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Polymer 43 (2002) 1541–1547

polymer

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Polymer Communication

Study on antibacterial *O*-carboxymethylated chitosan/cellulose blend film from LiCl/*N*, *N*-dimethylacetamide solution

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Received 28 August 2001; received in revised form 19 October 2001; accepted 19 October 2001

Abstract

Two types of *O*-carboxymethylated chitosan (*O*-CMCh)/cellulose polyblends were prepared by mixing cellulose LiCl/*N*,*N*-dimethylacetamide (DMAc) solution with *O*-CMCh aqueous solution (I) or DMAc emulsion (II) and their corresponding films (I and II) were regenerated in water. The (*O*-CMCh)/cellulose films obtained were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and wide-angle X-ray-scattering (WAXS). FTIR analyses showed that amino groups of *O*-CMCh were not affected during the film formation. SEM observations indicated that the *O*-CMCh/cellulose polyblend displayed a heterogeneous microstructure. *O*-CMCh microdomains dispersed in the cellulose matrix of the blend film. Blend film I showed a better dispersion of the *O*-CMCh microdomains than blend film II did. DSC and WAXS analyses suggested that, for both two kinds of the blend films, the addition of *O*-CMCh did not significantly influence the crystallinity and thermal properties of cellulose. The antibacterial activity of the films against *Escherichia coli* (*E. coli*) was also measured via optical density method. Both blend films I and II exhibited satisfying antibacterial activity against *E. coli*, even the *O*-CMCh concentration was only 2 wt%. Due to the coagulation effect of water on the polyblend, *O*-CMCh water solution is suitable for the preparation of the blend film with low *O*-CMCh concentration, while *O*-CMCh DMAc emulsion should be selected when high *O*-CMCh concentration is needed.

Keywords: Cellulose; *O*-carboxymethylated chitosan; Blend film

1. Introduction

Chitosan, a copolymer of glucosamine and *N*-acetylglucosamine units linked by 1-4 glucosidic bonds, is obtained by *N*-deacetylation of chitin, which is the second most naturally occurring biopolymer (after cellulose) [1]. Chitosan is a biocompatible polymer reported to exhibit a great variety of useful biological properties such as anticholesteremic [2] and ionsequestering actions [3]. Recently, the antibacterial and antifungal activities of chitosan have been followed with great interest. Chitosan inhibits the growth of a wide variety of bacteria and fungi [4–11] showing broad spectra of antibacterial activity, high killing rate and low toxicity toward mammalian cells [12,13]. Chitosan ($pK_a = 6.8$), however, exhibits its antibacterial activity only in an acidic medium because of its poor solubility above pH 6.5. Thus, water-soluble chitosan derivatives soluble to both acidic and basic physiologic circumstances may be good candidates for the polycationic biocide. When chitosan is changed into *O*-carboxymethylated chitosan (*O*-CMCh) by introducing $-CH_2COOH$ onto $-OH$ along the chitosan molecular chain, its antibacterial activity becomes much stronger [11]. Moreover, since *O*-CMCh can be dissolved in a wide pH scale ranging from 3 to 11 [14], it has a much broader application as an antibacterial agent than chitosan does.

Because of the antibacterial activity and little skin reaction over a wide range of biomedical investigation, chitosan and its derivatives have been used in the antibacterial next-to-skin fabrics. Cellulose fiber is people's favorite undergarment materials due to the safety to human body and the comfort resulted from its high moisture-retentivity. The molecular structures of cellulose, chitosan and *O*-CMCh are very similar (c.f. Fig. 1), which is expected to give high compatibility between cellulose and chitosan or *O*-CMCh, so blending cellulose and chitosan or *O*-CMCh is expected to be a useful method to introduce antibacterial activity into cellulose fiber.

An antibacterial fiber CHITOPOLY (Fuji Tex. Japan) is a blend fiber composed of polynosic and chitosan microparticles with a mean size less than 5 μm which were blended in ripening viscose via a mechanical blend method. Some research activities on its antimicrobial abilities,

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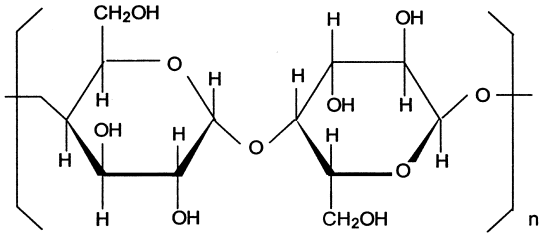
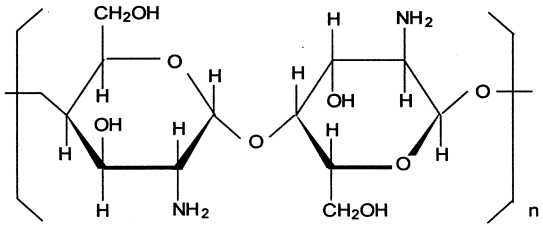
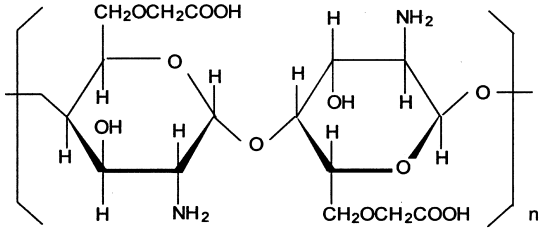
0032-3861/02/$ - see front matter
PII: S0032-3861(01)00699-1



(a) Cellulose



(b) Chitosan



(c) O-CMCh

Fig. 1. Structures of cellulose, chitosan and *O*-CMCh.

biological degradability, mechanical properties and applications have been reported [7,15–17]. In our previous study [18], a chitosan/*N*,*O*-CMCh/viscose rayon hybrid fiber was prepared. This fiber exhibits excellent antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, and the good moisture-retentivity of viscose rayon was not affected by the addition of chitosan.

Though the viscose process is a widely used solution-spinning process of cellulose fiber, where the carbon disulfide applied is very harmful and hydrogen sulfide generated during the process as a byproduct is a notorious air pollutant. Therefore, several promising solvent systems such as LiCl/*N*,*N*-dimethylacetamide (DMAc), dimethylformamide, and *N*-methylmorpholine *N*-oxide (NMMO)/H_2O have been utilized and corresponding fibers were developed [19–23].

In order to introduce the latest environment friendly cellulose spinning progress into the manufacture of antibacterial cellulose-based fiber, and to search a suitable formula of the spinning solution, two types of *O*-CMCh/cellulose polyblends were prepared by using *O*-CMCh aqueous solution (I) and DMAc emulsion (II) in this paper. The corresponding blend films were formed and characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and wide-angle X-ray-scattering (WAXS). The antibacterial activities of the films against *E. coli* were also studied.

2. Experimental

2.1. Materials

Chitosan (molecular weight 1.08×10^6; the degree of deacetylation 0.85), provided by Qingdao Medicine Institue, China, was depolymerized via γ irradiation degradation to a lower molecular weight of 2.0×10^5. Cellulose was obtained from Tianjin Rayon Factory (Tianjin, China). Its viscosity-average degree of polymerization (DP) was 612, which was determined in cupriethylene diamine solution at 25 °C [24]. *E. coli* (8099) was provided by School of Biology Science, NanKai University, Tianjin, China, and stored at 4 °C.

2.2. Preparation and characterization of O-CMCh

To synthesize *O*-CMCh, 15 g chitosan and 9 g monochloroacetic acid were suspended in 150 ml sodium hydroxide solution (42%, by weight). The system reacted at 0 °C for 48 h and then the pH was adjusted to 1.0 with hydrochloric acid. After filtration, the solid product was washed with methanol for two times. The *O*-CMCh yielded was dried in an oven at 60 °C. The degree of substitution determined by pH titration was 0.86 [25].

O-CMCh was characterized by FTIR with a Nicolet 560 E.S.P. spectrometer. After being dried completely at 50 °C, *O*-CMCh powders were mixed with paraffin paste and FTIR spectra were recorded.

2.3. Preparation of cellulose solution

Ten grams of cellulose was pulverized and dipped in 200 ml DMAc, heated to 150 °C and stirred for 30 min. Then the cellulose was filtered and dissolved in LiCl/DMAc solution (LiCl concentration is 9 wt%) under stirring.

2.4. Preparation of blend films

Blend films I and II were prepared as follows:

Blend film I. 10 g *O*-CMCh powder was dissolved in 90 g de-ionized water. A certain amount of *O*-CMCh water solution was drip fed to cellulose solution under violent stirring. Then the mixture was spread on a glass plate while its films

were regenerated at ambient temperature in de-ionized water. Finally, the films were dried at 50 °C in an oven.

Blend film II. The pH of the *O*-CMCh water solution was adjusted to 14 with 1 M NaOH under vigorous agitation to evolve *O*-CMCh, and the *O*-CMCh water emulsion was obtained. This water emulsion was washed with DMAc for several times so that water could be replaced by DMAc. The *O*-CMCh water emulsion was thus changed into *O*-CMCh DMAc emulsion. Since that almost no *O*-CMCh was lost in this process, the concentration of *O*-CMCh in the DMAc emulsion could be calculated. Like *O*-CMCh water solution, a certain amount of the DMAc emulsion was also blended with cellulose solution and then films were regenerated in water according to the similar method described earlier.

2.5. Characterization

The blend films I and II with *O*-CMCh concentration of 6 wt% were chosen as the representative samples for the following measurements.

FTIR spectra of the blend films were obtained with a Nicolet 560 E.S.P. spectrometer. After being dried completely at 50 °C, the samples could be used for FTIR analysis.

SEM observations were made on the films. Films were coated with a small amount of gold and examined with a Philips SL-30 SEM.

A differential scanning calorimeter (DSC), Model Perkin–Elmer DSC7, was used. Each sample (5–10 mg) was run at a scanning rate of 5 °C/min under nitrogen atmosphere. The temperature for the first scan ranged from 20 to 160 °C. Then the samples were quenched and scanned the second time with the temperature ranging from −50 to 300 °C.

WAXS experiments were performed for the films by a D/max-2500 X-ray diffractometer. The X-ray source was Ni-filterd Cu Kα radiation (25 kV, 10 mA). Samples were scanned from 5 to 40° 2θ at a scanning rate of 4° 2θ/min.

2.6. Antibacterial assessment

Antibacterial activity of the blend films against *E. coli* was evaluated by using the optical density method described as follows. A representative bacteria colony was picked off, placed in a nutrient broth (peptone 10 g, beef extract 3 g, NaCl 3 g in distilled water 1000 ml; pH 7.0) and incubated at 37 °C for 24 h. Then the obtained fresh culture where bacteria cells grew luxuriantly was ready for antibacterial test. 0.2 ml of the fresh culture was inoculated into the medium (9.8 ml) containing cellulose film (reference) or blend film (0.1 g) and incubated in a shaking bed (150 rpm) at 37 °C for 24 h. During incubation, turbidity of the medium was measured at 610 nm for six times with a spectrophotometer (756MC UV–Vis, Shanghai, China).

3. Results and discussion

3.1. FTIR analysis

FTIR spectra of the samples are shown in Fig. 2. The FTIR spectrum of cellulose film (reference) shows ca. 1375 cm^{-1} peak contributing to the C–O–H bond. The adsorption of hydroxyl group is the wide adsorption band observed ranging from ca. 3400 to 3230 cm^{-1}. For *O*-CMCh, peaks at ca. 1616 and 1506 cm^{-1} are identified as the adsorption of $-NH_3^+$, and ca. 1732 cm^{-1} peak is attributed to C=O in carboxy groups of *O*-CMCh. The IR spectra of blend film I and blend film II are almost identical and very similar to that of cellulose. Weak peaks at ca. 1768 and 1751 cm^{-1} (c.f. Fig. 2c and d) are assigned to the C=O of *O*-CMCh contained in the blend films. The adsorption of C=O shifted to a higher frequency indicating that there was some interaction between *O*-CMCh and cellulose in blend films which improved the compatibility between them. $-NH_3^+$ peaks at ca. 1616 and 1506 cm^{-1} were also found in spectra of blend films I and II, illustrating that the amino groups of *O*-CMCh were not affected during the blend formation. Since the antibacterial activities of chitosan and *O*-CMCh are resulted from the $-NH_3^+$ on the molecular chain [11], the existence of $-NH_3^+$ ensured films to exhibit antibacterial activity.

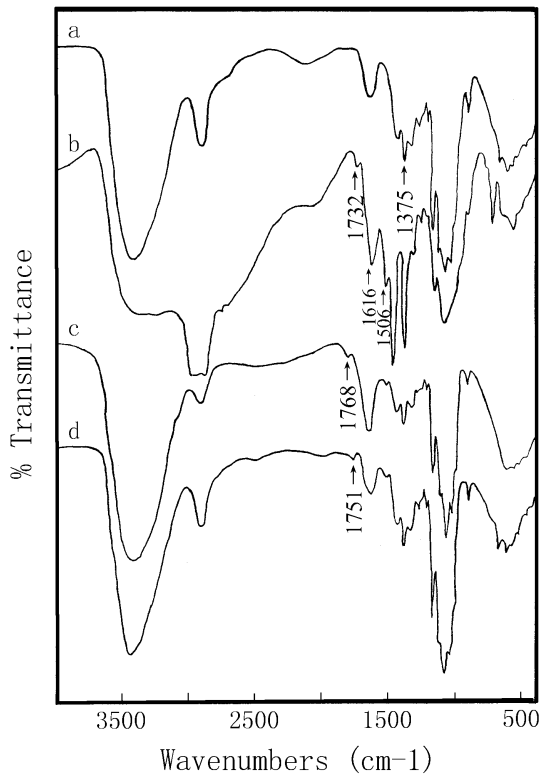


Fig. 2. FTIR spectra of cellulose film (a), *O*-CMCh (b), blend film I (c), and blend film II (d).

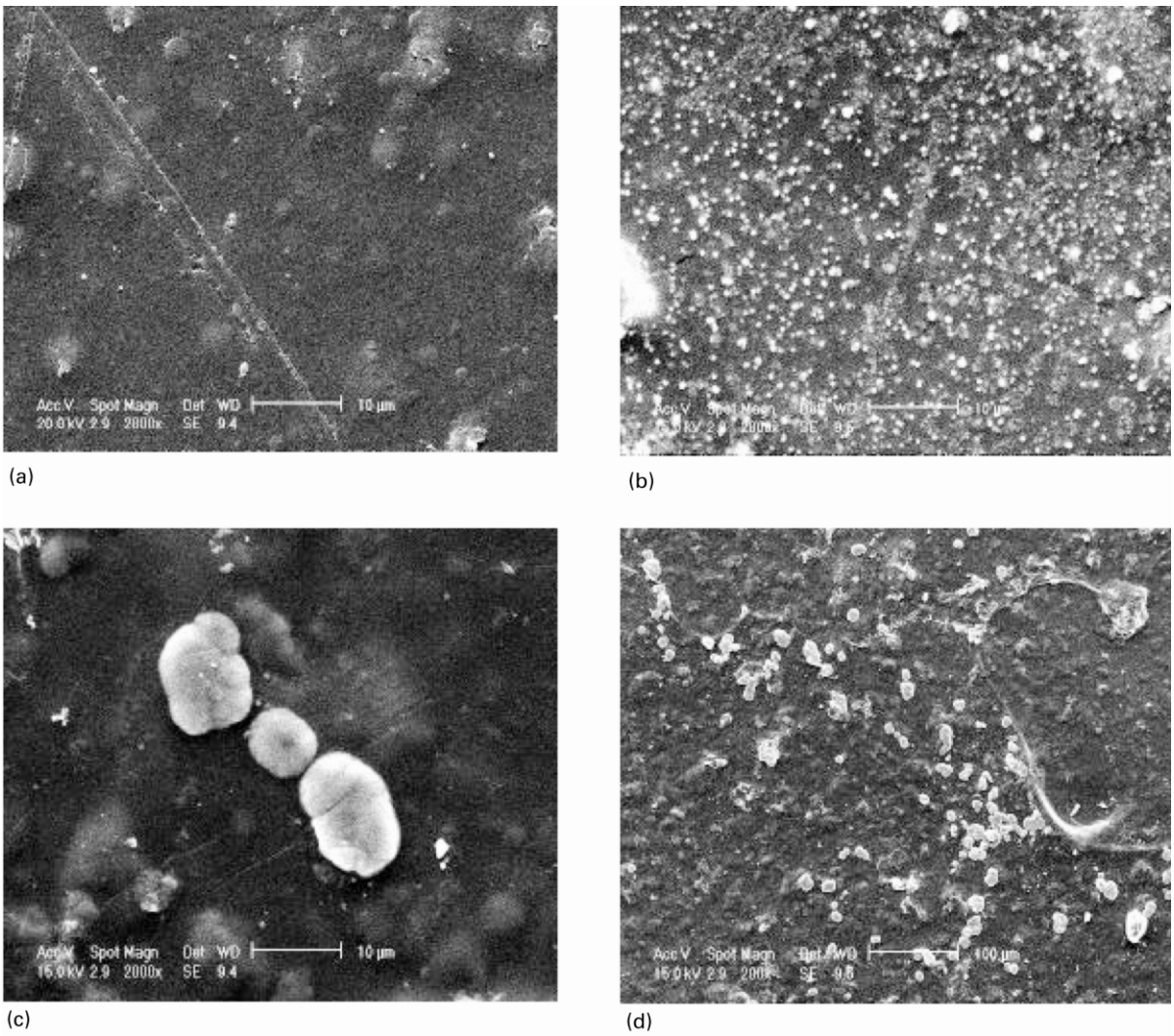


Fig. 3. SEM photographs of cellulose film (a), blend film I (b), and blend film II (c,d).

3.2. SEM observation

SEM microphotographs of the surfaces of cellulose film (reference) and blend films are shown in Fig. 3. Fig. 3a displays the surface of cellulose film, which is relatively flat. Unlike cellulose film, there are heterogeneous surfaces for blend films. One can see microphase separation on the surface, i.e. *O*-CMCh microdomains dispersed within cellulose matrix. For blend film I, the sizes of microdomains are less than 1 μm (c.f. Fig. 3b), in comparison with that of blend film II being 6–10 μm (c.f. Fig. 3c and d).

From the SEM analyses, a conclusion can be drawn that, for the above *O*-CMCh/cellulose blend films, blend film I shows a better degree of dispersion of the *O*-CMCh microparticles than blend film II does. This is because that the dispersion degree depends on the *O*-CMCh dispersion system before being added to cellulose solution. In *O*-CMCh aqueous solution, *O*-CMCh disperses at molecule level, while in DMAc emulsion *O*-CMCh exists as microaggregations. This difference leads to the varying dispersion degree in the blend films. According to SEM analyses, the *O*-CMCh aqueous solution is superior to the DMAc emulsion for the polyblend preparation. But this hypothesis acts only when the *O*-CMCh concentration in the polyblend is low. Since water is a coagulating agent for the cellulose LiCl/DMAc solution, the addition of a large amount of water may affect the rheology properties of the cellulose solution and even result in coagulation. Therefore, *O*-CMCh DMAc emulsion would be an appropriate choice when high *O*-CMCh concentration is needed. Generally speaking, it is acceptable to blend 20 ± 5 ml *O*-CMCh aqueous solution into 1 l cellulose solution. If the *O*-CMCh aqueous solution needed is greater than this ratio, *O*-CMCh DMAc emulsion should be a suitable selection.

3.3. DSC analysis

The thermal transitions of cellulose film (reference) and

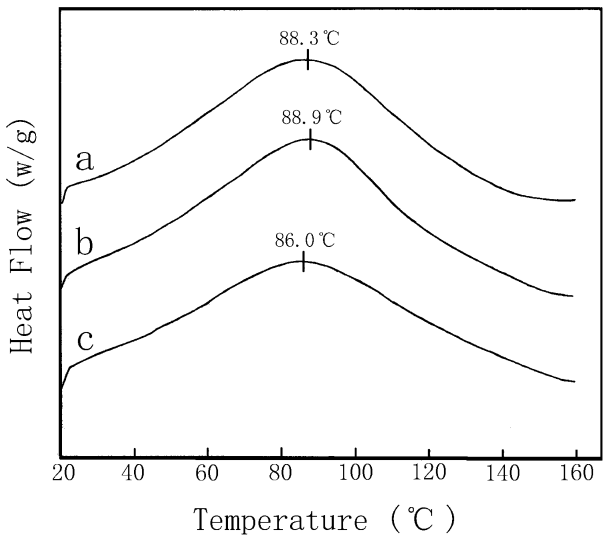


Fig. 4. DSC spectra of the first scan of cellulose film (a), blend film I (b), and blend film II (c).

blend films were determined by DSC analysis. For the first scan, the temperature was raised from 20 to 160 °C (Fig. 4), ensuring the samples did not decompose. Due to a certain amount of free water contained in the samples, all spectra give a significant transition at about 90 °C. The identity of this transition peak implies the good moisture-retentivity of cellulose was not affected by the addition of *O*-CMCh.

After the first scan, the free water was eliminated and the thermal transitions of each film could be seen clearly in the spectra of the second scan (Fig. 5). The three spectra in Fig. 5 are almost the same, indicating that there was no significant change in thermal property caused by adding *O*-CMCh into cellulose. For cellulose reference specimen, a transition is observed at 169.2 °C (Fig. 5a), so do the blend films I and II with *O*-CMCh concentration of 6 wt% (Fig. 5b and c). This similarity reveals the good compatibility between *O*-CMCh and cellulose in the blend films. When the temperature reached 250 °C, all the three films decomposed seriously as the temperature increased. What is more, there isn't any significant difference among the decomposition peak temperatures of the three films. Compared with cellulose film, both blend films I and II didn't exhibit obvious change in thermal stability.

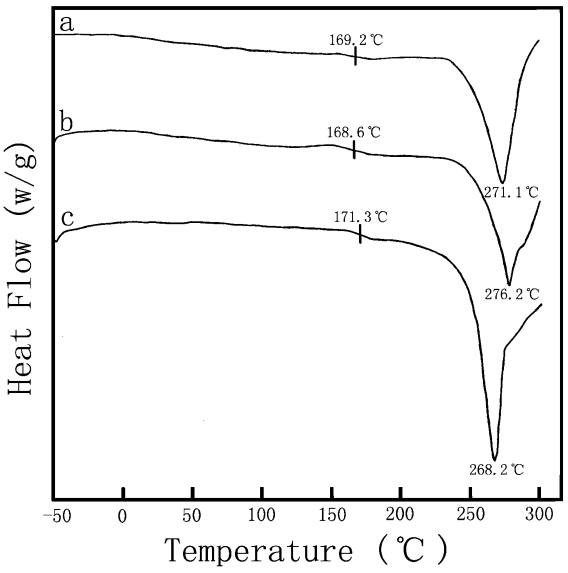


Fig. 5. DSC spectra of the second scan of cellulose film (a), blend film I (b), and blend film II (c).

3.4. Wide-angle X-ray analysis

X-ray scattering patterns of *O*-CMCh, cellulose film and blend films are shown in Fig. 6. There is a strong peak in the diffractogram of *O*-CMCh powder at 2θ being 31.7° (c.f. Fig. 6a) indicating the high degree of crystallinity of *O*-CMCh. Regenerated cellulose fiber and film like viscose rayon or mercerized natural cellulose generally show a diffraction pattern for cellulose II at $2\theta = 12°$ for (101), 20° for ($10\bar{1}$), and 21.7° for (002) [26]. Fig. 6c and d, the diffractograms of blend film I and blend film II, show nearly no difference from that of cellulose one (Fig. 6b). This may prove that the small amount of *O*-CMCh existing does not influence the crystallinity of cellulose. No peak is found at around 31.7° in the diffractograms of blend films. This

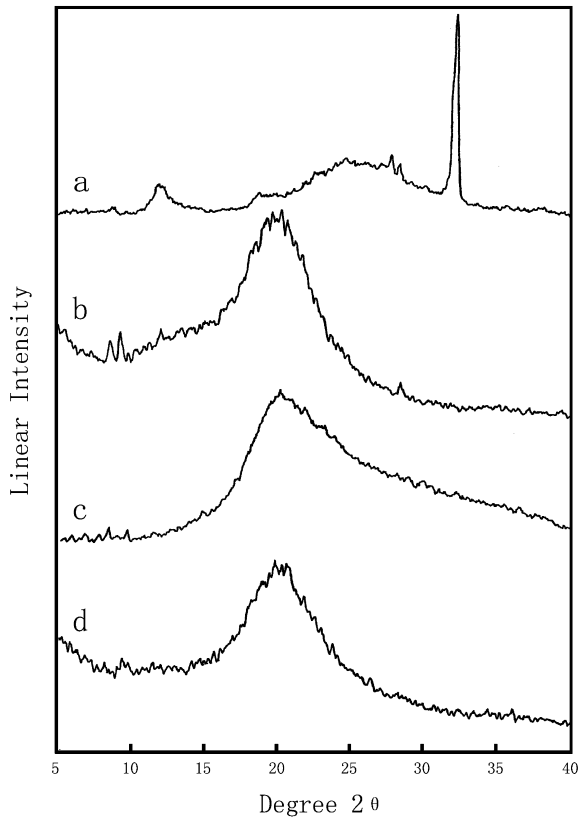


Fig. 6. WAXS diffractograms of *O*-CMCh (a), cellulose film (b), blend film I (c), and blend film II (d).

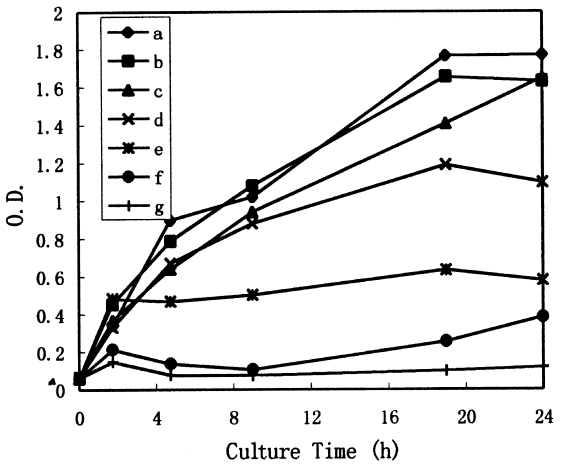


Fig. 7. OD versus culture time of medium (a), cellulose film (b), and blend film I with different *O*-CMCh concentrations (c–g) against *E. coli* (*O*-CMCh concentrations in c–g are 0.5, 1, 2, 3, and 6%, respectively).

indicates that the *O*-CMCh does not form its own crystalline region in polyblends and maintains amorphous state during film formation.

3.5. Antibacterial assessment

Figs. 7 and 8 demonstrate curves of optical density (OD) versus culture time for the blend film I and blend film II against *E. coli*, respectively. Because the bacterial cell is opaque, the medium became turbid as the bacteria propagated. Therefore, the optical density can be used as a criterion measuring the antibacterial activity of the films. The smaller the OD of the medium, the higher was the antibacterial activity of the film.

According to Figs. 7 and 8, both blend film I and blend film II exhibit significant antibacterial activity. Compared to the pure medium and cellulose film, OD of blend films are much lower. Moreover, with an increase in concentration of *O*-CMCh in the film, OD values decrease accordingly. In other words, the antibacterial activity of blend films may enhance if the *O*-CMCh concentration is raised. From Figs. 7 and 8, one can see that both blend film I and blend film II provide satisfying antibacterial activity, even the *O*-CMCh concentration was only 2 wt%.

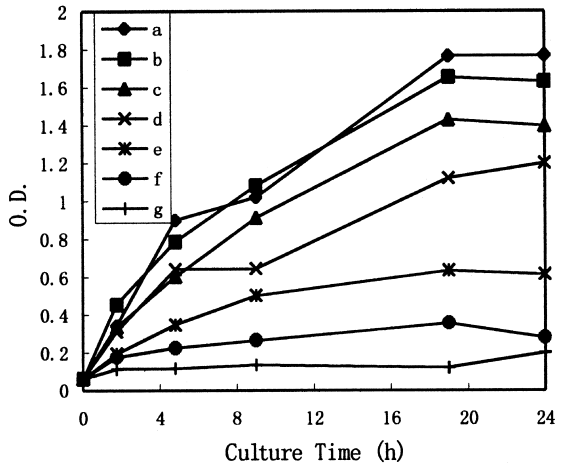


Fig. 8. OD versus culture time of medium (a), cellulose film (b), and blend film II with different *O*-CMCh concentrations (c–g) against *E. coli* (*O*-CMCh concentrations in c–g are 0.5, 1, 2, 3, and 6%, respectively).

4. Conclusions

In this paper, two types of *O*-CMCh/cellulose blend films were prepared by blending cellulose LiCl/DMAc solution with *O*-CMCh water solution or DMAc emulsion and regenerated in water. Amino groups of *O*-CMCh from which the antibacterial activity resulted were not affected during polyblend formation. The *O*-CMCh/cellulose polyblend was characterized by a heterogeneous microstructure. *O*-CMCh microdomains dispersed in the cellulose matrix of the blend film. Blend film I, which was made from *O*-CMCh water solution, showed a better dispersion of the *O*-CMCh microparticles than blend film II prepared from *O*-CMCh DMAc emulsion. For both two kinds of the blend films, the addition of *O*-CMCh didn't influence the crystallinity and thermal properties of cellulose significantly. Moreover, both blend films I and II exhibit satisfying antibacterial activity against *E. coli*, even the *O*-CMCh concentration was only 2 wt%. Because of the coagulation effect of water on the polyblend, *O*-CMCh water solution is suitable for the preparation of the blend film with low *O*-CMCh concentration, while *O*-CMCh DMAc emulsion should be chosen when high *O*-CMCh concentration is needed.

Acknowledgements

The authors are grateful for the financial support of the National Nature Science Foundation of China through the grant 50173019.

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