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Preparation and Blood Compatibility of Oxidized-chitosan Films

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Abstract: Chitosan membrane was modified by the selective oxidization of chitosan molecules on its surface with NO_2 gas. FTIR spectra indicated there were plenty of –COOH and $–COO^-$ groups on the modified membrane surface. The SEM study showed the modified membrane surface was rough rather than smooth as chitosan membrane. All antithrombosis test, hemolysis test and blood cell morphology observation with SEM revealed that modified chitosan membranes have superior blood compatibility to chitosan.

Keywords: Chitosan, blood compatibility, modified chitosan membrane, nitrogen dioxide.

Chitosan, a (1→4)-linked 2-amino-2-deoxy-β-D-glucan, is prepared by N-deacetylation of chitin, which is the main structural component of crab and shrimp shells. Due to its biocompatibility and biodegradability[1], chitosan has been developed for a variety of biomedical applications including wound dressings and drug delivery systems[2, 3], *etc*. However, chitosan, having common characteristics of a polycation electrolyte, could easily adsorb erythrocytes and thrombocytes that carried negative charges on their surfaces to form thrombus or cause hemolysis and had poor blood compatibility[4]. So it is essential to improve its biocompatibility with human tissues. S. Hirano[5] ***et al.*** have synthesized polysulphate chitosan derivatives and determined its antithrombin activity and have made wet spun chitosan-collagen blend fibers for improving blood compatibility of chitosan fibers[6]. T. Chandy[7] found that the chitosan, cross-linked with heparin using glutaraldehyde as crosslinking agent, had well blood compatibility. Our previous paper[8] reported that the hydromethyl group of 2-amino-2-deoxy-D-glucose unit in chitosan could be selectively oxidized to carboxyl group with NO_2 gas to form 6-carboxy chitosan, which was expected to improve its compatibility. However, our preliminary studies indicated both polysulphate chitosan derivatives and 6-carboxy chitosan have lower intrinsic viscosities and poor abilities of forming fibers or films.

In the present paper, chitosan and modified chitosan membranes were obtainded as follows. Casting chitosan/acetic acid (0.5 mol/L) solution onto a glass plate to form a uniform thin film, the membrane was removed from the plate by soaking in 0.1 mol/L phosphate buffer (pH=7.4) after solvent evaporation. After washing with water and then with ethanol, drying at room temperature, finally chitosan membrane with about

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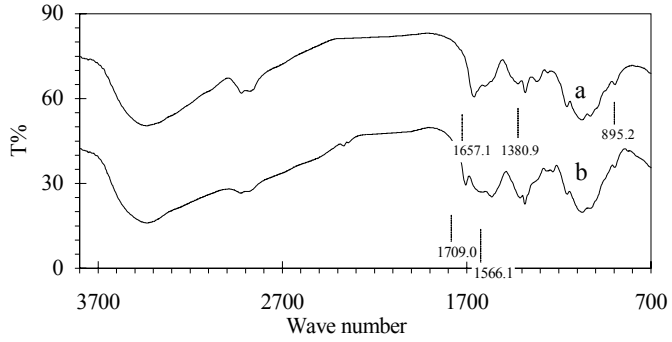
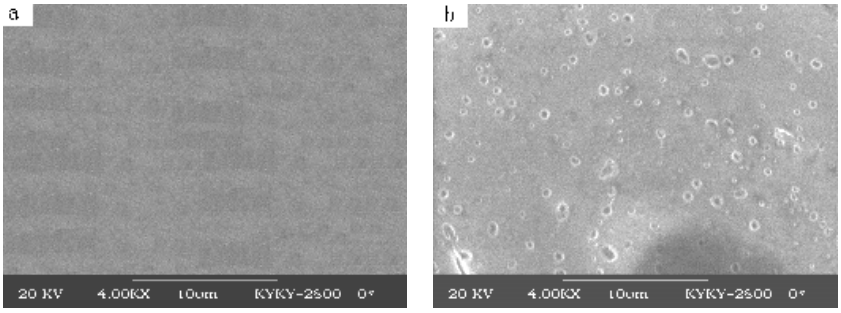
Figure 1 The FTIR spectra of chitosan film and modified chitosan film



a: chitosan film; b: modified chitosan film (exposure to NO_2 gas for 36.0 h)

Figure 2 The SEM pictures of the films

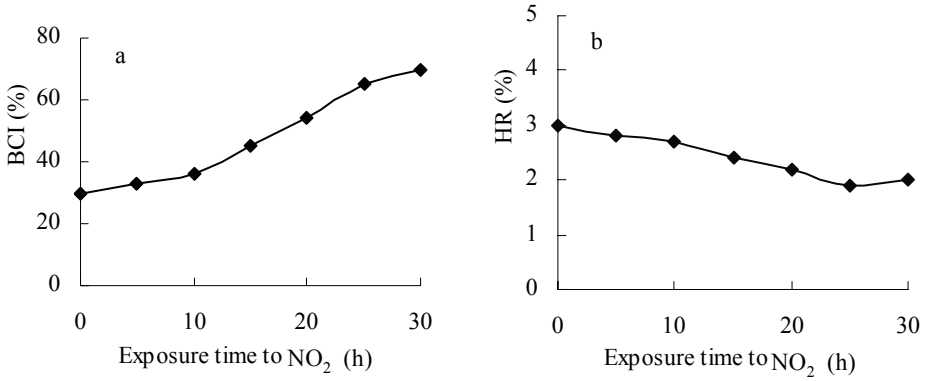


a: chitosan film; b: modified chitosan film (exposure toNO_2 gas for 24.0 h)

0.15 mm thin was obtained. And the modified films were obtained by exposing chitosan films whose surfaces were moistened to NO_2 atmosphere at room temperature for some times. During the exposure process, only the hydromethyl groups of 2-amino-2-deoxy-D-glucose units in chitosan on the surface were selectively oxidized to carboxyl groups with NO_2 gas to form 6-carboxy chitosan, which did not greatly decrease the tensile strength of the film.

The FTIR spectra were recorded with a SHIMADZU FTIR-8900 infrared spectrophotometer using KBr pellet method. As shown in **Figure 1**, the FTIR spectrum reveals that the modified chitosan film (**Figure 1 b**) had a new carbonyl ν-C=O at 1709.0 cm^{-1} comparing with chitosan (**Figure 1 a**). Since a part of –COOH and $–NH_2$ groups in the same molecule converted into $–COO^-$ and $–NH_3^+$ groups, a new ν_{as}-COO^- at 1566.1 cm^{-1} was observed too, which indicated that a portion of hydromethyl groups on the surface of chitosan film have truly been oxidized to carboxyl groups.

Figure 3 Effects of the chitosan films with different degree of oxidation on BCI (a) and HR (b)



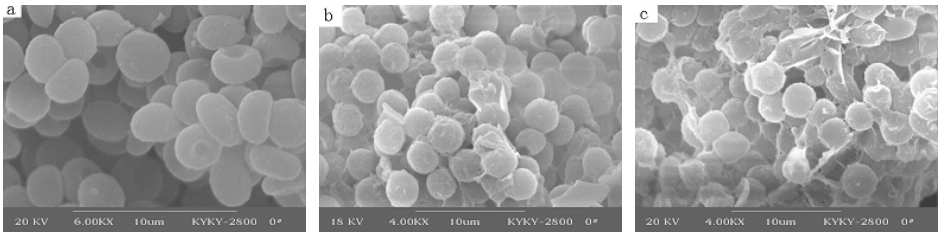
The morphology of the films was evaluated by scanning electron microscopy using a KYKY-2800 (china) instrument. As shown in **Figure 2,** the modified film surface was more rough (**Figure 2 b**) than chitosan (**Figure 2 a**). With increasing the exposure time to NO_2, the speckles on the surface of the modified film increased and the tensile strength of the film decreased. The reason was that NO_2 gas could easily dissolve in the tiny water-drops, which was produced in the oxidation process on the film surface, to form nitric acid so that chitosan molecules might dissolve in nitric acid, and it caused the surface or inside of the film was oxidized easily.

Both antithrombotic property and hemolytic property of the films were evaluated by anticoagulantic index (BCI) and hemolytic ratio (HR) according to E. Imai's method[9] using anticoagulanted sheep blood, respectively. As to modified chitosan films, the more exposure time to NO_2 gas they took, the higher degree the films were oxidized. The relationship between BCI or HR and the degree of oxidization of modified film could be demonstrated by the BCI or HR variety with the exposure times to NO_2 gas and were shown in **Figure 3.** The BCI value increased as the degree of oxidization of the films was raised (**Figure 3 a**). The reason was follows. When the modified films contacted with blood (pH= 7.35~7.45), the –COOH groups on the membrane surface converted into $–COO^-$ groups. Thus the plenty of negative charges on the membrane surface might prevent blood cells to load on it because of the electrostatic repulsion between chitosan membrane surface and blood cells. Then the coagulating of blood cells could be avoided. Though both chitosan and modified chitosan membranes have low HR values, it was obvious that the HR values of modified membranes decreased as the degree of oxidization increased (**Figure 3 b**). The reason was that chitosan membrane carried trace of positive charges on its surface, which were produced by amino protonation. When the membrane contacted with blood, the electrostatic attraction between chitosan membrane surface and erythrocyte membrane containing anionic glycoproteins induced curvature off erythrocyte membrane, ultimately leading to rupture and release haemoglobin. As for modified chitosan membranes, because of plenty of negative charges on its surface, the attraction between chitosan membrane

surface and erythrocyte membrane could be decreased or avoided and so did the hemolysis. This result was clearly visualized by SEM (**Figure 4**), too. Being incubated with chitosan or modified chitosan films for 4.0 h, blood cells were damaged more seriously by chitosan film than did by modified film.

In conclusion, the modified chitosan membranes have superior blood compatibility to chitosan. The higher the degree of oxidization of the membrane is, the better the blood compatibility of the membrane is.

Figure 4 SEM pictures of sheep blood cell after exposure to membranes for 4.0 h. (a) control; (b) exposure to modified chitosan film; (c) exposure to chitosan film



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