Adsorption of Guanosine, Cytidine, and Uridine on a β-Cyclodextrin Derivative Grafted Chitosan

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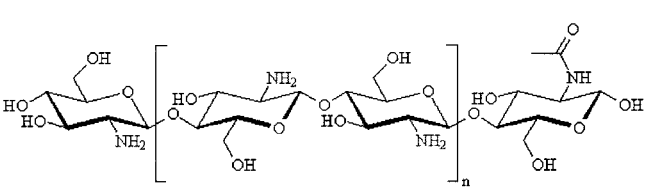
ABSTRACT: A β-cyclodextrin derivative grafted chitosan (CDD-C) was synthesized with chitosan and carboxymethyl-β-cyclodextrin (β-CD). Its structure was characterized by elemental, infrared spectra, and X-ray diffraction analyses. The degree of substitution by the carboxymethyl-β-CD moiety achieved 0.27 with the addition of DMF to the reaction solution. The results are in agreement with the expectations. The static adsorption properties for guanosine, cytidine, and uridine were studied. Experimental results demonstrated that CDD-C had higher adsorption capability for guanosine than cytidine and uridine, and the adsorption capacity for guanosine was 74.20 mg/g. The adsorption capacity was greatly influenced by pH, time, and temperature. The introduction of chitosan enhanced the adsorption ability and adsorption selectivity of β-CD for guanosine. This novel derivative of chitosan is expected to have wide applications in separation, concentration, and analysis of guanosine, cytidine, and uridine in biological sample. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 3050–3055, 2007

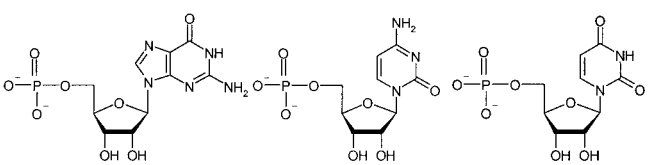
Key words: chitosan; carboxymethyl-β-cyclodextrin; guanosine; cytidine; uridine; adsorption

INTRODUCTION

Chitosan is available in a variety of useful forms and its unique chemical and biological properties make it a very attractive biomaterial. It is extensively used in many types of applications such as treatment of wastewater[1] and chromatographic support.[2] In those applications, chitosan's key properties are biocompatibility, nontoxicity (its degradation products are natural metabolites), and solubility in moderated acidic aqueous solutions. Studies on chemical modification of chitosan have been extensively performed to introduce novel functions into this biomaterial for wound-healing activity,[3,4] gene delivery,[5] cell culture,[6] tissue engineering,[7,8] and drug delivery.[9,10] Therefore, chitosan is receiving great attention as novel functional materials.



chitosan



guanosine cytidine uridine

Despite their interesting biological properties, utilization has been scarcely developed. In the meantime, commercial or practical use of chitosan has been confined to the unmodified forms. For a breakthrough in utilization, chemical modification to introduce a variety of functional groups will be a key point. For this purpose, more fundamental studies on chemical modification will be required. Until now, much work has been reported on the chemical modification of chitosan. Most studies have been published in reviews and books.[11–16] Chitosan allows specific chemical modifications since it has primary amine groups at the C-2 position and primary alcoholic groups at the C-6 position of its monomeric units. These reactive sites enable the grafting of a large variety of properly functionalized molecules.[17,18]

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide built from seven D-glucose units and is formed during the enzymatic degradation of starch and related compounds.[19] The D-glucose units are covalently linked together by 1,4 linkages to form torus-like structures.[20] The secondary hydroxyl groups at the 2- and 3-positions of the glucose units are on one side of the

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torus, and all the primary hydroxyl groups at the 6-positions of the glucose units are on the other side of the ring. The secondary side is shown to be the most important side of CD in binding studies.[21,22] Recent biotechnological advancements have resulted in dramatic improvements in CD production, which has lowered their production cost. This has led to the availability of highly purified CDs, which are well suited as pharmaceutical excipients. CDs are widely used in basic research and industrial processes for the encapsulation of unstable or volatile substances.[20,23–28] CD has the merit of a hydrophobic cavity, which is easy to assemble with other molecules. Chitosan has the merit to degrade slowly in organism. Therefore, grafting CD molecules into chitosan-reactive sites may lead to a molecular carrier that possess the cumulative effects of inclusion, size specificity, and transport properties of CDs as well as the controlled release ability of the polymeric matrix.[29] The products obtained by CD grafting to chitosan using different methods and their inclusion ability, sorption, and controlled release properties have been studied extensively.[30–35]

In this study, a β-cyclodextrin derivative of chitosan (CDD-C) was synthesized with chitosan and carboxymethyl-β-CD via one step. The static adsorption properties for guanosine, cytidine, and uridine were studied. Experimental results demonstrated that the CDD-C had higher adsorption capability for guanosine than cytidine and uridine.

EXPERIMENTAL

Materials

β-Cyclodextrin (β-CD) of reagent grade was recrystallized twice in water and dried for 12 h in vacuum at 100°C. Chitosan was purchased from Qingdao Baicheng Biochemical (Qingdao, China). Its degree of deacetylation was 88.03%, and the viscosity average molecular weight was 2.0×10^5. Guanosine, cytidine, and uridine were obtained from Sigma (USA). The other reagents were of analytical grade and used without further purification.

Measurements

Infrared spectra were recorded as KBr pellets from 4000 to 450 cm^{-1} on a Nicolet 5DX Fourier transform infrared spectrophotometer at a resolution of 4 cm. Wide-angle X-ray diffraction (WAXD) patterns were obtained by monitoring the diffraction angle 2θ from 1° to 70° using a Rigaku (D/MAX, 2500×) diffractometer. The diffractometer was equipped with a Cu Kα (l = 0.1542 nm) radiation, produced under the conditions of 40 kV and 100 mA. Elemental analysis was determined with a PerkinElmer automatic instrument. Differential thermal analysis (DTA) was carried out on a Netzsch STA 409 simultaneous thermal analyzer (Netzsch Italiana, Verona, Italy). The samples (4–15 mg) were accurately weighed in platinum pans (Netzsch) and heated at a scanning rate of 10°C/min.

Determination of guanosine, cytidine, and uridine

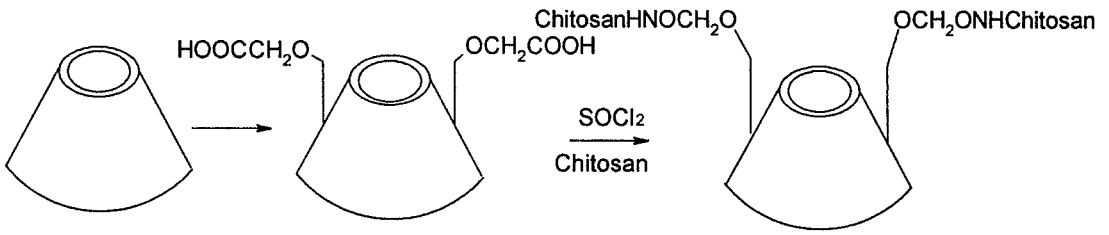
The concentration of guanosine, cytidine, and uridine was determined with a 756MC UV–vis spectrophotometer (Shanghai, China). The individual standard was dissolved in ethanol and diluted to form solutions of 10, 20, 30, 40, 50, 60, 70, and 80 mg/L. The determination was performed at 260 nm. The data of the content (X) of guanosine, cytidine, or uridine and absorbance (Y) formed a standard curve, namely $Y = 0.195 + 29.12\ X$ ($r = 0.9995$), $Y = 0.221 + 47.00\ X$ ($r = 0.9997$), and $Y = 0.155 + 61.50\ X$ ($r = 0.9996$), respectively.

Preparation of CDD

Carboxymethyl-β-CD was synthesized according to the method reported by Zhou et al.[36] The yield was 22% from the native β-CD. Elemental analysis: C, 40.61%; H, 5.14%. FAB-MS, m/z, 1573 ($[M + 1]^+$). The degree of substitution was 0.27.

Preparation of CDD-C

In a typical procedure (Scheme 1), the CDD-grafted chitosan (CDD-C) was synthesized as follows: 30 mL of the SOCl2 and 2.000 g of the CDD were stirred for 12 h at 72°C. About 0.5000 g of chitosan, 60 mL of DMF, and 20 mL of pyridine were then added to the reaction solution. The reaction solution was stirred for 12 h at 90°C. Then, brown solid was attained and



Scheme 1 Synthesis of a chitosan derivative (CDD-C).

washed with distilled water, acetone, and anhydrous ethyl alcohol in proper order for many times. The objective product (1.7136 g) was attained after dried with 5% acetic acid.

Adsorption experiments

Fifty milliliters of an ethanol solution of 150 mg/L guanosine or cytidine or uridine with different pH value, and 50 mg of CDD-C was put into an Erlenmeyer flask. After the flask was shaken at 150 rpm at different temperature for 6 h, 3 mL of the solution was removed and the concentrations of guanosine, cytidine, and uridine were determined according to the method described in Determination of Guanosine, Cytidine, and Uridine. The solution was returned to the flask after the measurement. The adsorption capacity of CDD-C on guanosine, cytidine, and uridine was measured and calculated according to following equation:

$$Q = \lfloor (C_0 - C_f)\ V/1000 \rfloor / M$$

where Q is an adsorption capacity of CDD-C (mg/g), C_0 the initial concentration (mg/L) of guanosine, cytidine, and uridine, C_f the final concentration (mg/L) of guanosine, cytidine, and uridine, V the volume of solution tested (mL), and M the mass of dried CDD-C (g). The adsorption capacity is an important parameter to evaluate chitosan derivative for application as an absorbent.

Adsorption isotherm models

Fifty milliliters of ethanol solution of 10–200 mg/L guanosine or cytidine or uridine with pH 3.0, 50 mg of CDD-C, and chitosan for comparison were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm at 30°C for 6 h, 3 mL of the solution was removed and the concentrations of guanosine, cytidine, and uridine were determined according to the method described in Determination of Guanosine, Cytidine, and Uridine.

RESULTS AND DISCUSSION

Characterization of CDD-C

FTIR analysis

CDD-C was prepared from chitosan and β-CD. Infrared spectroscopy was used to follow the chemical structure of the produced derivatives. The IR spectra [Fig. 1(c) and (d)] clearly showed that the process has no significant influence on the structure of chitosan. Any change in the FTIR spectrum in this region can be directly attributed to a change in the carbonyl group environment, such as binding with other group.

The FTIR spectrum of CDD-C was compared with those of β-CD, CDD, and chitosan (Fig. 1). The infrared spectra of chitosan and its derivatives showed a wide band appeared from 3600 to 2400 cm^{-1} due to NH_2 and OH stretching vibration [Fig. 1(c,d)]. The relative absorbance the band at 3400 cm^{-1}, which is characteristic to OH group ratio of the absorbance at the subscript wave number 3400 cm^{-1} to the absorbance of wave number at 1325 cm^{-1}, which corresponds to the CH rocking of the ring, is higher than that chitosan (Table I). The spectrum of CDD-C showed peaks ascribable to both CDD (O—H, 3398 cm^{-1}, C=O, 1715 cm^{-1}, C—O—C, 1088 cm^{-1}), and the chitosan skeleton (N—H, 2400–3500 cm^{-1}, and N—H, 1599 cm^{-1}). The wave number of C=O group changed largely from CDD to CDD-C. It may be that

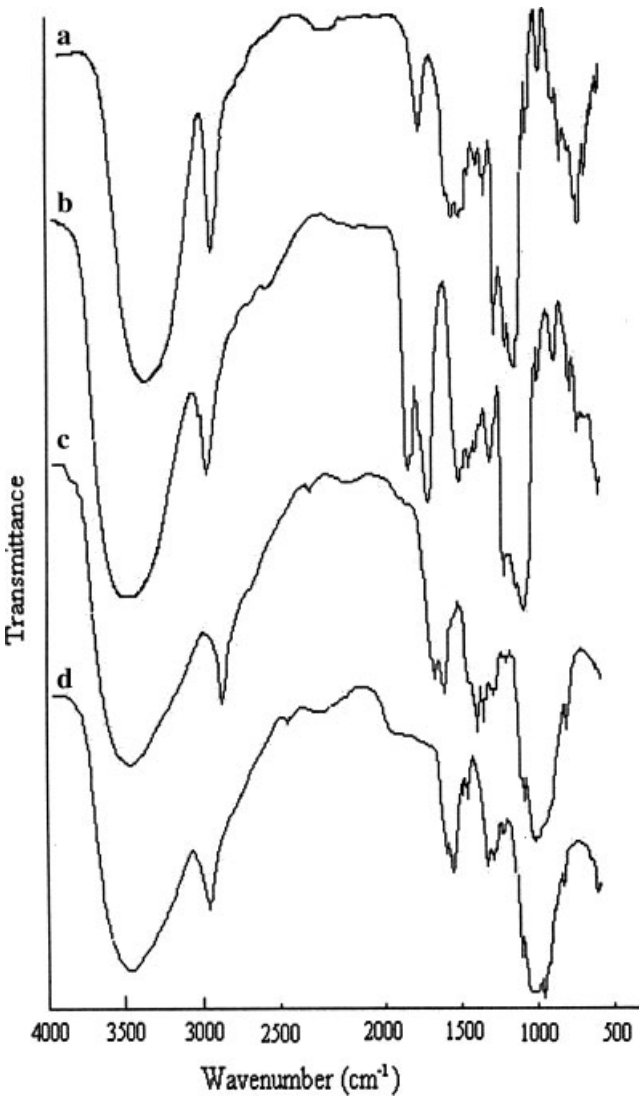


Figure 1 FTIR spectra of (a) β-CD, (b) CDD, (c) chitosan, and (d) CDD-C.

TABLE I
FTIR Characteristic of Chitosan and Its Derivatives

	Wave number (cm^{-1})			
Material	OH	C—H	C=O	N—H/O—H
β-CD	3415	2927	–	3600–3100
CDD	3338	2928	1730	3600–3100
Chitosan	3440	2816	–	3600–2400
CDD-C	3398	2830	1715	3600–2400

C=O exited as COOH in CDD. But in CDD-C, C=O exited as CONH.

XRD analysis

Figure 2 showed the XRD patterns of chitosan and CDD-C. The XRD pattern of chitosan shows the characteristic peak at $2\theta = 10$ and 20. It can be found that the peak at $2\theta = 10$ disappeared, and the characteristic peak at $2\theta = 20$ decreased obviously in CDD-C. The decrease in crystallinity of CDD-C was resulted from the deformation of the strong hydrogen bond in the original chitosan because of the substitution of amino and hydroxyl groups by CDD. The low crystallinity of CDD-C indicated that they are more amorphous than chitosan.

Thermal analysis

The characterization of the complexes in the solid state was performed by thermal analysis. The DTA curves of CDD-C, β-CD, and chitosan were illustrated in Figure 3. CDD exhibited a broad peak near 90°C [Fig. 3(b)], which can be traced to the release of water. CDD-C exhibited a broad peak near 116°C. Chitosan displayed an endothermic transition at about 237°C

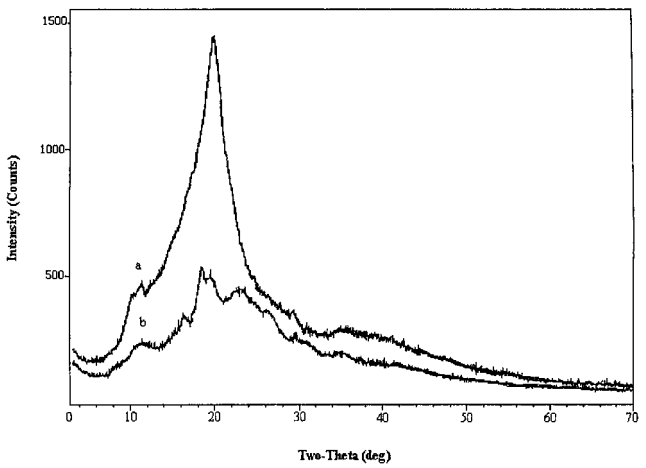


Figure 2 X-ray diffraction patterns of chitosan (a) and CDD-C (b).

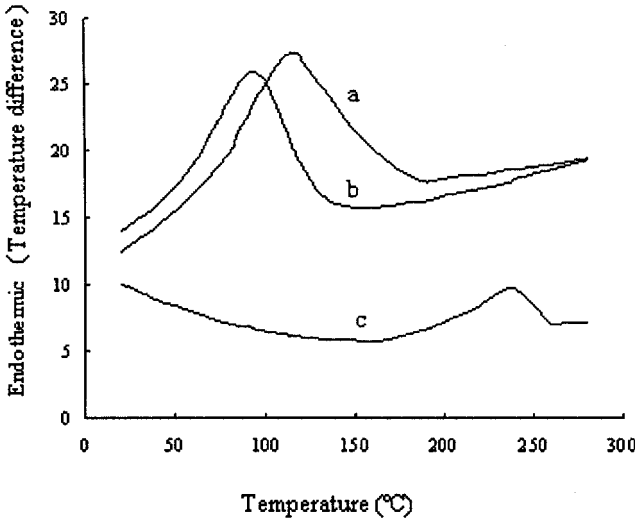


Figure 3 DTA curves of CDD-C (a), CDD (b), and chitosan (c).

(DTA curve) corresponding to its decomposition temperature. This transition disappeared in the DTA thermogram of the CDD-C.

Adsorption behavior of CDD-C

Effect of pH value on adsorption capacity of CDD-C

Fifty milliliters of ethanol solution of 150 mg/L guanosine or cytidine or uridine with different pH value, namely 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, and 9.50 and 50 mg of CDD-C were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm at 30°C for 6 h, the concentrations of guanosine, cytidine, and uridine were determined according to the method described in Determination of Guanosine, Cytidine, and Uridine.

Figure 4 showed the effect of pH value on adsorption capacity of CDD-C. With the increase of pH value ($\gg$3.0), the adsorption capacity of CDD-C decreased rapidly. The influence of the pH is given as a function of the sorption capacity (mg/g). The optimum pH appeared to be around pH 2.5–3. Sorption efficiency (and sorption capacity) increased from pH 1 to pH 3 for guanosine [Fig. 4(a)]. But, it appeared that the increase of pH value (pH 1–3) was significantly less important for uridine and cytidine [Fig. 4(b,c)]. A maximum was reached at pH 3 for guanosine, uridine, and cytidine. Above pH 3, sorption capacity decreased again, but it appeared that the decrease was significantly more important for guanosine, uridine, and cytidine than from pH 1 to pH 3. At pH 4, the sorption capacity decreased by less than 20% for guanosine, uridine, and cytidine. The decrease exceeded 50% at pH 7. It appeared that the grafting allowed the influence of the pH to be decreased due

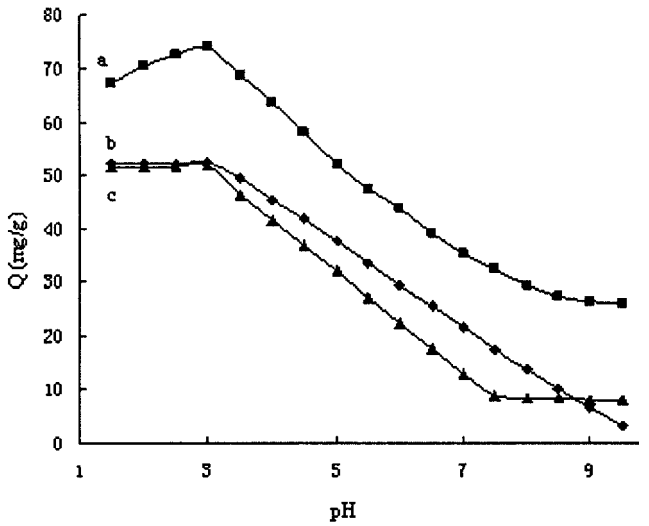


Figure 4 Effect of pH on adsorption by CDD-C (initial concentration of guanosine, cytidine, and uridine is 150 mg/L, vibrating time is 6 h, and dosage of CDD-C is 50 mg). (a) guanosine, (b) uridine, and (c) cytidine (30°C).

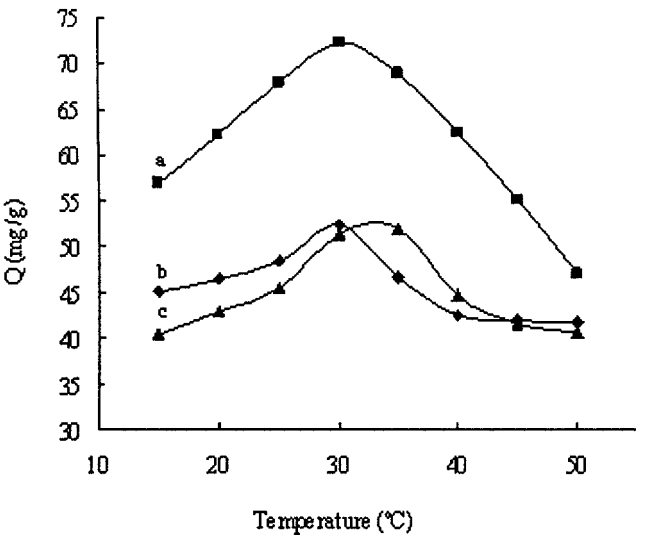


Figure 5 Effect of temperature on adsorption by CDD-C (initial concentration of (a) guanosine, (b) cytidine, and (c) uridine is 150 mg/L, vibrating time: 6 h, pH: 3.0, and dosage of CDD-C is 50 mg).

to the partial change in the sorption mechanism instead of a pure ion exchange mechanism with cross-linked material. And also guanosine, uridine, and cytidine are strongly sensitive to the pH. The sorption mechanism may relate to the pKa of guanosine ($pKa_1 = 2.4$), uridine ($pKa_1 = 9.5$), and cytidine ($pKa_1 = 4.5$). The optimum pH was 3.0. It is also interesting to note that sorption capacity was significantly higher for guanosine at pH 3.0 than uridine and cytidine.

Effect of temperature on adsorption capacity of CDD-C

Fifty millililers of ethanol solution of 150 mg/L guanosine or cytidine or uridine with pH 3.0 and 50 mg of CDD-C were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm at different temperature, namely 15, 20, 25, 30, 35, 40, 45, and 50°C for 6 h, the concentrations of guanosine, cytidine, and uridine were determined according to the method described in Determination of Guanosine, Cytidine, and Uridine. Figure 5 showed the effect of temperature on adsorption capacity of CDD-C. With the increase of temperature (15–30°C), the adsorption capacity of CDD-C increased obviously. However, after 30°C, increasing of temperature resulted in decreasing the adsorption capacity of CDD-C. It may be that stability of complex of CDD-C with guanosine, cytidine, and uridine reduced under high temperature. With increasing of temperature, the binding capacity between CDD-C and guanosine (or cytidine and uridine) decreased. Thus, it affected the adsorption effect of CDD-C on guanosine, cytidine, and uridine. The optimum temperature was 30°C.

Adsorption kinetics of CDD-C

Simple batch adsorption kinetics experiments of CDD-C were carried out. Fifty milliliters of ethanol solution of 150 mg/L guanosine or cytidine or uridine with pH 3.0 and 50 mg of CDD-C was put into an Erlenmeyer flask. After the flask was shaken at 150 rpm at 30°C for different time, namely 1, 2, 3, 5, 6, 7, 8, and 9 h, the concentrations of guanosine, cytidine, and uridine were determined according to the method described in Determination of Guanosine, Cytidine, and Uridine. The adsorption kinetics was showed in Figure 6. The results demonstrate that the adsorption

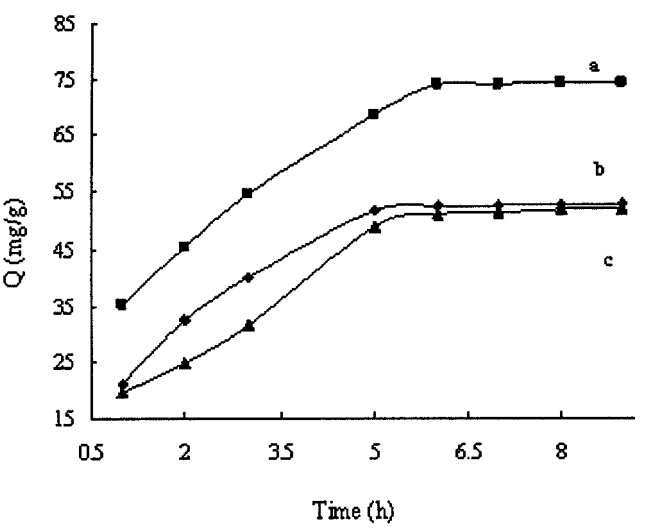


Figure 6 Effect of time on adsorption by CDD-C (initial concentration of (a) guanosine, (b) cytidine, and (c) uridine is 150 mg/L, temperature: 30°C, pH: 3.0, and dosage of CDD-C is 50 mg).

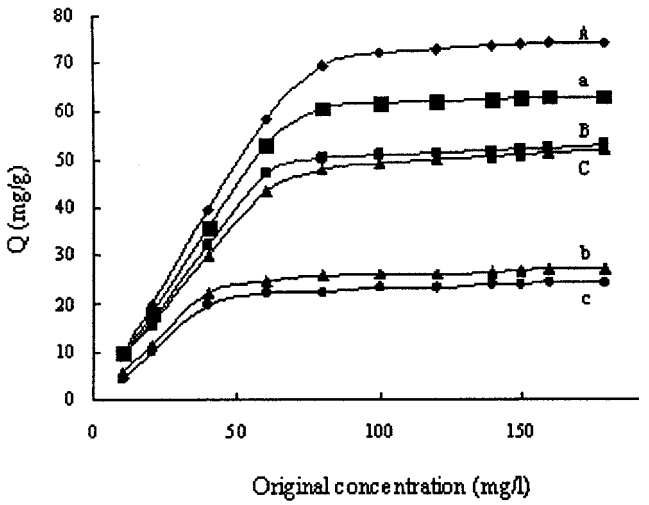


Figure 7 Adsorption isotherm of CDD-C and chitosan for guanosine, cytidine, and uridine. A, B, and C: adsorption capacity of CDD-C versus initial concentrations; a, b, and c: adsorption capacity of chitosan versus initial concentrations; A and a for guanosine; B and b for cytidine; C and c for uridine.

of CDD-C is fast. Specifically, CDD-C showed a high adsorption velocity for guanosine in 2 h and the adsorption reaches equilibrium in about 6 h; while the adsorption of uridine and cytidine needs 5 h to reach equilibrium. Guanosine and cytidine showed similar kinetic behavior, but different for uridine. In the first 3 h, the adsorption velocity for guanosine and cytidine was faster, but the adsorption velocity for uridine was slower. The adsorption of CDD-C reaches the equilibrium essentially after 6 h.

Adsorption isotherm models

Fifty milliliters of ethanol solution of 20–180 mg/L guanosine or cytidine or uridine with pH 3.0, 50 mg of CDD-C and chitosan for comparison were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm at 30°C for 6 h, the concentrations of guanosine, cytidine, and uridine were determined according to the method described in ''Determination of guanosine, cytidine, and uridine'' above. Figure 7 showed the adsorption isotherm of CDD-C and chitosan for guanosine, cytidine, and uridine via static balance combination method. CDD-C [Fig. 7(A)–(C)] has higher adsorption capability for these three compounds than chitosan [Fig. 7(a)–(c)]. However, CDD-C has more excellent adsorption capability for guanosine than cytidine and uridine. The adsorption capability of CDD-C for guanosine, cytidine, and uridine were 74.20, 53.01, and 51.93 mg/g, respectively. But, the adsorption capability of chitosan for guanosine, cytidine, and uridine were only 63.04, 27.25, and 24.49 mg/g, respectively.

CONCLUSIONS

A novel β-CD derivative was synthesized by the reaction between chitosan and carboxymethyl-β-CD. Its structure was characterized by FTIR spectra, elemental, X-ray diffraction, and thermal analyses, which was shown in an agreement with the expected results. The introduction of chitosan enhanced the adsorption ability and adsorption selectivity of β-CD for guanosine. This novel derivative of chitosan is expected to have wide applications in separation, concentration, and analysis of nucleotides in biological sample.

References

1. Crini, G. Prog Polym Sci 2005, 30, 38.
2. Shi, T. C.; Jing, Y. M.; Sui, D. X. J Chromatogr 1996, 742, 107.
3. Kweon, D. K.; Song, S. B.; Park, Y. Y. Biomaterials 2003, 24, 1595.
4. Khnor, E.; Lim, L. Biomaterials 2003, 24, 2339.
5. Sato, T.; Ishii, T.; Okahata, Y. Biomaterials 2001, 22, 2075.
6. Mao, J. S.; Liu, H. F.; Yin, Y. J.; Yao, K. D. Biomaterials 2003, 24, 1621.
7. Gingras, M.; Paradis, I.; Berthod, F. Biomaterials 2003, 24, 1653.
8. Wang, Y. C.; Lin, M. C.; Wang, D. M.; Hsieh, H. J. Biomaterials 2003, 24, 1047.
9. Schnurch, A. B.; Hornof, M.; Zoidl, T. Int J Pharm 2003, 260, 229.
10. Prabaharan, M.; Mano, J. F. Drug Deliv 2005, 12, 41.
11. Kurita, K. Prog Polym Sci 2001, 26, 1921.
12. Gupta, K. C.; Kumar, M. N. V. R. JMS-Rev Macromol Chem Phys 2000, 40, 273.
13. Kumar, M. N. V. R. React Funct Polym 2000, 46, 1.
14. Sashiwa, H.; Aiba, S.-i. Prog Polym Sci 2004, 29, 887.
15. Prabaharan, M.; Mano, J. F. Carbohydr Polym 2006, 63, 153.
16. Varma, A. J.; Deshpande, S. V.; Kennedy, J. F. Carbohydr Polym 2004, 55, 77.
17. Grcic, J. F.; Voinovich, D.; Moneghini, M.; Lacan, M. B.; Magarotto, L.; Jalsenjak, I. Eur J Pharm Sci 2000, 9, 373.
18. Rossi, S.; Ferrari, F.; Bonferoni, M. C.; Caramella, C. Eur J Pharm Sci 2000, 10, 251.
19. Buschmann, H. J.; Knittel, D.; Schollmeyer, E. J Inclusion Phenom Macrocyclic Chem 2001, 40, 169.
20. Al, E. M.; Valle, M. D. Process Biochem 2004, 39, 1033.
21. Pablo, R. S. R.; Isasi, J. R.; Gaitano, G. G. J Photochem Photobiol A 2005, 173, 248.
22. Rozou, S.; Michaleas, S.; Vyza, E. A. J Chromatogr A 2005, 1087, 86.
23. Wang, T. L.; Yuan, L. L.; Li, F. Y. J Chromatogr A 2003, 1013, 19.
24. Yang, J.; Lin, H. J.; Huang, H. Y. Anal Chim Acta 2005, 530, 213.
25. Jursic, B. S.; Patel, P. K. Tetrahedron 2005, 61, 919.
26. Fakayode, S. O.; Swamidoss, I. M.; Busch, M. A.; Busch, K. W. Talanta 2005, 65, 838.
27. Baglole, K. N.; Boland, P. G.; Wagner, B. D. J Photochem Photobiol A 2005, 173, 230.
28. Fini, P.; Castagnolo, M.; Catucci, L.; Cosma, P.; Agostiano, A. Thermochim Acta 2004, 418, 33.
29. Auzely, R.; Rinaudo, M. Macromolecules 2001, 34, 3574.
30. Jiménez, V.; Belmar, J.; Alderete, J. B. J Inclusion Phenom Macrocyclic Chem 2003, 47, 71.
31. Prabaharan, M.; Mano, J. F. Macromol Biosci 2005, 5, 965.
32. Venter, J. P.; Kotze, A. F.; Auzely-Velty, R.; Rinaudo, M. Int J Pharm 2006, 313, 36.
33. Auzély-Velty, R.; Rinaudo, M. Macromolecules 2002, 35, 7955.
34. Maestrelli, F.; Garcia-Fuentes, M.; Mura, P.; Alonso, M. J. Eur J Pharm Sci 2006, 63, 79.
35. Jug, M.; Becirevic-Lacan, M. Eur J Pharm Sci 2004, 21, 251.
36. Zhou, Y. H.; An, W. T.; Zhang, G. M.; Shuang, S. M. J Shanxi Univ 2005, 28, 276.