Synthesis of a New Chitosan Immobilized with β-Cyclodextrins and Its Adsorption Properties for Bilirubin

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ABSTRACT: A new chitosan derivative of chitosan immobilized with β-cyclodextrins (CTC) has been synthesized by the reaction of chitosan and allyl-substituted β-cyclodextrins (ASC). Its structure has been proved by Fourier transform infrared spectral analysis, X-ray powder diffraction analysis, and chemical analysis. Its adsorption of bilirubin has been also studied. By the experiment, it has been found that this new product has good adsorption ability on bilirubin. At initial 2 h, the adsorption increased largely. The adsorption mechanism was also dealt with. It predicted that this new chitosan derivative could have application in removing the bilirubin. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 99: 1264–1268, 2006

Key words: chitosan; immobilized; β-cyclodextrins; unconjugated bilirubin; adsorption

INTRODUCTION

Chitosan derived from the polysaccharide is a low cost, renewable, and marine polymer, because the source of chitin comes from the shells of crustaceans, for instance, shrimp, lobsters, and crabs; it is the most plentiful natural polymer after cellulose. Chitosan has wide application in biomedical fields, e.g., as an immobilization and encapsulation agent of toxic organic matter,[1] as a bacteriostatic agent,[2] and as a drug delivery vehicle,[3] because of its biocompatibility.

β-Cyclodextrins (β-CDs) have been used in mimicking enzymes in the food industry, pharmaceutical industry, agriculture, chemical separation, and in other fields. β-CDs have inclusion ability for many sorbents, because of its proper hydrophobic torus.[4–6] By introduction of β-CDs onto polymer matrix, β-CDs polymers can be gained, which have inclusion ability with many substances. Previously, we have made the products of chitosan modified by β-CDs to adsorb radiative iodine and pollutant *p*-dihydroxybenzene effectively.[7–8]

Excess free bilirubin (BR) can deposit in tissues, especially in the brain, and can result in hyperbilirubinemia. Some people have researched this by removing BR with active charcoal, ion exchange, and polymeric adsorbents. Some researchers have studied chitosan modified by different ways, so that the modification was expected to bring expected adsorption properties for BR at higher BR concentration.[9–10]

Here, we have also prepared a chitosan derivative with β-CDs, with a new structure. Its preparation is simple. This new and easily prepared product has been studied for its adsorption property for BR at lower BR concentration. It is expected that this new material would have application in removing BR.

EXPERIMENTAL

Material

Chitosan, whose degree of deacetylation (DD) was calculated to be 85% from amino content, was purchased from Yuhuan Organisms Co. Ltd. (Hangzhou, Zhejiang Province, China). The DD was determined by acid–base titration. The deacetylation calculation was performed as follows:[11]

Chitosan consists of the units of β-(1,4)-2-acetamido-2-deoxy-D-glucose (formula weight, $M_1 = 203$) and β-(1,4)-2-amino-2-deoxy-D-glucose (formula weight, $M_2 = 161$). DD can be obtained by the actual amino content of chitosan divided by the ideal amino content by acid–base titration method. The ideal amino content was calculated as follows:

$$\text{Ideal amino content}(\%) = 16/161 \times 100 = 9.94$$

About 0.4 g of chitosan sample was dissolved into 30 mL standard solution of hydrochloric acid. A little indicator was added into the solution. Then, it was titrated until end point with a standard solution of sodium hydroxide. The actual amino content was calculated as follows:

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$$\text{Actual amino content}(—NH_2)\% = \frac{(C_1V_1 - C_2V_2)0.016}{G(100 - W)} \times 100$$

where C_1 is the concentration of standard solution of hydrochloric acid (mol/L), V_1, the volume of standard solution of hydrochloric acid (mL), C_2, the concentration of standard solution of sodium hydroxide (mol/L), V_2, the volume of consumed standard solution of sodium hydroxide (mL), G, the weight of sample (g), W, water content of sample (%), and 0.016 is the amino content equivalent of 1 mL of 1 mol/L hydrochloric acid (g).

Every sample was performed in triplicate. DD was calculated from amino content as follows:

$$D\ D(\%) = \frac{(—NH_2)}{9.94} \times 100$$

β-CDs were recrystallized, vacuum-dried at 100°C for 8–10 h over P_2O_5 before use. BR was obtained from Chinese Pharmaceutical Shanghai Chemicals Co. Other chemicals were of analytical grade.

Apparatus

FTIR spectra were measured on a Shimadzu FTIR 8000 Series spectrophotometer. Wide-angle X-ray diffraction patterns were recorded with the use of nickel-filtered CuKα radiation produced by a Rigaku (D/MAX, 111A) diffractometer. The visible spectrum data of BR were made by UV-1601 ultraviolet–visible spectrometer (Toshiba).

Preparation of chitosan immobilized with β-CDs

The reaction scheme for the synthesis of chitosan immobilized with β-CDs (CTC) is shown in Figure 1.

Allyl-substituted β-CD (ASC) was synthesized by the reaction of allyl bromide and β-CDs in anhydrous dimethyl sulfoxide according to Armstrong et al.[12] Dried β-CD (6.67 g) was dissolved in anhydrous dimethylated sulfoxide (200 mL) in a three-necked flask. After dissolution, 3.36 g of powdered NaOH was added to β-CD solution. Approximately, 1 mL allyl bromide in 10 mL anhydrous dimethylated sulfoxide was dropped into the flask slowly. After stirring for 24–48 h at room temperature, viscous ASC solution was formed. The mole ratio of allyl bromide and β-CDs was controlled as little more than 1 : 1.

One gram of chitosan was soaked in 100 mL of ASC solution for 24 h. In this solution, 1.5% $FeSO_4$ solution (12 mL) was added and nitrogen gas is passed. Then 20 mL 10% (v/v) H_2O_2 solution was also added into the solution. After stirring for 5 h at room temperature, CTC was filtered, and washed with acetone and water repeatedly. The light brown powder of CTC (1.2 g) was gained when dried to constant weight under infrared light. The real amount of β-CDs onto chitosan was calculated as about 170 μmol/g.

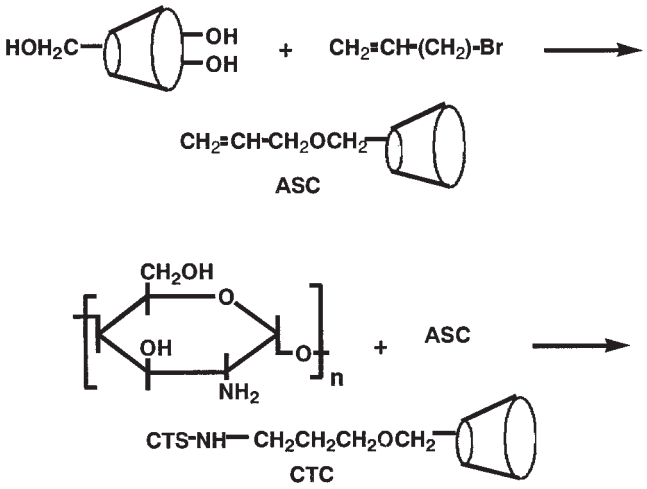

Figure 1 The reaction scheme for the synthesis of CTC.

Measurement of the apparent amount of β-CDs onto chitosan

Plotting of standard curve was conducted according to the procedure reported previously.[13] The measurement was done as follows: CTC (25 mg) was hydrolyzed in 15 mL sulfuric acid (0.5 mol/L) and stirred for 10 h at 100°C. The solution was poured into a measuring flask and diluted to 50 mL. The glucose content was determined using a spectrometer at 490 nm. The β-CD apparent amount of immobilization was calculated as follows:

$$Q_{CD} = \frac{C \times 50 \times 1000}{180 \times 7 \times W}$$

where Q_{CD} is the apparent amount of immobilization (μmol/g), C, the concentration of glucose (μg/mL), and W is the weight of CTC (mg).

The chemical analysis of the apparent immobilization amount of β-CDs was 26.35 μmol/g. The real amount of immobilization of β-CDs is different from the apparent amount of β-CDs immobilization. It is possible that the glucose residues of β-CDs were not hydrolyzed completely owing to the formation of ether bonds.

Adsorption for BR of CTC

Twenty milligrams of CTC was added into 10 mL BR solution of different concentrations in the absence of light, and shaken in a thermostat (20 ± 1) °C biological oscillator for some hours. The BR solution was pre-

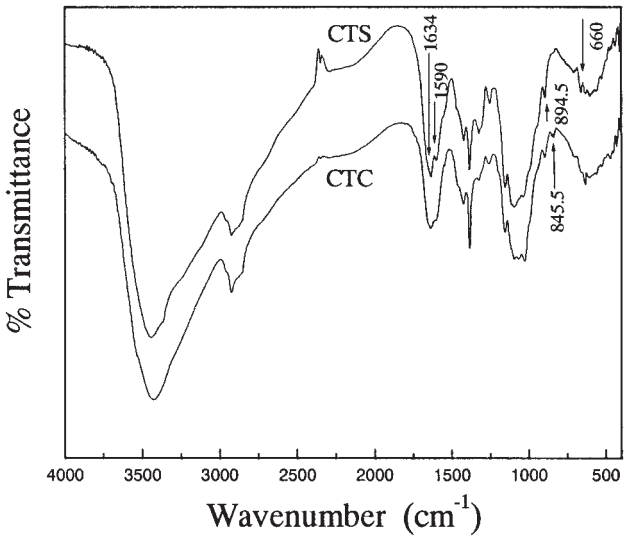

Figure 2 IR spectra of CTS and CTC.

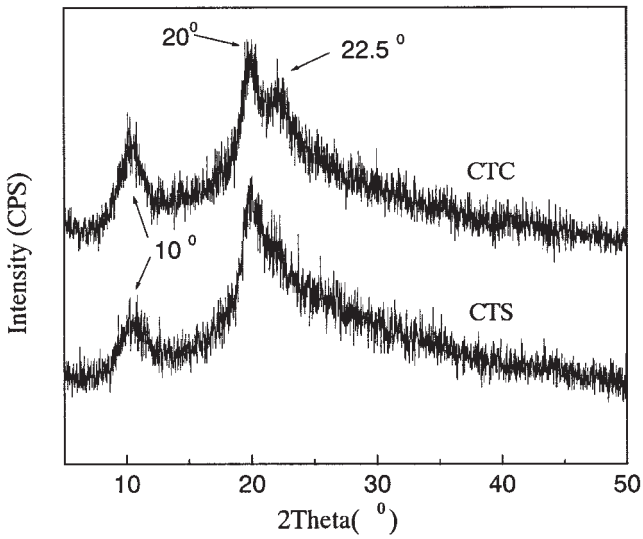

Figure 3 X-ray diffraction patterns of CTS and CTC.

pared with phosphate buffer. The adsorption capacity was measured at 438 nm by spectrophotometric analysis.

The adsorption capacity for BR by CTS or CTC was calculated respectively, as follows:

$$Q = \frac{V(C_0 - C_1)}{W}$$

where V is the volume of BR solution (mL), C_0, the concentration of BR (mg/mL), C_1, the concentration of BR solution after absorption (mg/mL), and W is the weight of adsorbent CTC or CTS (mg).

RESULTS AND DISCUSSION

Characterization of CTC

The infrared spectra of CTC and CTS were detected as shown in Figure 2. The peak at 894.5 cm^{-1} appeared as the group of the β-pyanyl vibration of CTS. This indicated that the ring of chitosan was not destroyed. In contrast with IR spectrum of CTS, the new peak at 845.5 cm^{-1} was ascribed to the introduction of β-CDs and its α-pyanyl vibration. The amino peak at 1590 cm^{-1} almost disappeared. This suggested that the amino group was involved in the reaction. The facts supported that the amino group of CTS has reacted with the double bond of ASC.

X-ray analysis

In Figure 3 the peak of CTC at 10°C increased when compared with CTS. The CTC peak at 20°C was similar to the peak of CTS. This is because a lot of hydroxyl groups of CDs has been introduced into the CTS matrix and formed new hydrogen bond action, although these groups could interrupt original regular crystalline structure. The X-ray data also supported that the ASC has reacted with chitosan.

Adsorption for BR OF CTC

The effect of adsorption time

In Figure 4, it can be seen that the time of adsorption equilibrium for BR by CTC is about 5 h. And the time of adsorption equilibrium for CTS was about 3 h. It was also seen that at the initial 2 h the adsorption rate was relatively high. It was benefited to actual application. From Figure 4, it was also found that the ad-

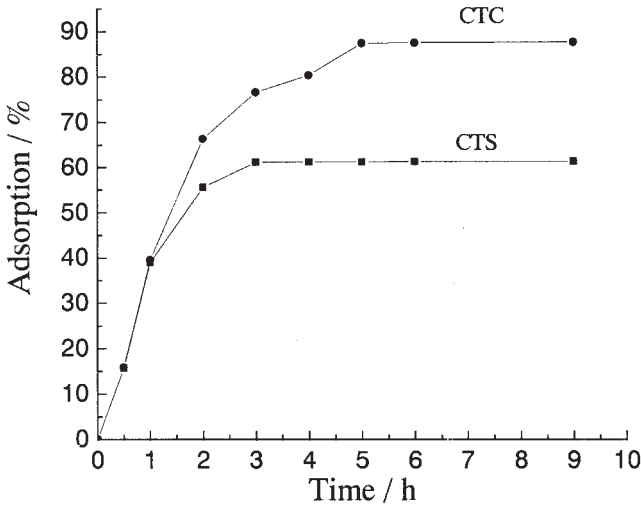

Figure 4 Influence of the adsorption time on the adsorption for BR. Adsorption conditions: BR solution, 10 mL (14.5 mg/L); adsorbent, 20 mg (pH 7.4, 20°C).

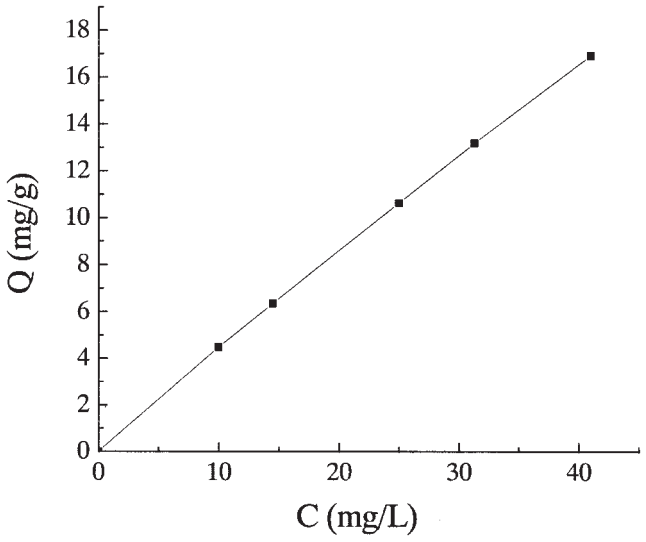

Figure 5 Influence of concentration of BR on the adsorption capacity for BR. Adsorption conditions: BR solution, 10 mL; adsorbent: 20 mg (pH 7.4, 20°C); adsorption time, 24 h.

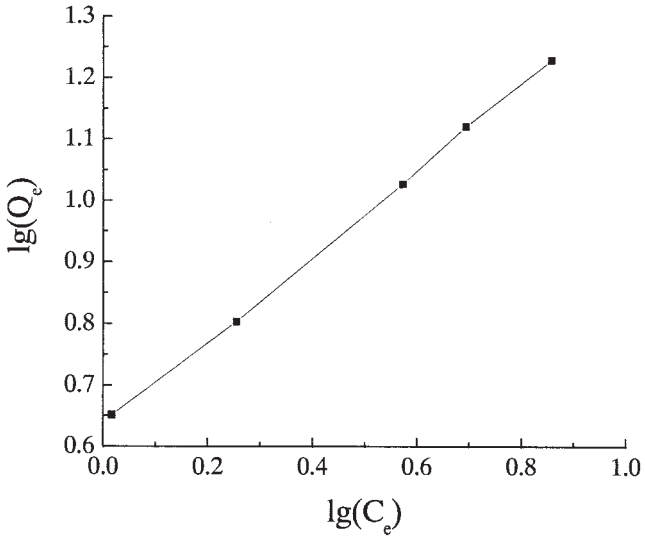

Figure 6 Freundlich isotherm linear plots of CTC for BR.

sorption capacity was 6.35 and 4.56 mg/g respectively, when the adsorption equilibrium has attained. As CTC contained the torus of β-CDs and more hydroxyl groups, the adsorption capacity for BR was bigger than chitosan itself.[14]

The effect of the concentration of BR on adsorption capacity

Figure 5 shows the influence of the concentration of BR on the adsorption capacity tested in vitro. The experimental results showed that the adsorption capacity for BR by CTC increased with an increase in concentration of BR.

Freundlich empirical formula was usually used to describe adsorption system.[15] The relation between balanced adsorption Q_e (mg/g) and concentration C_e (mg/L) was as follows:

$$Q_e = Q' C_e^{1/n}$$

A linear form of the Freundlich equation will yield the Freundlich constants Q' and denominator of exponent (n): $1g\ Q_e = 1g\ Q' + \frac{1}{n} 1g\ C_e$.

The data of lg Q_e and lg C_e were plotted in Figure 6. In Figure 6, the experimental data have been treated by Freundlich model. The relation of lg Q_e and lg C_e assumed linear. The BR concentrations on the adsorbent CTC will increase as long as there is an increase in the BR concentration in the liquid.

From Figure 6, the slope and intercept results got by Freundlich were $Q' = 4.3$, $n = 1.44$, and R (the relative index) = 0.99968. The adsorption for BR of CTC was accorded with Freundlich isothermal equation.

The IR of adsorption for BR of CTC

The IR spectra of the adsorbent adsorbing BR (CTC-BR), CTC, and BR are shown in Figure 7.

From Figure 7, it was seen that the acetyl amino group of CTC-BR peak appeared at a lower frequency (1618 cm^{-1}). The hydroxyl peaks shifted to lower frequency (1076 and 1018 cm^{-1}) and increased greatly compared with CTC. These facts indicated that there were mutual hydrogen bond actions among acetyl amino group and hydroxyl group of CTC and the amino and hydroxyl group of BR. The stretching vi-

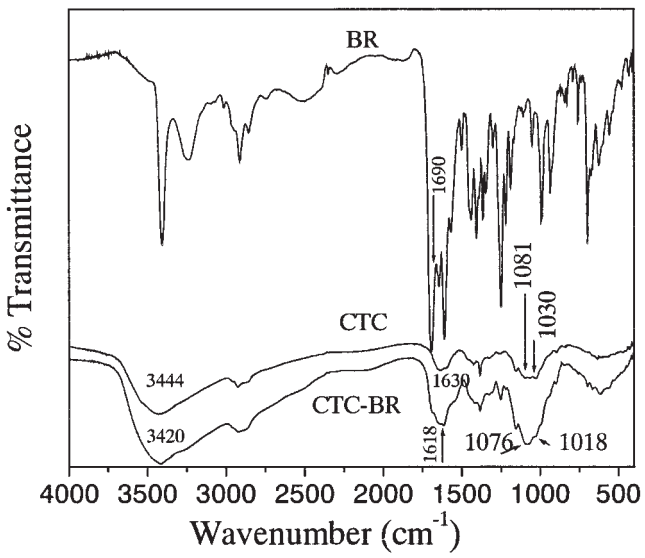

Figure 7 IR spectra of CTC, CTC-BR, and BR.

bration at 3444 cm^{-1} of —NH_2 group and —OH group, shifted to lower frequency (3420 cm^{-1}) and became wider. This also suggested that there was a strong hydrogen bond action between CTC and BR. At the same time, at CTC-BR spectrum, no new peaks, like carbonyl at 1690 cm^{-1} of BR, appeared. This has proved that BR adsorbed by CTC did not destroy the structure of CTC matrix structure.

CONCLUSIONS

A new adsorbent for BR, CTC, has been prepared by the reaction of chitosan and ASC. Its structure has been confirmed by infrared spectra analysis and X-ray diffraction analysis. The experimental results showed that this adsorbent for BR has a higher capacity (6.33 mg/g) than CTS at initial concentration 14.5 mg/L at 20°C. It was mainly derived from the torus of β-CDs on CTC and hydrogen bond between CTC and BR. The adsorption for BR of CTC was accorded with Freundlich isothermal equation. This new adsorbent might be applied as biomedical adsorbents in the removal of BR in medical administration.

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