 6.3 ppm. The amount of NMA linked to COS was determined from spectral integration of proton signals at peaks a, b and c, showing that about 1.5 molecules of NMA was

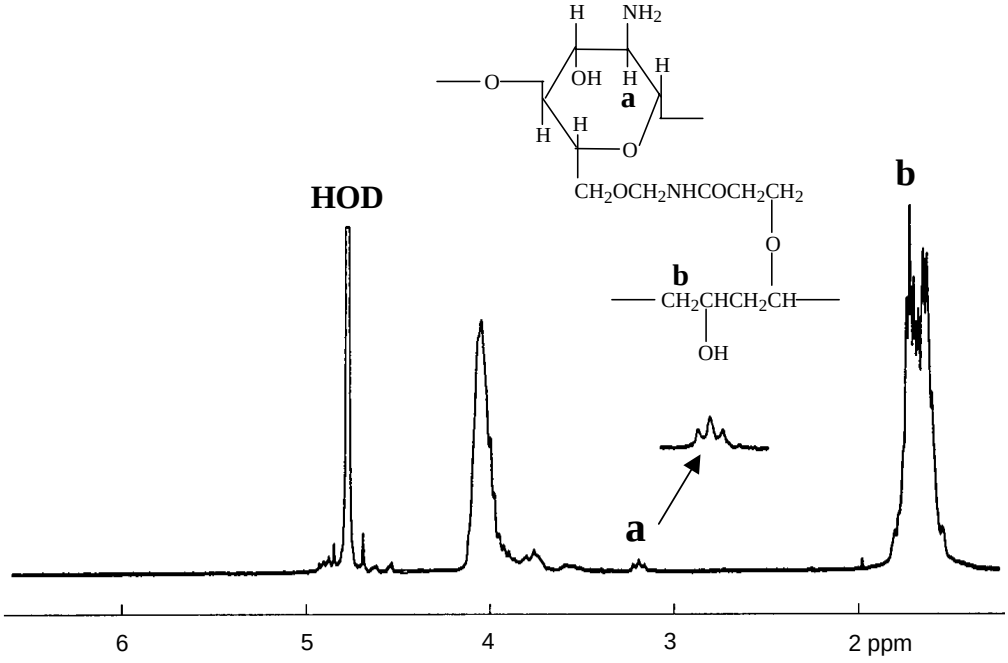

Fig. 4. ^{1}H NMR spectrum of PC (COS content 7.51%).

Table 1
Effect of reaction conditions on the COS content of PC. PVA/COS–NMA 0.85/0.15 (w/w)

PC	Time (h)	Temperature (°C)	NaOH concentration (mol/l H_2O)	COS content (wt%)
I	2	20	0.16	4.49
II	4	20	0.16	7.51
III	6	20	0.16	8.50
IV	8	20	0.16	8.23
V	6	15	0.16	4.20
VI	6	25	0.16	6.90
VII	6	30	0.16	3.50
VIII	6	20	0.08	4.49
IX	6	20	0.12	7.21
X	6	20	0.20	8.43

linked to each COS. In the ^{1}H NMR spectrum, the other proton signals except for a, b and c were not separated completely and difficult to interpret. Fig. 3 shows the ^{13}C NMR spectrum of COS–NMA. All carbon signals were separated completely and assigned [32,33]. Among these carbon signals, C-6′ and C-7 indicate the reaction between COS and NMA.

The second step is the reaction of COS–NMA with PVA in an alkaline medium. PVA undergoes a Michael addition reaction with compounds containing activated double bonds in an alkaline medium [34–36]. A double bond in conjugation with a carbonyl group is susceptible to nucleophilic attack. The ^{1}H NMR spectrum of PC is shown in Fig. 4. The peaks of the vinyl groups at 5.8 and 6.3 ppm in COS–NMA do not appear in the PC spectrum. The COS content was determined from spectral integration of proton signals at peaks a and b, representing wt% of COS in PC.

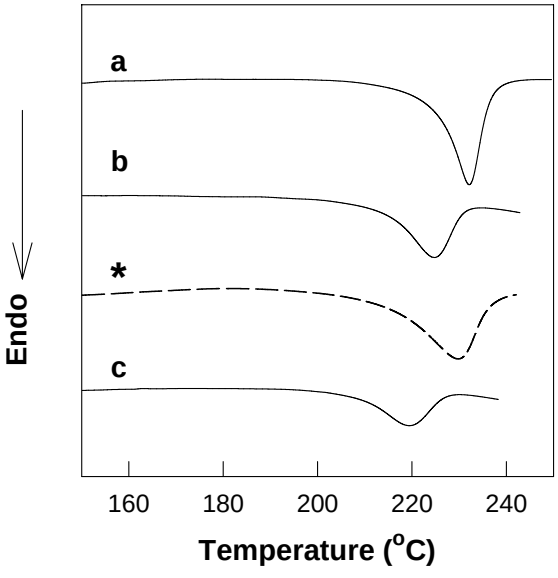

Fig. 5. DSC curves of PVA and PCs with different COS contents. (a) PVA; (b) COS content 4.49%; (c) COS content 7.51% (*) PVA/COS blend (weight ratio 92.49/7.51).

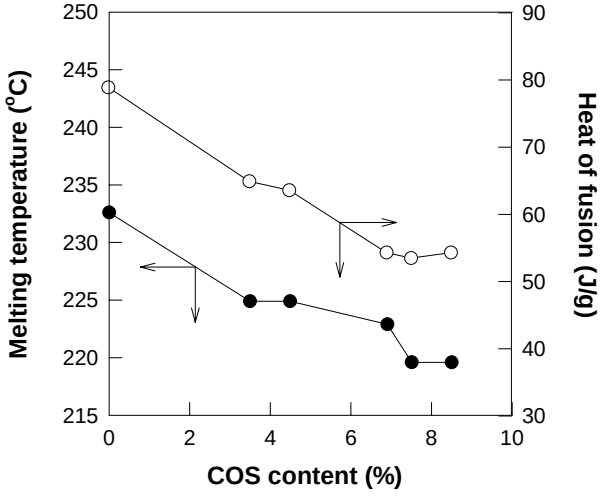

Fig. 6. Melting temperatures and heat of fusions of PVA and PCs with different COS contents.

Table 1 shows the effect of reaction conditions such as time, temperature, and sodium hydroxide concentration on the reaction of COS–NMA and PVA. It was observed that the reaction time more than 6 h had little effect on COS content and the accelerating effect of temperature on the addition reaction was more pronounced at 20°C. The sodium hydroxide concentration up to 0.16 mol/l H_2O increased the COS content significantly with a minor decrease in the COS content beyond that level. Therefore, increasing the reaction temperature and sodium hydroxide concentration beyond the optimum level may activate side reactions [36].

Thermal properties of the PC were examined by DSC. Fig. 5 shows the DSC curves of PVA and PC. In this figure, a thermogram of a PVA/COS blend (92.49/7.51) is also given for comparison with the data of PC. The PVA has a relatively large and sharp melting peak in comparison with that of the PC. With an increase in the COS content of PC, the endothermic peak of PC tends to broaden and shift to lower temperature. Fig. 6 shows the dependence of T_m and the heat of fusion of PC on the COS content, respectively. Heat of fusion was calculated from the thermogram area of the DSC curve. Both T_m and heat of fusion of PC became continuously lower as the COS content increased. The decrease in T_m and heat of fusion are generally caused by a reduction in the crystal thickness or the crystal perfection.

Wide-angle X-ray diffraction (WAXD) patterns of PVA and PC are shown in Fig. 7. The spectrum a shows a typical peak for PVA that appears at around $2\theta = 20°$. This peak is assigned to a mixture of (101) and (200) [37]. The PC peak intensity is lower and its shape is broader than that of PVA. Accordingly, we can see that the crystallinity and regularity of the crystalline region of PC is decreased. This is the same tendency as shown in the DSC thermogram.

PVA is a flexible chain crystalline polymer. The flexible PVA backbone is favorable for close molecular packing and crystallization. The decrease in the crystallinity of PC is due

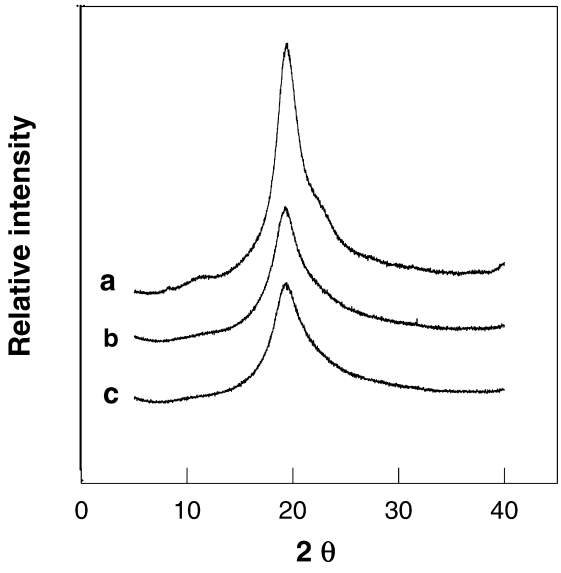

Fig. 7. X-ray diffractograms of PVA and PCs with different COS contents. (a) PVA; (b) COS content 4.49%; (c) COS content 7.51%.

to the COS side chain which can hinder and retard the crystallization of PC by its bulky and rigid structure. Therefore, the crystallinity and the regularity of the crystalline region of PC decreased with an increase of COS content.

The immobilized COS could produce some of the advantages of chitosan such as biocompatibility, antimicrobial activity, tissue repair property, hemostatic activity, and adsorption properties for metal ions. However, there is a possibility that the mechanical properties of PVA may be reduced.

PVA and PC films were prepared in order to investigate their mechanical properties. Fig. 8 shows tensile strengths and tensile moduli of the films. The tensile modulus of PC film was lower than that of PVA film due to its low crystallinity and decrease in regularity of its crystalline region. However, the tensile strength of PC film was higher than that of PVA film and it increased with an increase of COS

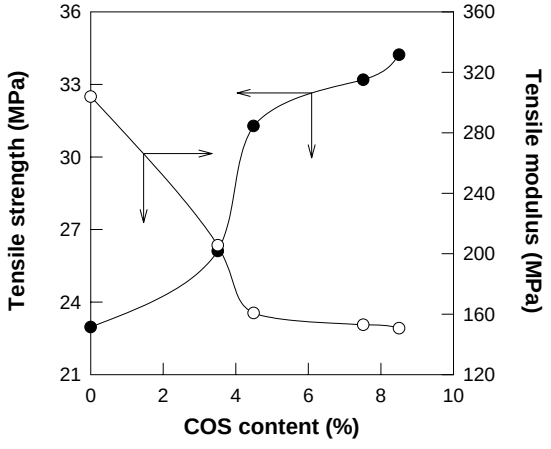

Fig. 8. Tensile strengths and moduli of PVA and PC films with different COS contents.

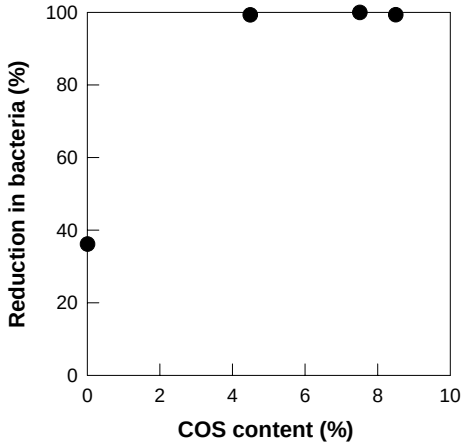

Fig. 9. Antimicrobial activity of PC.

content. Miya et al. [13] reported that the tensile strength of a PVA/chitosan blend was greater than the sum of the component values, suggesting that this enhancement of tensile strength should be caused by the hydrogen bonding between –OH and $-NH_2$ groups in chitosan and –OH groups in PVA. The enhancement of tensile strength of PC film may be due to intra- and inter-molecular interactions between PVA and COS in the PC similar to the blend system.

Chitosan is known as an antimicrobial polysaccharide probably due to the amino group in the C-2 position of the glucosamine residue. Fig. 9 shows the antimicrobial activity of PC as reduction in bacteria. The growth of bacteria was almost completely suppressed due to the COS immobilized on PVA. PC showed excellent antimicrobial activity. The enhanced antimicrobial property of PVA could be an important advantage in biomedical as well as fiber applications.

4. Conclusions

COS was immobilized onto PVA through two successive steps using NMA. The relative crystallinity of PC was lower than that of PVA due to the stiff molecular chain of COS immobilized on PVA. The tensile modulus of PC film was lower than that of PVA film due to its low crystallinity. However, the tensile strength of PC film was higher than that of PVA film, and it increased with increasing COS content. PC showed excellent antimirobial activity. The PC is considered to be a promising candidate for new biomaterial having various functional properties of chitosan coupled with good mechanical properties of PVA.

Acknowledgements

The authors wish to acknowledge the financial support of the Korea Research Foundation made in the program year of 1998.

References

[1] Sakurada I. Polyvinyl alcohol fibers, New York: Marcel Dekker, 1985 p. 3–9, 361–86.

[2] Masuda M. Recent advances in polyvinyl alcohol films. In: Finch CA, editor. Polyvinyl alcohol—developments, New York: Wiley, 1991. p. 403–22.

[3] Cholakis CH, Zingg W, Sefton MV. Effect of heparine–PVA hydrogel on platelets in a chronic canine arterio-venous shunt. J Biomed Mater Res 1989;23:417–41.

[4] Horiike S, Matsuzawa S. Application of syndiotacticity-rich PVA hydrogels to drug delivery system. J Appl Polym Sci 1995; 58:1335–40.

[5] Llanos GR, Sefton MV. Immobilization of poly(ethylene glycol) onto a poly(vinyl alcohol) hydrogel. 1. Synthesis and characterization. Macromolecules 1991;24:6065–72.

[6] Allan CR, Hadwiger LA. The fungicidal effect of chitosan on fungi of varying cell wall composition. Exp Mycol 1979;3:285–7.

[7] Kendra DF, Hadwiger LA. Characterization of the smallest chitosan oligomer that is maximally antifungal to Fusarium solani and elicits pisatin formation in Pisum sativum. Exp Mycol 1984;8:276–81.

[8] Okamoto Y, Shibazaki K, Minami S, Matsuhashi A, Tanioka S, Shigemasa Y. Evaluation of chitin and chitosan on open wound healing in dogs. J Vet Med Sci 1995;57:851–4.

[9] Rao SB, Sharma CP. Use of chitosan as a biomaterial: Studies on its safety and hemostatic potential. J Biomed Mater Res 1997;34:21–28.

[10] Seo H, Mitsuhashi K, Tanibe H. Antibacterial and antifungal fiber blended by chitosan. In: Brine CJ, Sandford PA, Zikakis JP, editors. Advances in chitin and chitosan, London: Elsevier Science, 1992. p. 34–40.

[11] Quchi T, Banba T, Fugimoto M, Hamamoto S. Synthesis and antitumor activity of chitosan carrying 5-fluorouracils. Makromol Chem 1989;190:1817–25.

[12] Peter MG. Application and environmental aspects of chitin and chitosan. J Macromol Sci: Pure Appl Chem A 1995;32:629–40.

[13] Miya M, Iwamoto R, Mima S. FT-IR study of intermolecular interactions in polymer blends. J Polym Sci: Polym Phys Ed 1984;22:1149–51.

[14] Nakatsuka S, Andrady AL. Permeability of vitamin B-12 in chitosan membranes. Effect of crosslinking and blending with poly(vinyl alcohol) on permeability. J Appl Polym Sci 1992;44:17–28.

[15] Chandy T, Sharma CP. Prostaglandin E_1-immobilized poly(vinyl alcohol)-blended chitosan membranes: blood compatibility and permeability properties. J Appl Polym Sci 1992;44:2145–56.

[16] Lee YM, Kim SH, Kim SJ. Preparation and characteristics of β-chitin and poly(vinyl alcohol) blend. Polymer 1996;37:5897–905.

[17] Hasegawa M, Isogai A, Kuga S, Onabe F. Preparation of cellulose–chitosan blend film chloral/dimethylformamide. Polymer 1994; 35:983–7.

[18] Hasegawa M, Isogai A, Onabe F, Usuda M. Dissolving state of cellulose and chitosan in trifluoroacetic acid. J Appl Polym Sci 1992;45:1857–63.

[19] Hasegawa M, Isogai A, Onabe F, Usuda M, Atalla RH. Characterization of cellulose–chitosan blend films. J Appl Polym Sci 1992;45:1873–9.

[20] Lim HH, Nam CW, Ko SW. Spinning and properties of cellulose/chitosan blend fibers. J Korean Fiber Soc 1997;34:444–50.

[21] Yang NC, Ko SW. Spinning and properties of cellulose/chitin blend fibers. J Korean Fiber Soc 1997;34:10–15.

[22] Mima S, Miya M, Iwamoto R, Yoshikawa S. Highly deacetylated chitosan and its properties. J Appl Polym Sci 1983;28:1909–17.

[23] Sannan T, Kurita K, Iwakura Y. Studies on chitin 2. Effect of deacetylation on solubility. Makromol Chem 1976;177:3589–600.

[24] Hirano S, Kondo Y, Fujii K. Preparation of acetylated derivatives of modified chito-oligosaccharides by the depolymerization of partially *N*-acetylated chitosan with nitrous acid. Carbohydr Res 1985; 144:338–41.

[25] Horton D, Philips KD. The nitrous acid deamination of glycosides and acetates of 2-amino-2-deoxy-D-glucose. Carbohydr Res 1973;30: 367–74.

[26] Petersen H. In situ formation of polymers. In: Mark H, Wooding NS, Atlas SM, editors. Chemical aftertreatment of textiles, New York: Wiley, 1971. p. 174–7.

[27] Gardon JL. A quantative study of crosslinking cotton with *N*-methylol acrylamide. J Appl Polym Sci 1961;5:734–51.

[28] Kamel M, Hebeish A, Allan M, Al-Aref A. Creation of reactive centers on cotton. II. J Appl Polym Sci 1973;17:2725–38.

[29] Kamel M, Hebeish A, Al-Aref A. Creation of reactive centers on cotton: Part V. Simultaneous dyeing and finishing of acrylamidomethylated cotton fabrics. Textile Res J 1975;45:131–5.

[30] Ibrahim NA, Abo-Shosha MH. Novel cation-exchange composites resulting from polymerization/cross-linking of acrylic acid/*N*-methylolacrylamide mixtures with cellulose. J Appl Polym Sci 1993;49:291–8.

[31] Kubota N, Eguchi Y. Facile preparation of water-soluble *N*-acetylated chitosan and molecular weight dependence of its water-solubility. Polym J 1997;29:123–7.

[32] Martinou A, Bouriotis V, Stokke BT, Varum KM. Mode of action of chitin deacetylase from Mucor rouxii on partially *N*-acetylated chitosans. Carbohydr Res 1998;311:71–8.

[33] Guibal E, Milot C, Eterradossi O, Gauffier C, Domard A. Study of molybdate ion sorption on chitosan gel beads by different spectrometric analyses. Int J Biol Macromol 1999;24:49–59.

[34] Fessenden RJ, Fessenden JS. Organic chemistry, 4. Belmont, CA: Brooks-Cole, 1990 p. 719–721.

[35] Feit BA, Zilkha A. Kinetics and mechanism of cyanoethylation of alcohols. J Org Chem 1963;28:406–10.

[36] Hashem MM, Bach E, Kesting W, Hebeish AA, Schollmeyer E. Synthesis of chemically bonded poly(vinyl alcohol)–starch composite. Angew Makromol Chem 1995;230:189–204.

[37] Nishio Y, Manley RS. Cellulose/poly(vinyl alcohol) blends prepared from solutions in *N,N*-dimethylacetamide lithium chloride, Macromolecules 1988;21:1270–7.