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Preparation and properties of chitosan derivative/poly(vinyl alcohol) blend film crosslinked with glutaraldehyde

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ABSTRACT

Novel crosslinked blend films of N-(2-hydroxy)propyl-3-trimethylammonium chitosan chloride (HTCC) and poly(vinyl alcohol) (PVA) were prepared by mixing their aqueous solutions followed by crosslinking with glutaraldehyde (GA). The effects of the GA content and ratio of HTCC to PVA on the appearance, swelling, gel content, morphology and antibacterial activities of blend films were studied and a possible mechanism of crosslinking was proposed based on the results. It was found that the equilibrium degree of swelling (ESD) increased with the increasing content of HTCC, but decreased with the content of GA. The ESD was a maximum (212%) when the ratio of HTCC/PVA/GA was 60/40/2 (wt). Antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* of the blend films were weakened slightly by crosslinking, but still showed substantial antibacterial activity. These results demonstrate that, not only the PVA, but also the HTCC reacted with GA. It was the amino groups on HTCC that was un-substituted by quaternary ammonium salt, which reacted with the aldehyde groups on GA.

1. Introduction

Chitosan, an aminopolysaccharide, is composed of β,1-4 glucosidic bonds. Due to its unique polycationic nature, chitosan and its derivatives have been proposed for various applications in biomedical, food, agricultural, biotechnological and pharmaceutical fields (Arvanitoyannis, Nakayama, & Aiba, 1998; Cardile et al., 2008; Feng & Huang, 1997; Wang, Turhan, & Gunasekaran, 2004). The antibacterial activity of chitosan and its antibacterial mechanism have been researched extensively. The six main antibacterial mechanisms have been proposed as follow: (1) interactions between the positively charged moieties on the chitosan molecules and the negatively charged ones on the microbial cell outer membranes leads to changes in the cell membrane structure and permeability inducing the leakage of proteinaceous and other intracellular constituents; (2) chitosan acts as a chelating agent that selectively binds trace metals and subsequently inhibits the production of toxins and microbial growth; (3) chitosan activates several defense processes in the host tissue, acting as a water binding agent and inhibits various enzymes; (4) low molecular weight chitosan penetrates the cytosol of the microorganisms and, through the binding of chitosan with DNA, and results in the interference with the synthesis of mRNA and proteins; (5) chitosan, on the surface of the cell, can form an impermeable polymeric layer which alters the cell permeability and prevents nutrients from entering the cell; and (6) finally, chitosan can adsorb the electronegative substances in the cell and flocculate them, it disturbs the physiological activities of the microorganism leading to their death (Muzzarelli et al., 2000; Vallapa et al., 2011).

In general, chitosan of high molecular weight can only be dissolved in acid solution. Solutions made from chitosan in dilute acid solution often need a repeated washing process to neutralize the acid, which restricts its applications thus the chemical modification of chitosan to improve its water-solubility is desirable. A series of water soluble compounds have been synthesized, hydroxyethylated, N-carboxymethylated, N-alkylated, oxidated, degradation of chitosan N, O-sulfated, oxygen inorganic acid esterified and quaternary ammonium cationizated chitosan derivatives (Chen, Wang, Liu, & Park, 2002; Ji et al., 2009; Ma et al., 2007). N-[(2-hydroxy-3-trimethyl-ammonium)-propyl] chitosan chloride (HTCC) could be prepared by reacting chitosan with glycidyl trimethylammonium chloride (GTMAC). The introduction of N-trimethylated quaternary ammonium salt group would greatly weaken the hydrogen bonds between chitosan molecule chains, and improve the water-solubility, antibacterial activity, moisture absorption and retention capabilities of chitosan. In addition, the reagents used in the prepa-

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ration of HTCC were of low cost, and the processing was relatively simple. This method has been widely used in the production of chitosan derivatives (Chiellini, Cinelli, Chiellini, & Imam, 2004; Jia, shen, & Xu, 2001; Kim, Choi, Chun, & Choi, 1997; Qin et al., 2004).

Poly(vinyl alcohol) (PVA) is one kind of hydrophilic semi-crystalline polymer. It has been widely used in biomedical applications because of its non-toxicity, biocompatibility, excellent chemical resistance and mechanical strength (Peppas & Wright, 1998).

Up to now, much work has been done on the preparation and study of chitosan/chitosan derivatives and PVA blend. Arvanitoyannis, Kolokuris, Nakayama, Yamamoto, and Aiba (1997) have prepared the blend of chitosan/PVA plasticized with sorbitol and sucrose for using as a food packaging material, and studied the effect of plasticizer on the structure, thermal, mechanical property and CO_2 permeability and also reviewed the preparation, physical properties, and potential as food packaging materials of this kind of blend based on natural and synthetic macromolecules (Arvanitoyannis, 1999). Some studies have focused on the applying of a series of crosslinking methods, e.g. irradiation, freeze-thawing and chemical methods. These studies include the combination of two crosslinking methods to prepare the hydrogel of PVA and chitosan, and the results showed that hydrogels made by irradiation followed by freeze-thawing show larger swelling capacity and mechanical strength, higher thermal stability, lower water evaporation rate, and are less turbid than those made by pure freeze-thawing and freeze-thawing followed by irradiation. Hydrogels made by irradiation alone cannot be used as wound dressing due to their poor mechanical strength. (Shen, Ruan, & Ga, 2009; Yang, Liu, Chen, Yu, & Zhu, 2008). The chemical crosslinking method has the advantages of improving the mechanical strength, thermal stability, keeping the intrinsic opaque appearance and swelling capacity (Bai, Xie, & Zhu, 2007). Most of studies focused on the effect of chemical crosslinking on the mechanical, thermal and swelling properties of PVA/chitosan blend, but the changes in antibacterial activity as a result of crosslinking have been seldom studied. Although some studies had demonstrated that the amino groups on chitosan might react to the aldehyde groups on GA through the FTIR spectrum, the changing of the absorption bands of N–H and characteristic peak of other groups were not so obvious, and there was no other evidence to support this mechanism (Milosavljević et al., 2009).

Our research aims at prepare the HTCC and HTCC/PVA blend films crosslinked by glutaraldehyde (GA), investigate the swelling properties, degree of crosslinking and antibacterial activities, and explore the crosslinking mechanism.

2. Experiments

2.1. Materials

Chitosan (K-03) used in this work was provided by FUNAKOSHI Co. (Japan). The viscosity-average molecular weight ($\overline{M}_v$) was 35,000 and the degree of deacetylation was 90%. PVA was purchased from Sinopharm Chemical Reagent Co., Ltd. (China) with a degree of polymerization of 1750 ± 50. GA was prepared in the lab. Analytical-grade sodium hydroxide (NaOH), acetic acid (CH_3COOH), isopropanol and GA aqueous (25 wt%) were used without further purification. Nutrient broth and agar culture medium (Hangzhou Microbial Reagent Co., Ltd., China), agar powder (Sinopharm Chemical Reagent Co., Ltd., China), meat-extract (Dainippon Pharmaceutical Co., Ltd., Japan) and polypeptone (Wako Pure Chemical Industries, Ltd., Japan) were used for the antibacterial activity test.

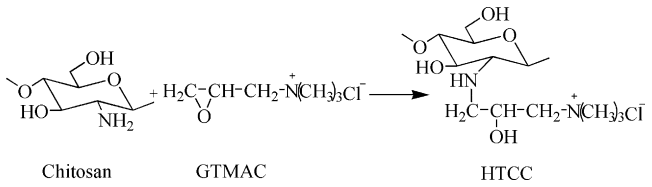

Fig. 1. Synthetic scheme of HTCC.

2.2. Sample preparation and measurement

2.2.1. Preparation of HTCC

Chitosan was dissolved in a 2 wt% acetic acid solution, and then the solution was adjusted with NaOH solution (0.1 mol/L) to pH = 9, kept for 6 h to allow sedimentation. The precipitate was then filtered out and dissolved in isopropanol, and heated to 60 °C under a nitrogen atmosphere. GTMAC was then added into the solution, and stirred for 6 h at 80 °C. After that the clear viscous solution was cooled to ambient temperature and deposited in isopropanol, then the filtered out precipitate was washed three times by isopropanol and dried at 80 °C. The synthetic scheme was represented in Fig. 1. Hydrogen atoms of $-NH_2$ on chitosan were substituted by the quaternary ammonium salt group, and the proportion of hydrogen atom being substituted was expressed by the degree of substitution (DS).

To measure the DS of HTCC, a certain quality of HTCC was dissolved in deionized water, and Cl^- in solution was titrated with silver nitrate solution using potassium chromate as the indicator. DS of the quaternary ammonium salt groups was calculated as following:

$$\mathrm{DS}\% = \frac{VM}{VM + (W - VM \times 314)/161} \quad (1)$$

where W is the weight of HTCC in grams, V (mL) and M (mol/L) are the volume and concentration of silver nitrate solution used for titration, respectively. The numbers 314 and 161 corresponded to the molecular weight of the repeat structural unit of HTCC and CS. When the hydrogen atoms of $-NH_2$ on chitosan were fully substituted, the DS of HTCC was 200%, since there were two hydrogen atoms on each amino group which could be substituted by the quaternary ammonium salt groups. And the measured DS of HTCC was 124%, which indicates that 76% of the hydrogen atoms of $-NH_2$ on chitosan were not substituted.

2.2.2. Preparation of sample films

PVA pellets were added into deionized water (PVA/water = 1/10 in weight) and dissolved under continuous stirring at 100 °C. Then the HTCC was added into the PVA solution, and an absolutely clear mixture was obtained for a while. After that the diluted GA aqueous was added into the flask, and the mixture was stirred for 15 min at 70 °C. Then the solution was cooled down and poured into the dish, dried at ambient temperature to form the crosslinked PVA–HTCC film. Pure PVA films and HTCC films crosslinked with GA were also prepared with the same method.

2.2.3. Measurement of the swelling properties of the films

The swelling properties of PVA–HTCC blend films in buffer solutions (pH = 7.4) at 37 °C were studied. The swelling degree (SD) and equilibrium swelling degree (ESD) of films with different contents of HTCC and GA were determined gravimetrically. Dried films of appropriate size were weighed and then immersed in NaH_2PO_4–Na_2HPO_4 buffer solutions, and NaOH solutions were used to adjust pH to be 7.4. Sodium chloride was used to adjust the ionic strength of the solutions to be 0.15 mol/L. To ensure complete equilibration, the samples were allowed to swell for