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Adsorption of Nucleotides on β-Cyclodextrin Derivative Grafted Chitosan

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Abstract: A novel β-cyclodextrin derivative (CCD-C) was synthesized with chitosan and carboxymethyl-β-cyclodextrin. Its structure was characterized by elemental analysis, infrared spectra analysis, and X-ray diffraction analysis. The adsorption properties for guanosine 5′-monophosphate, cytidine 5′-monophosphate and uridine 5′-monophosphate were studied. Experimental results demonstrated that CCD-C had higher adsorption capability for guanosine 5′-monophosphate, and that the adsorption capacity for guanosine 5′-monophosphate 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-cyclodextrin for guanosine 5′-monophosphate. This novel derivative of chitosan is expected to have wide applications in the separation, concentration and analysis of nucleotides in biological samples.

Keywords: chitosan, carboxymethyl-β-cyclodextrin, nucleotides, adsorption.

Introduction

Chitosan is available in a variety of useful forms and its unique chemical and biological properties make it very attractive biomaterials. It is extensively used in many types of applications such as treatment of wastewater,[1] chromatographic support.[2] In those applications, chitosan's key properties are biocompatibility, non-toxicity (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 are receiving greater attention as novel functional materials.

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 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 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 more 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 microencapsulation of unstable or volatile substances.[23-29] CD has the merit of a hydrophobic cavity, which is easy to assemble with other molecules. Chitosan has the merit of degradation 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.[30] The products

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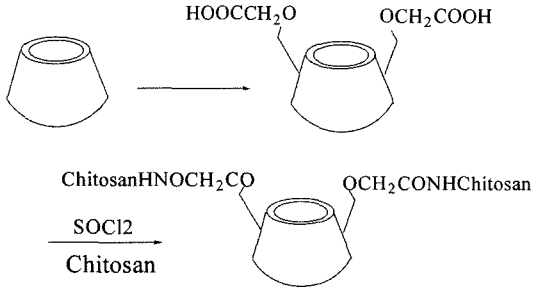
obtained by CD grafting to chitosan using different methods and their inclusion ability, sorption and controlled release properties have been studied extensively.[31-36]

In this study, a novel β-cyclodextrin derivative of chitosan (CCD-C) was synthesized with chitosan and carboxymethyl-β-cyclodextrin via one step. The static adsorption properties for guanosine 5′-monophosphate (GMP), cytidine 5′-monophosphate (CMP) and uridine 5′-monophosphate (UMP) were studied. Experimental results demonstrated that CCD-C had higher adsorption capability GMP.



Scheme I. Synthesis of a chitosan derivative (CCD-C).

Experimental

Materials. β-Cyclodextrin (β-CD) of reagent grade was recrystallized twice from water and dried for 12 h in vacuum at 100 °C. Chitosan was purchased from Qingdao Baicheng Biochemical Corp (Qingdao, China). Its degree of deacetylation was 88.03%, and the viscosity average-molecular weight was 2.0×10^5. Guanosine 5′-monophosphate (GMP), cytidine 5′-monophosphate (CMP) and uridine 5′-monophosphate (UMP) 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 1-70° using a Rigaku (D/MAX, 2500X) diffractometer. The diffractometer was equipped with a CuKα (l=0.1542 nm) radiation, produced under the conditions of 40 kV and 100 mA. Elemental analysis was determined with a Perkin-Elmer automatic instrument. Differential thermal analysis (DTA) was carried out on a Netzsch STA 409 simultaneous thermal analyzer (Netzsch Italiana, Verona, Italy).

Determination of GMP, CMP, and UMP. The concentration of GMP, CMP, and UMP were 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, 80 mg/L. The determination was performed with a 756MC UV-vis spectrophotometer at 260 nm. The data of the content (X) of GMP, CMP or UMP and absorbance (Y) formed a standard curve, namely Y=0.195+29.12X (r=0.9995), Y=8.221+47.00X (r=0.9997), and Y=3.155+61.50X (r=0.9996), respectively.

Preparation of CCD. Carboxymethyl-β-cyclodextrin (CCD) was synthesized according to the method reported by Zhou *et al.*[37] 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 0.42.

Preparation of CCD-C. In a typical procedure, the CCD-grafted chitosan (CCD-C) was synthesized as follows: 30 mL of the $SOCl_2$ and 2.000 g of the CCD were stirred for 12 h at 72 °C. 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 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 mL of an ethanol solution of 150 mg/L GMP or CMP or UMP with different pH value and 50 mg of CCD-C were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm/min at different temperature for 6 h, 3 mL of the solution was removed and the concentrations of GMP, CMP, and UMP were determined according to section of Determination of GMP, CMP, and UMP. The solution was returned to the flask after the measurement. The adsorption capacity of CCD-C on GMP, CMP, and UMP was measured and calculated according to following equation:

$$Q = [(C_0 - C_f)\, V \times 1000] / M \qquad (1)$$

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

Adsorption Isotherm Models. Fifty mL of an ethanol solution of 10-200 mg/L GMP or CMP or UMP with pH 3.0, 50 mg of CCD-C and chitosan for comparison were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm/min at 30 °C for 6 h, 3 mL of the solution was removed and the concentrations of and the concentrations of GMP, CMP, and UMP were determined according to section of Determination of GMP, CMP, and UMP.

Results and Discussion

Characterization of CCD-C.

FTIR Analysis: Chitosan undergoes different reactions to produce derivatives used as additives in paper sheets. CCD-C was prepared from chitosan and β-CD. Infrared spectroscopy

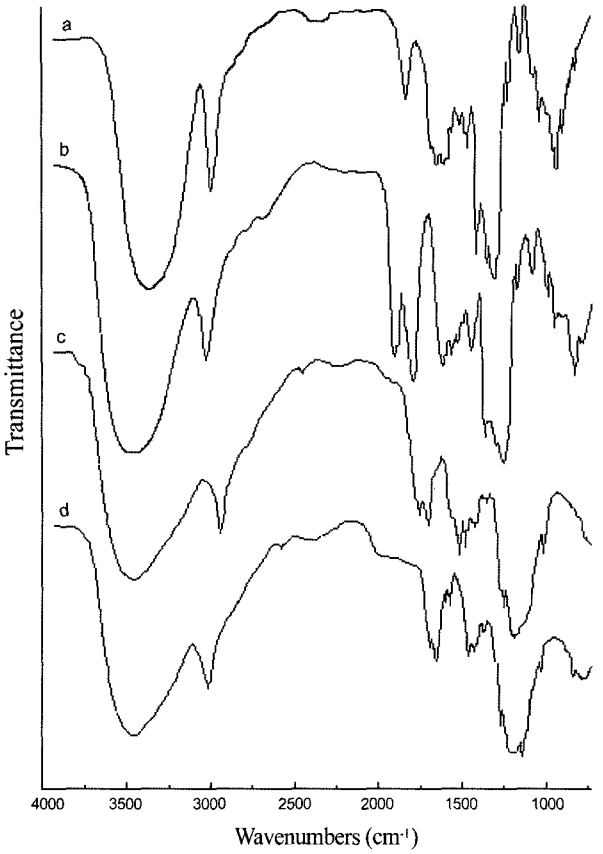


Figure 1. FTIR spectra of (a) α-CD, (b) CCD, (c) chitosan, and (d) CCD-C.

was used to follow the chemical structure of the produced derivatives.

The FTIR spectrum of CCD-C was compared with those of β-CD, CCD, and chitosan (Figure 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 (Figure 1(c) and 1(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 CCD-C showed peaks

Table I. FTIR Characteristic of Chitosan and Its Derivatives

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

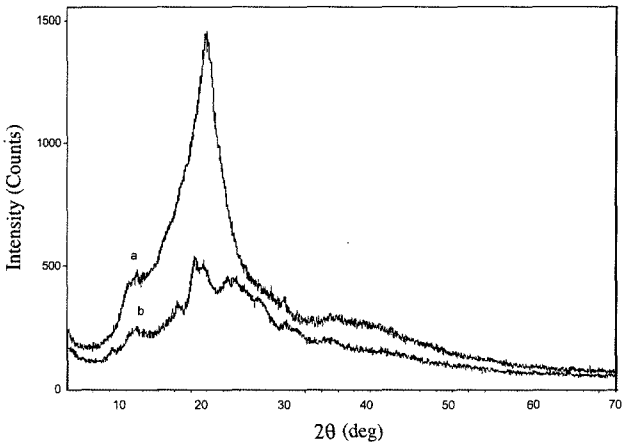


Figure 2. X-ray diffraction patterns of chitosan (a) and CCD-C (b).

ascribable to both CCD (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 wavenumber of C=O group changed largely from CCD to CCD-C. It may be that C=O exited as COOH in CCD. But in CCD-C, C=O exited as CONH.

XRD Analysis: Figure 2 showed the XRD patterns of chitosan and CCD-C. The XRD pattern of chitosan shows the characteristic peak at 2θ=10 and 20°. It can be found that the peak at 2θ=10° disappears, and the characteristic peak at 2θ=20° decreases obviously in CCD-C. The decrease in crystallinity of CCD-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 CCD. The low crystallinity of CCD-C indicates they are more amorphous than chitosan.

Thermal Analysis: Differential thermal analysis (DTA) was carried out on a Netzsch STA 409 simultaneous thermal analyzer. The samples (4-15 mg) were accurately weighed in platinum pans (Netzsch) and heated at a scanning rate of 10 °C/min. The characterization of the complexes in the solid state was performed by thermal analysis. The DTA profiles of CCD-C, β-CD, and chitosan were illustrated in Figure 3. β-CD exhibited a broad peak near 90 °C, which can be traced to the release of water. CCD-C exhibited a broad peak near 116 °C. Chitosan displayed an endothermic transition at about 237 °C (DTA curve) corresponding to its boiling temperature. This transition disappeared in the DTA thermogram of the CCD-C.

Adsorption Behavior of CCD-C.

Effect of pH Value on Adsorption Capacity of CCD-C: Fifty mL of an ethanol solution of 150 mg/L GMP or CMP or UMP 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 9.5, and 50 mg of CCD-C were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm/min at 30 °C for 6 h,

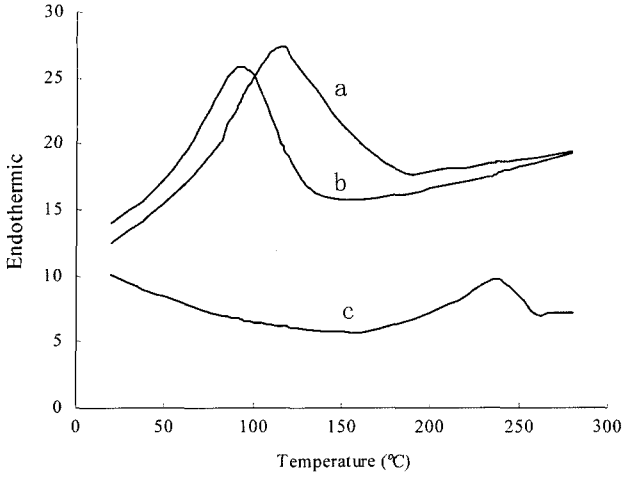


Figure 3. DTA thermogram of CCD-C (a), CCD (b), and chitosan (c).

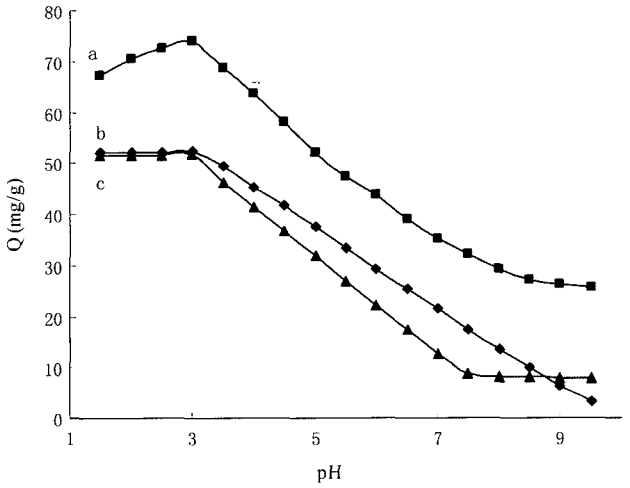


Figure 4. Effect of pH on adsorption by CCD-C (initial concentration of GMP, CMP, and UMP is 150 mg/L, vibrating time: 6 h, dosage of CCD-C is 50 mg) (a. GMP, b. UMP, c.CMP).

the concentrations of GMP, CMP, and UMP were determined according to section of Determination of GMP, CMP, and UMP.

Figure 4 showed the effect of pH value on adsorption capacity of CCD-C. With the increase of pH value (>3.0), the adsorption capacity of CCD-C decreased rapidly. The influence of the pH is given as a function of the relative residual concentration (C_f/C_0) and sorption capacity (mg/g). The optimum pH appeared to be around pH 2.5-3. Sorption efficiency (and sorption capacity) strongly increased from pH 1 to 3 for GMP. But it appears that the increase was significantly less important for UMP and CMP from pH 1 to 3. A maximum was reached at pH 3 for GMP, UMP, and CMP. Above pH 3, sorption capacity decreased again, but it appears that the decrease was significantly more important for GMP, UMP, and CMP than from pH 1 to 3. At pH 4, the sorption capacity decreased by less than 20% for GMP, UMP, and CMP. The decrease exceeded 50% at pH 7. It appears that the grafting allowed the influence of the pH to be decreased due to the partial change in the sorption mechanism instead of a pure ion exchange mechanism with cross-linked material. And also GMP, UMP, and CMP are strongly sensitive to the pH. The optimum pH was 3.0. It is also interesting to note that sorption capacity was significantly higher for GMP at pH 3.0 than with UMP and CMP.

Effect of Temperature on Adsorption Capacity of CCD-C: Fifty mL of an ethanol solution of 150 mg/L GMP or CMP or UMP with pH 3.0 and 50 mg of CCD-C were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm/min at different temperature, namely 15, 20, 25, 30, 35, 40, 45, and 50 °C for 6 h, the concentrations of GMP, CMP, and UMP were determined according to section of Determination of GMP, CMP, and UMP. Figure 5 showed the effect of temperature on adsorption capacity of CCD-C. With the increase of temperature (15-30 °C), the adsorption capacity of CCD-C increased obviously. However after 30 °C, increasing of temperature resulted to the adsorption capacity of CCD-C decrease. It may be that stability of complex of CCD-C with GMP, CMP, and UMP reduced under high temperature. Thus it affected the adsorption of CCD-C. The optimum temperature was 30 °C.

Adsorption Kinetics of CCD-C. Simple batch adsorption kinetics experiments of CDD-C were carried out. Fifty mL of an ethanol solution of 150 mg/L GMP or CMP or UMP with pH 3.0 and 50 mg of CCD-C were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm/min at 30 °C for different time, namely 1, 2, 3, 5, 6, 7, 8, and 9 h,

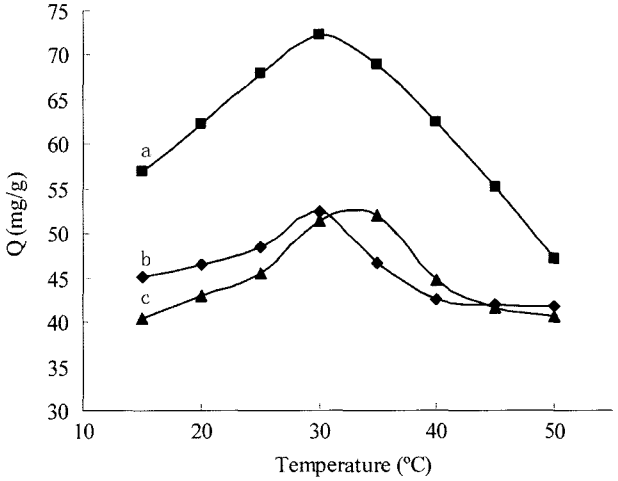


Figure 5. Effect of temperature on adsorption by CCD-C (initial concentration of GMP, CMP, and UMP is 150 mg/L, vibrating time: 6 h, pH: 3.0, dosage of CCD-C is 50 mg) (a. GMP, b. UMP, c.CMP).

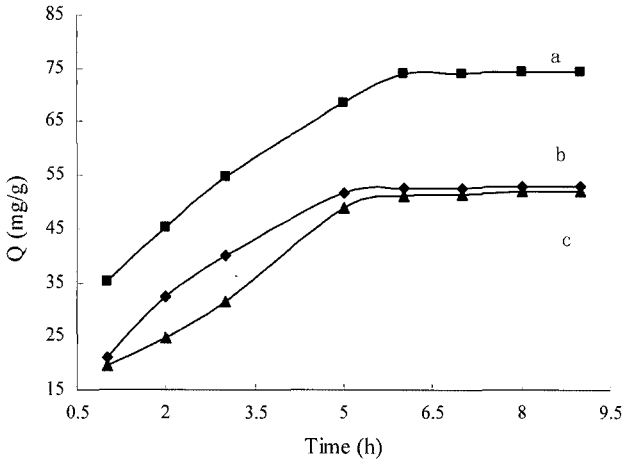


Figure 6. Effect of time on adsorption by CCD-C (initial concentration of GMP, CMP, and UMP is 150 mg/L, temperature: 30 °C, pH: 3.0, dosage of CCD-C is 50 mg) (a. GMP, b. UMP, c.CMP).

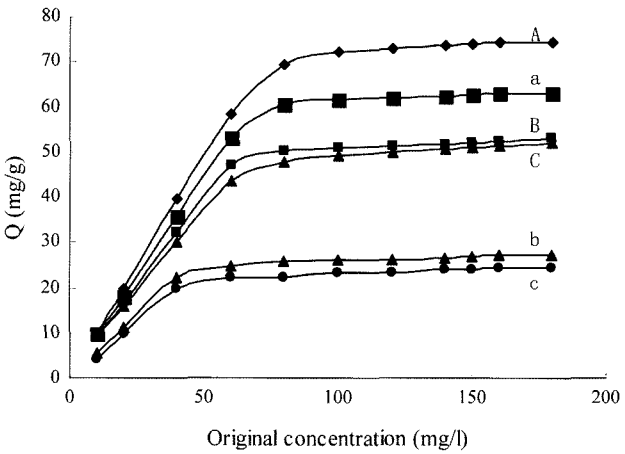


Figure 7. Adsorption isotherm of CCD-C and chitosan for GMP, CMP, and UMP. A, B, and C: Adsorption capacity of CCD-C *vs.* initial concentrations; a, b, and c: Adsorption capacity of chitosan *vs.* initial concentrations; A and a for GMP: B and b for CMP: C and c for UMP.

the concentrations of GMP, CMP, and UMP were determined according to section of Determination of GMP, CMP, and UMP. The adsorption kinetics were showed in Figure 6. The results demonstrate that the adsorption of CDD-C is fast. Specifically, CCD-C shows a high adsorption velocity for GMP in 2 h and the adsorption reaches equilibrium in about 6 h; while the adsorption of UMP and CMP needs 5 h to reach equilibrium. GMP and CMP show similar kinetic behavior, but different for UMP. In the first 3 h, the adsorption velocity for GMP and CMP is faster. But the adsorption velocity for UMP is slower. The adsorption of CCD-C reaches the equilibrium essentially after 6 h.

Adsorption Isotherm Models. Fifty mL of an ethanol solution of 20-180 mg/L GMP or CMP or UMP with pH 3.0, 50 mg of CCD-C and chitosan for comparison were put into an Erlenmeyer flask. After the flask was shaken at 150 rpm/min at 30 °C for 6 h. Figure 7 showed the adsorption isotherm of CCD-C and chitosan for GMP, CMP, and UMP via static balance combination method. CCD-C (Figure 7(A), (B) and (C)) has higher adsorption capability for these three compounds than chitosan (Figure 7(a), (b) and (c)). However, CCD-C has a more excellent adsorption capability for GMP than CMP and UMP. The adsorption capability of CCD-C for GMP, CMP, and UMP was 74.20, 53.01, and 51.93 mg/g, respectively. But the adsorption capability of chitosan for GMP, CMP, and UMP was 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 CCD. Its structure was characterized by FTIR spectra analysis, elemental analysis, X-ray diffraction analysis, and thermal analysis, which was shown in agreement with expected results. The introduction of chitosan enhanced the adsorption ability and adsorption selectivity of β-CD for GMP. This novel derivative of chitosan is expected to have wide applications in separation, concentration and analysis of nucleotides in biological sample.

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