Study on β-Cyclodextrin Grafting with Chitosan and Slow Release of Its Inclusion Complex with Radioactive Iodine

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ABSTRACT: β-CD-2-CTS was synthesized by β-cyclodextrin reacting with *p*-toluenesulfonyl chloride, then grafting with chitosan. The infrared spectra analysis and ^{13}C NMR confirmed that β-cyclodextrin reacted with *p*-toluenesulfonyl chloride at the 2-position carbon atom in the substituted glucose unit of β-cyclodextrin and formed β-CD-2-OTs. In the ^{13}C NMR of β-CD-2-OTs, the characteristic peak of the 2-postion carbon atom in the substituted glucose unit of β-cyclodextrin appeared at 78.43 ppm. β-CD-2-CTS was characterized with infrared spectra analysis and X-ray diffraction. In the infrared spectra of β-CD-2-CTS, the characteristic peak of α-pyanyl vibration of β-CD was at 848.6 cm^{-1}. The characteristic peak of β-pyanyl vibration of CTS was at 894.9 cm^{-1}. The X-ray diffraction analysis showed that the peak at $2\theta = 20°$ decreased greatly in β-CD-2-CTS. The polymer inclusion complex of β-CD-2-CTS with iodine was prepared and its inclusion ability was studied. The experimental results showed that a nice bit of iodine was included with β-CD-2-CTS and formed a stable inclusion complex. After the subcutaneous implantation of the polymer inclusion complex of β-CD-2-CTS with $^{131}I_2$ in rats, $^{131}I_2$ exhibited the property of slow release. $^{131}I_2$ in the blood of rats decreased slowly. $^{131}I_2$ in the blood of rats maintained approximately half of maximum for 70 days later, and maintained much higher radioactivity in the organs of rats compared to the inclusion complex of β-CD with $^{131}I_2$, too. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 2414–2421, 2001

Key words: cyclodextrin; chitosan; graft; inclusion complex; characterize; slow release

INTRODUCTION

Cyclodextrins (CD) are cyclic oligosaccharides consisting of 6 (in α-), 7 (inβ-), or 8 (in γ-)glucose units linked together by 1,4 linkages to form torus-like structures. All the secondary hydroxyl groups at the 2- and 3-positions of the glucose units are on one side of the torus, and all the primary hydroxyl groups at the 6-positions of the glucose units are on the other side of the ring.[1] Cyclodextrins have gained prominence in recent years because their cavity, which is hydrophobic in nature, is capable of binding aromatic and other small organic molecules, and therefore provide ideal binding sites.[2] Selective functionalization at the 6-position is relatively easy. However, the secondary side is shown to be the more important side of cyclodextrin in binding studies.[3]

Chitin is a polysaccharide that is widely spread among marine and terrestrial invertebrate and lower forms of a plant kingdom.[4] Chitosan is a polyaminosaccharide, normally obtained by alkaline deacetylation of chitin. Chitosan's availability in a variety of useful forms and its unique chemical and biological properties make it a very attraction biomaterial. It is extensively used in

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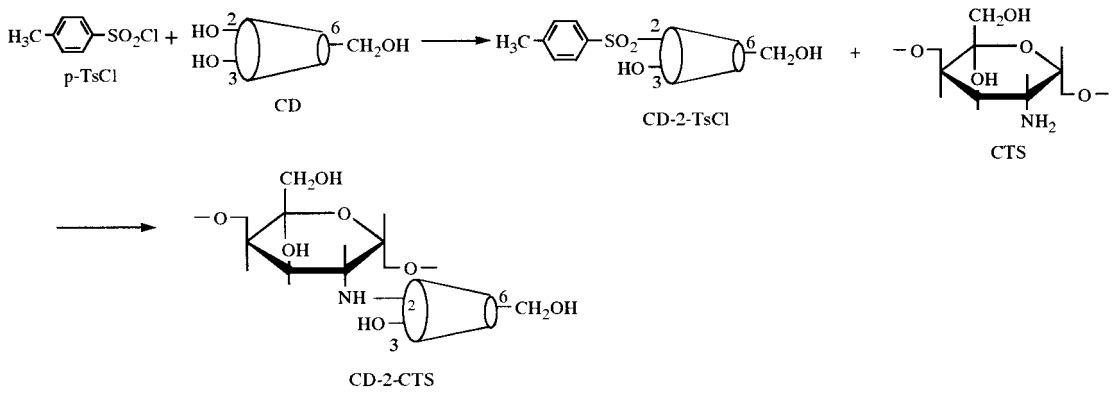


Figure 1 Reaction scheme for the synthesis of β-CD-2-CTS.

many types of applications such as treatment of wastewater,[5] chromatographic support,[6] enzyme immobilization,[7] wound-healing dressing,[8] dental application,[9] adhesion bandages for surgery,[10] and drug-delivery system.[11–13] In those applications, Chitosan's key properties are biocompatibility, nontoxcity (its degradation products are natural metabolites).

Cyclodextrin has the merit of a hydrophobic cavity, which is easy to assemble with other molecules. Chitosan has the merit of degradation slowly in organism. The aim of this paper is to develop a potential candidate for a slow-release system by chitosan grafting with cyclodextrin and including other appropriate drugs.

EXPERIMENTAL

Material

Chitosan (CTS), whose degree of deacetylation was calculated to be 88% from amino content, was prepared by N-deacetylation of chitin from shrimp shells and was used after passage through a 200 mesh sieve. *p*-Toluenesulfonyl chloride (TsCl), solid iodine, and β-cyclodextrin (β-CD) were purchased from the First Chemical Factory in Shanghai (China). $^{131}I_2$ was supplied by China Nuclear Energy Institute. All inorganic compounds were reagent grade, and all solvents and available organic materials were chemical reagent grade used without purification except that solid iodine and ethanol were analytical reagent grade.

Male mature Wistar rates for slow-release investigation, whose average weights were 183 ± 3 g, were supplied by China Military Medicinal Institute.

Measurement

Infrared spectra was measured on a Shimadzu FTIR 8000 Series spectrophotometer. Wide-angle X-ray diffraction (WAXD) patterns were recorded with the use of nickel-filtered CuKα radiation produced by a Rigaku (D/MAX, 111A) diffractometer. ^{13}C NMR was conducted using Bruker MSL-400 model NMR spectrometer; proton and frequencies were 400 and 100 MHz, respectively. Radioactivity was counted by Beckman Ls-9800 liquid scintillation spectrometer.

Preparation of β-CD-2-CTS

The reaction scheme for the synthesis of β-CD-2-CTS is shown in Figure 1. Preparation of β-CD-2-OTs was conducted according to the procedure[14] reported previously. The yield of β-CD-2-OTs was 45%.

Powdered chitosan (3.0 g) was swelled in 150 mL dimethylformamide (DMF), then B-CD-2-OTs(5.0g) was dissolved in 20 mL DMF, was slowly dropped into the chitosan solution. The mixture was stirred for 48 h at 50°C, filtered, washed with water, and dried to give 3.9 g yellow β-CD-2-CTS.

Inclusion Procedure for β-CD-2-CTS with Iodine and Absorption Procedure for CTS with Iodine

The concentration of iodine in ethanol solution was determined by titration with aqueous sodium thiosulfate. The adsorption of iodine or inclusion of iodine was performed by immersing precisely weighed amount of chitosan (about 0.1 g) or β-CD-2-CTS (about 0.1 g) in 50 mL ethanol solution of different iodine concentrations and stirred for 24 h at 25°C. Then, the mixture was filtered. The

concentration of iodine solution after filtering was determined by using a spectrometer at 514 nm. The amount of absorbed iodine by CTS or included iodine by β-CD-2-CTS was calculated respectively as follows:

$$Q_1 = \frac{V(C_0 - C_1)}{W_1} \quad (1)$$

$$Q_2 = \frac{V(C_0 - C_2)}{W_2} - Q_1 \quad (2)$$

where V is the volume of iodine solution (mL), C_0 is the concentration of iodine solution before absorption or inclusion (g/mL), C_1 is the concentratrion of iodine solution after absorption (g/mL), W_1 is the weight of CTS (g), C_2 is the concentration of iodine solution after inclusion (g/mL), W_2 is the weight of β-CD-2CTS (g), Q_1 is the amount of absorbed iodine by CTS (g iodine/g CTS), Q_2 is the amount of included by β-CD-2-CTS with iodine.

Evaporation of the Included and Absorbed Iodine

The inclusion complex of β-CD-2-CTS with iodine and the absorption complex of CTS with iodine were placed in airer to evaporate the contained ethanol. Until their weights were constant at room temperature, the inclusion complex of β-CD-2-CTS with iodine and the absorption complex of CTS with iodine were moved under infrared light at 45°C. Their amounts were precisely weighted before being evaporated under infrared light. During evaporation, they were weighted precisely every interval 20 min. The loss amount of iodine of the inclusion complex of β-CD-2-CTS with iodine was estimated from the decrease of every twice weights during evaporation. The loss amount of iodine of the absorption complex of CTS with iodine was calculated as the inclusion complex of β-CD-2-CTS with iodine.

Slow-Release Procedure for the Inclusion Complex of β-CD-2-CTS with $^{131}I_2$

The preparation of inclusion complexes of β-CD-2-CTS and β-CD with $^{131}I_2$ was performed according to the procedure previously described in this paper. The inclusion complexes of β-CD-2-CTS and β-CD with $^{131}I_2$ was dissolved in 0.5% acetic acid to prepare 600 μci/mlr solutions.

Two groups of 16 Wistar male mice were used for slow-release investigation. One group (as the controlled group) of 8 mice was implanted with the inclusion complex of β-CD-2–CTS with $^{131}I_2$. The other group of 8 mice was implanted with the inclusion complex of β-CD with $^{131}I_2$. The radioactive solutions of the inclusion complexes with $^{131}I_2$ were injected into the back muscle of mice with the dose of 60 mci/mice.

Ten-microliter blood samples were collected from the eyeground veins of mice at each interval 7 days and were centrifuged for 10 min at 4°C and 3000 rpm. Ten-milliter plasma samples were separated from blood samples and treated by adding 0.1 mL HCHO, H_2O_2, and a drop of octanoic acid. The radioactivity was counted by adding 5 mL liquid scintillation cocktail into the treated plasma samples.

Eight mice of each group were killed after 70 days and dissected. Ten micrograms of wet tissue of each organ was collected and treated as the treatment of plasma samples. The radioactivity of each organ was counted as plasma sample.

All samples were analyzed using a liquid scintillation counter with efficiencies of 74% for $^{131}I_2$.

RESULTS AND DISCUSSION

Characterization of Structure of β-CD-2-OTs and β-CD-2-CTS

β-CD-2-OTs was successfully prepared by the reaction of the monotosylation of cyclodextrin. β-CD-2-OTs was a white powder. It was easily dissolved in water, methanol, dimethysulfoxide, and dimethylformaamide.

Infrared Spectra Analysis and ^{13}C NMR Analysis of β-CD-2-OTs

The Fourier transform infrared spectras of β-CD and β-CD-2-OTs were shown together in Figure 2.

In the IR spectra of β-CD-2-OTs, the characteristic peak of β-pyanyl vibration of β-CD was at 848.6 cm^{-1}, the characteristic peak of benzene cycle backbone (Ts groups) vibration appeared at 1604.7 cm^{-1} and the bending vibration of benzene cycle (Ts groups) appeared at 694.3 cm^{-1}. These evidences indicated β-CD had reacted with TsCl.

The chemical shifts (ppm) of β-CD-2-OTs gave peaks at 20.60 (Me), 125.20, 127.10, 129.00, and 130.32 for the aromatic carbons and the six normal peaks for cyclodextrin and the peaks for the substituted glucose unit as follows:

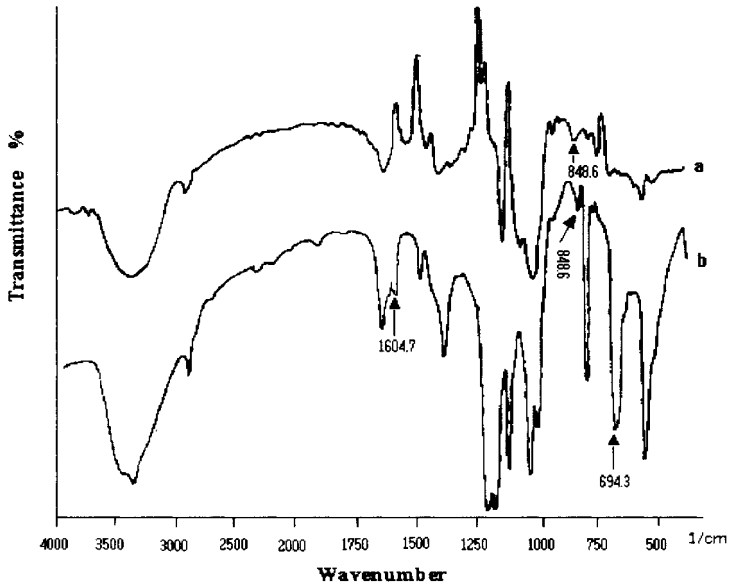
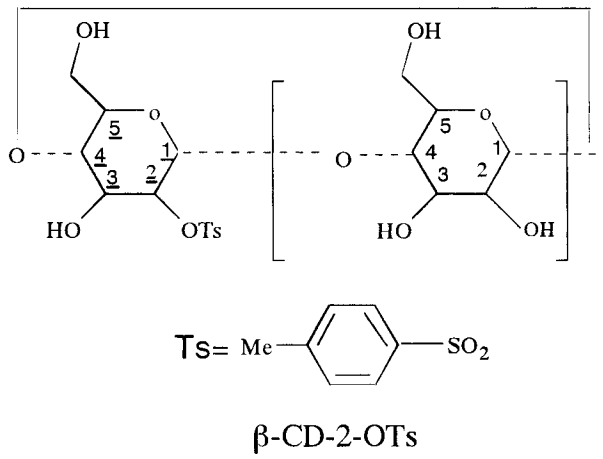


Figure 2 IR spectra of (a) β-CD and (b) β-CD-2-OTs.

C-1 (101.25) C-2 (72.08) C-3 (71.60) C-4 (80.38) C-5 (71.05) C-6 (59.59)

C-1 (99.02) C-2 (78.43) C-3 (70.9) C-4 (79.00) C-5 (68.6) C-6 (59.52)



β-CD-2-OTs

As elegantly explained by Breslow,[14] a large downfield chemical shift of C-2 and a small upfield chemical shift of C-3 and no change in the shift of C-6 of the substituted glucose unit with respect to unsubstituted glucose units clearly indicated that the substituent was at the 2-position of cyclodextrin.

IR spectra and ^{13}C NMR spectra confirmed the substitution had taken place at the 2-position. The product was β-CD-2-OTs.

Infrared Spectra Analysis and X-ray Diffraction Patterns of Analysis of β-CD-2-CTS

The IR spectra of β-CD, CTS, and β-CD-2-CTS were shown together in Figure 3. As β-CD and CTS both were carbohydrates, they had some similar groups. When β-CD grafted with CTS, the IR spectra peaks of most groups of β-CD would be covered by the same groups of CTS. So, the IR spectra of β-CD-2-CTS was similar to CTS. But the characteristic peak of the β-pyanyl vibration

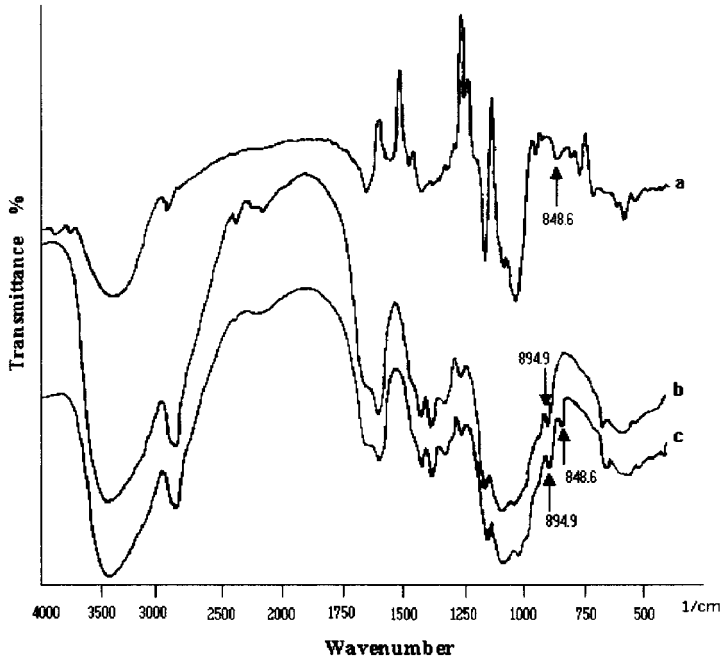


Figure 3 IR spectra of (a) β-CD, (b) CTS, and (c) β-CD-2-OTs.

of CTS at 894.9 cm^{-1} and the characteristic peak of α-pyanyl vibration of β-CD at 848.6 cm^{-1} both appeared in the IR spectra of β-CD-2-CTS. Furthermore, the characteristic peak of benzene cycle backbone vibration (Ts groups of β-CD-2-OTs) at 1604.7 cm^{-1} and the bending vibration of benzene cycle (Ts groups of β-CD-2-OTs) at 694.3 cm^{-1} disappeared respectively in the IR spectra of β-CD-2-CTS owing to Ts groups leaving from β-CD-2-OTs when β-CD-2-OTs reacted with CTS. These supported β-CD grafted with CTS.

The X-ray diffraction patterns of CTS and β-CD-2-CTS were shown together in Figure 4. The WAXD pattern of CTS showed the characteristic peaks at

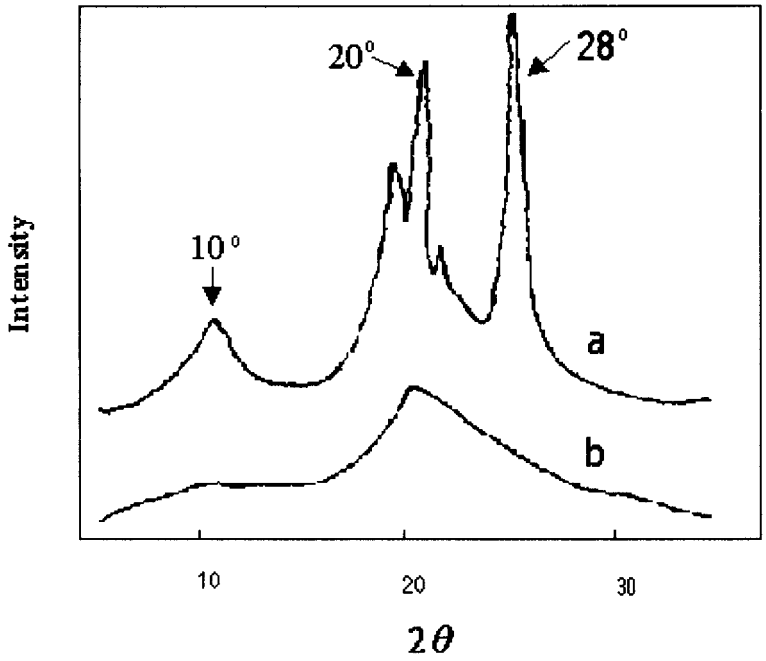


Figure 4 X-ray diffraction patterns of (a) CTS and (b) β-CD-2-CTS.

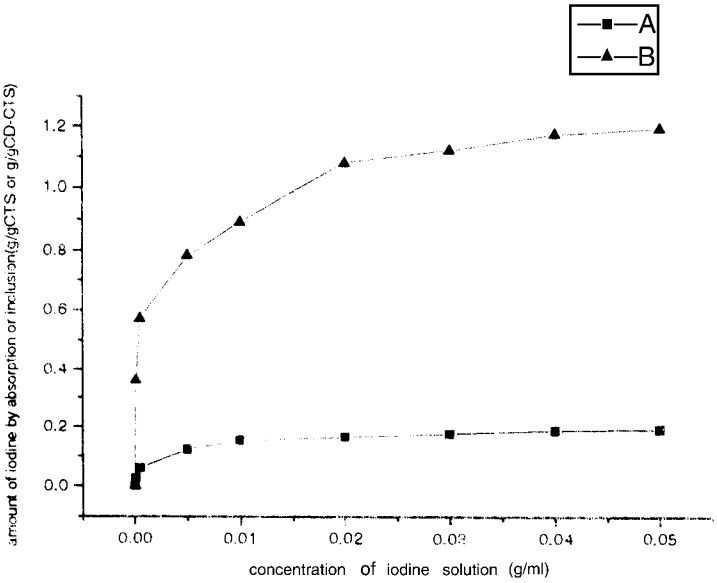


Figure 5 Adsorption of CTS with iodine (A) and inclusion of β-CD-2-CTS with iodine (B).

$2\theta = 10°, 20°, 28°$. Noting that the peaks at $2\theta = 10°$, 28° disappeared and the peak at $2\theta = 20°$ decreased greatly in β-CD-2-CTS. It was thought that the decrease in crystallinity of β-CD-2-CTS was attributed to the deformation of the strong hydrogen bond in the chitosan backbone as the amino groups were substituted by β-CD. β-CD-2-CTS gave a low crystallinity, indicating that it was considerably more amorphous than CTS.

Inclusion Ability of β-CD-2-CTS with Iodine

Figure 5 showed that the amount of the absorbed iodine by CTS was much less than the amount of the included iodine by β-CD-2-CTS, and Figure 6 exhibited that, after CTS absorbing iodine, most of iodine was evaporated soon under infrared light. On contrary, after β-CD-2-CTS including with iodine, only a little amount of iodine was

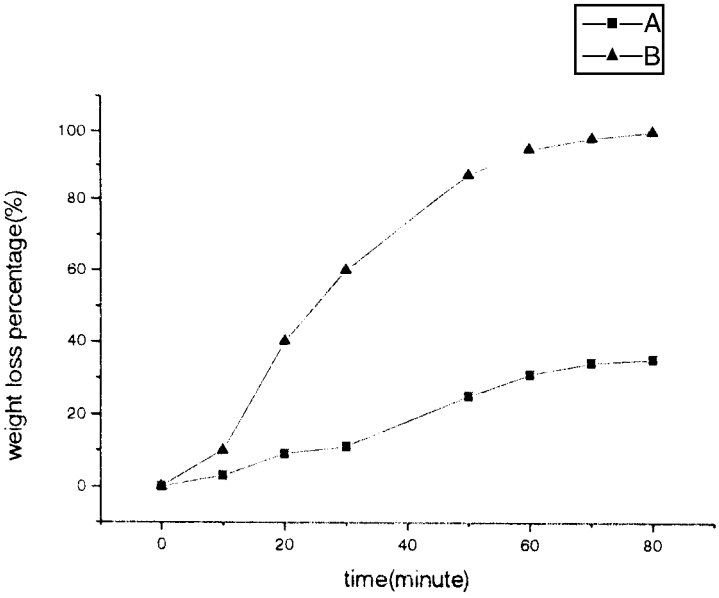


Figure 6 Evaporation of included iodine with β-CD-2-CTS (A) and the adsorbed iodine with CTS (B).

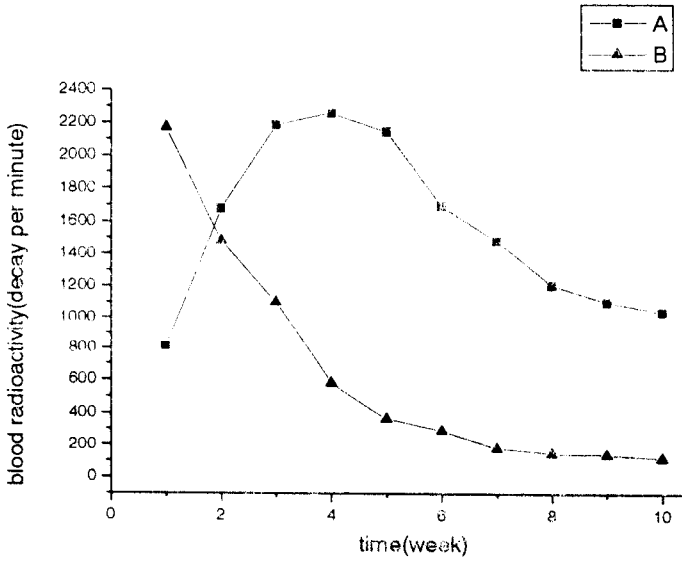


Figure 7 Blood radioactivity in rats after the subcutaneous implantation of the inclusion complex of β-CD-2-CTS with $^{131}I_2$ (A) and the inclusion complex of CD with $^{131}I_2$ (B).

evaporated slowly under infrared light. This indicated there was dramatically difference between the inclusion and absorption of iodine. The stronger inclusion ability of β-CD-2-CTS with iodine was caused by the special hydrophobic cavity structure of β-CD-2-CTS. β-CD was not only formed a 1:1 complex with iodine, but it also could form a 1:2 complex with iodine. CTS only had a little adsorbing ability of iodine. The absorption of iodine was considered to be caused by $n - \delta$ charge transfer[15] between amino groups of CTS and iodine molecules. It was thought that the inclusion of iodine was different from the absorption of iodine and the ability of inclusion of iodine was stronger than the ability of absorption of iodine.

Slow Release of the Inclusion Complex of β-CD-2-CTS with $^{131}I_2$ in Rates

Figure 7 exhibited the blood radioactivity in rates after the subcutaneous implantations of the inclu-

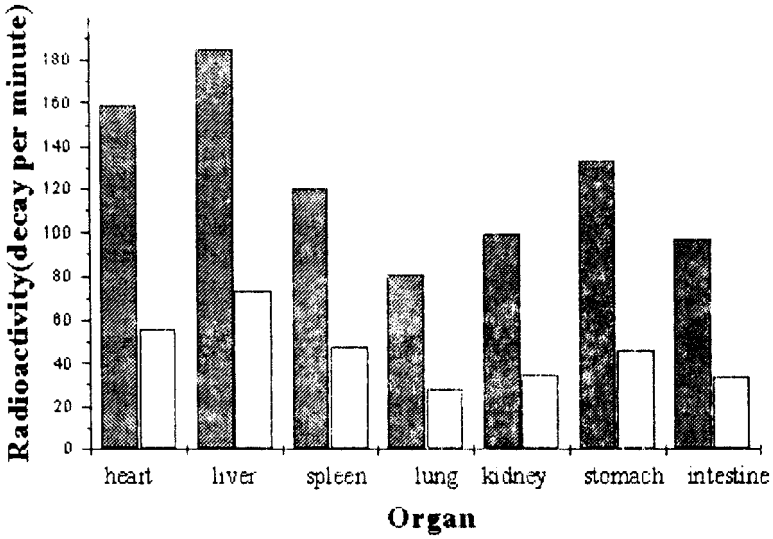


Figure 8 Body distribution of radioactivity after the implantation of the inclusion complexes of β-CD-2-CTS with $^{131}I_2$ (a) and β-CD with $^{131}I_2$ (b) in rats.

sion complex of β-CD-2-CTS with $^{131}I_2$ and the inclusion complex of β-CD with $^{131}I_2$. The radioactivity in the blood increased to the maximum 2253 decay per minute (dpm) after the subcutaneous implantation of the inclusion complex of β-CD-2-CTS with $^{131}I_2$ for 4 weeks, then decreased slowly. After 10 weeks, the radioactivity in the blood decreased to 1064 dpm. But, the radioactivity in the blood increased to maximum 2168 dpm after the subcutaneous implantation of the inclusion complex of β-CD with $^{131}I_2$ for a week, then decreased sharply. After 10 weeks, the radioactivity in the blood decreased to 116 dpm. The reason that caused this difference probably was that β-CD was easy to hydrolyze by amylase in organism and $^{131}I_2$ with the inclusion complex of β-CD was released speedily. No evidence was shown that $^{131}I_2$ with the inclusion complex of β-CD had the property of slow release. The slow-release of $^{131}I_2$ with the inclusion complex of β-CD-2-CTS was remarkable due to the slow degradation of CTS in organism.

After the subcutaneous implantations of the inclusion complexes of β-CD-2-CTS with $^{131}I_2$ and β-CD with $^{131}I_2$ in rates, the body distributions of radioactivity in organs of rates were shown in Figure 8. After $^{131}I_2$ was metabolized in organism for 10 weeks, the remains of $^{131}I_2$ with β-CD-2-CTS inclusion were much more than with β-CD inclusion in organs of rates. The difference was attributed to the slow degradation of CTS polymer, which resisted the release of the included $^{131}I_2$ with β-CD-2-CTS and increase the accumulation of $^{131}I_2$ in organs.

These indicated that β-CD-2-CTS had some property of slow release.

CONCLUSION

β-CD-2-CTS was synthesized by β-cyclodextrin reacting with *p*-toluenesulfonyl chloride, then grafting with chitosan. The polymer inclusion complex of β-CD-2-CTS with iodine was prepared. The experimental results showed that a nice bit of iodine was included with β-CD-2-CTS and formed a stable inclusion complex. After the subcutaneous implantation of the polymer inclusion complex of β-CD-2-CTS with $^{131}I_2$ in rates, $^{131}I_2$ exhibited the property of slow release. The amount of $^{131}I_2$ in the blood decreased slowly and maintained approximately half of maximum for 70 days later. Much higher radioactivity in the organs for 70 days later maintained comparing to the inclusion complex of β-CD with $^{131}I_2$, too.

REFERENCES

1. Bender, M. L.; Komiyama, M. Cyclodextrin Chemistry; Springer-Verlag, New York, 1978.
2. Souza, D.; Bender, M. L Acc Chem Res 1987, 20, 146.
3. Van Betten, R. C.; Secbastian, J. F.; Clowers, G. A.; et al. J Am Chem Soc 1976, 69, 3242.
4. Yao, K.; Peng, T.; Yin, Y.; Xu, M. Macromol Chem Phys C 1995, 35, 155–180.
5. Itani, T.; Nakalima, C.; Sungkan, I. E. J Environ Sci Health Part A Environ Sci Eng Toxic 1995, 30, 669–674.
6. Shi, T.C.; Jing, Y. M.; Sui, D. X.; et al. J Chromatogr 1996, 742, 107–112.
7. Krajewska, B.; Leszko, M.; Zoborska, W. J Chem Tech Biotecnol 1990, 48, 337–350.
8. Shahabeddin, L.; Damour, O.; Bethod, F.; et al. J Mater Sci Mater Med 1191, 2, 222–226.
9. Muzzarell, R.; Biagini, G.; Paugnaloni, A.; et al. Biomaterials 1989, 11, 598–603.
10. Needleman, J. G.; Martin, G. P.; Smales, F. C. J Clin Periodontal 1998, 25, 774–882.
11. Hirano, S.; Tobetto, K.; Hasagawn, M.; Matsuda, N. J Biomed Mater Res 1980, 14, 477.
12. Miyazaki, S.; Ishii, K.; Nadai, T. Chem Pharm Bull 1981, 29(10), 3067.
13. Hirano, S.; Ohe, Y.; Metsuda, N. Carbohydr Res 1978, 60(2), 404.
14. Ueno, A.; Bresolw, R. Tetrahedron Lett 1982, 23, 3451.
15. Shigeno, Y.; Takemot, K. J Appl Polym Sci 1980, 25, 731–738.