Synthesis of Chitosan C6-Substituted Cyclodextrin Derivatives with Tosyl-Chitin as the Intermediate Precursor

Yu Chen,[1] Yanchun Ye,[2] Liye Wang,[2] Yanwen Guo,[2] Huimin Tan[1]

[1]*School of Material Science and Engineering, Beijing Institute of Technology, Beijing 100081, People's Republic of China*
[2]*School of Science, Beijing Institute of Technology, Beijing 100081, People's Republic of China*

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ABSTRACT: A novel method was developed for the preparation of chitosan derivative with a high substitution of cyclodextrins at 6-OH positions. Tosylated chitin at 6-OH positions (6-OTs-Chitin) was prepared and then displaced by a monoamino β-cyclodextrin derivative (Mono-[6-(2-amino-ethyl)-amino-6-deoxy]-β-cyclodextrin, CDen) via nucleophilic substitution of the tosyl group to afford the 6-OH substituted cyclodextrin derivatives (Chitin-en-CD). Further removal of acetyl groups on the chitin main chain yielded the C6 substituted cyclodextrin derivatives (CTS-en-CD). The structures of the products were characterized by FTIR and their crystallinity properties were studied by XRD. The thermal stability of the product was studied by TGA. The optimal conditions for nucleophilic substitution of CDen to 6-OTs-Chitin were evaluated and the highest substitution capacity of Chitin-en-CD was found to be 93.97 μmol/g. Substitution capacity of the corresponding CTS-en-CD was 128.68 mol/g, which is much higher than the literature value. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: chitin; chitosan; β-cyclodextrin; tosylated; nucleophilic substitution

INTRODUCTION

The amino group (2-NH_2) on the pyranose of chitosan allows for chemical modification and also gives chitosan great physiological activities, making chitosan a well studied functional polymer.[1–3] Cyclodextrins are made up of several sugar molecules linked into a ring structure, where the exterior is hydrophilic and the interior is hydrophobic with a cavity that can accommodate hydrophobic molecules. Their structural and amphiphilic properties have become the focus of supramolecular chemistry as a class of host macrocyclic compounds.[4–6] Thus, the incorporation of cyclodextrin onto chitosan chains may effectively combine their features and lead to supramolecular biological macromolecules with unique properties. Immobilization of cyclodextrin on chitosan are well studied,[7,8] but in most cases the modifications were made on 2-NH_2, which is not conducive to further amino group modification. If cyclodextrin can be immobilized on chitosan at the 6-OH position while preserving the 2-NH_2 group, this can greatly expand the applications of such derivatives. For 6-OH grafted cyclodextrin derivatives of chitosan, the antibacterial, tissue repair, enzyme immobilizing properties of 2-NH_2 of chitosan and the including properties of cyclodextrin on 6-OH of chitosan could be combined, and the derivates show promising applications in the fields of medicine, environment protection, molecular identify, etc. In this regard, many studies have explored modification routes aimed at the 6-OH of chitosan.[9–12] Since the 6-OH group has a lower reactivity than the 2-NH_2, preparation of 6-OH grafted cyclodextrin derivatives was more difficult and yielded very low substitution degree (<70 μmol/g). In comparison, derivatives of chitosan immobilized with cyclodextrin at the 2-NH_2 position prepared by condensing aldehyde-functionalized cyclodextrin derivatives via the Schiff reaction have a substitution degree of 240 μmol/g.[13]

Since the tosyl group is a good electrophile and leaving group, tosylation of the 6-OH group has been used to prepare tosyl-chitin intermediates for synthesizing other chitin derivatives.[14–16] Consequently, sodium iodide, sodium borohydride, and potassium thioacetate have been used as nucleophiles to displace the tosyl group and yielded the corresponding iodo-chitin, deoxy-chitin, and acetylthio-chitin derivatives. Similarly, Khor and coworker synthesized chitin derivatives of 6-*O*-ethyl benzoate-chitin, 6-*O*-carboxyphenyl-chitin, 6-deoxydiethyl-malonate-chitin, 6-deoxydi(carboxy)-methyl-chitin, and 6-deoxydiethyl phosphite-chitin by nucleophilic

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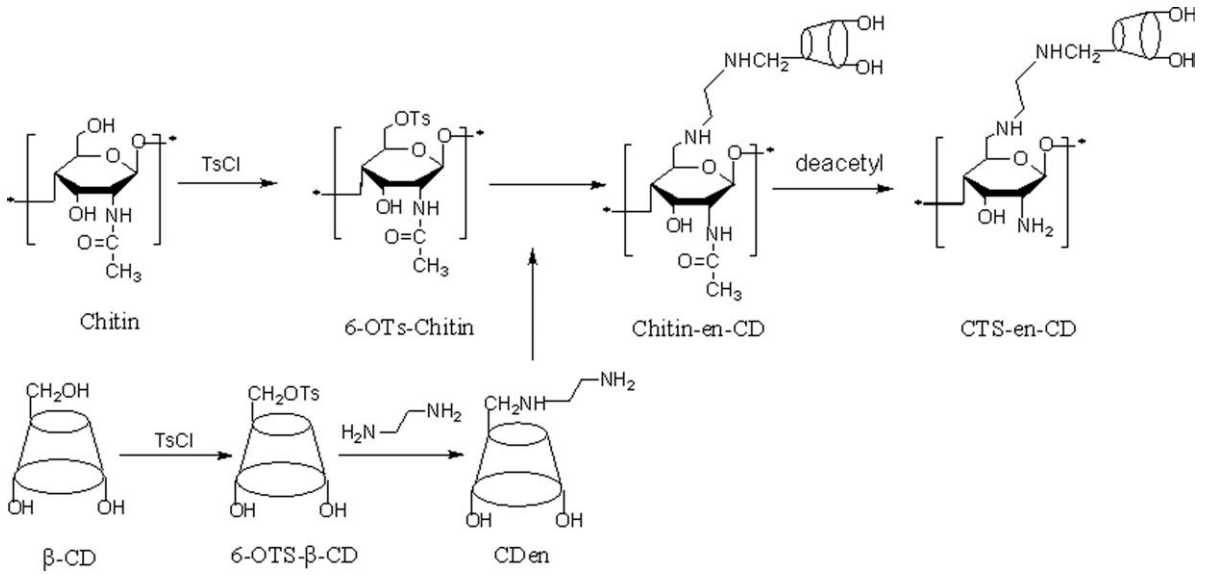

Figure 1 The nucleophilic substitution reaction routes of immobilization of β-CD on the C6 position of chitosan.

substitution of the tosyl-chitin intermediate precursor.[17] Surprisingly, studies based on this nucleophilic substitution route for the preparation of chitin and chitosan derivates have been less explored.

In this study, 6-OH groups on chitin were tosylated and then displaced by a monoamino substituted cyclodextrin derivative via nucleophilic substitution. Further removal of the acetyl groups on chitin afforded chitosan with a high substitution of supramolecular cyclodextrins at 6-OH positions. The main reaction route is shown in Figure 1.

EXPERIMENTAL

Materials

Chitin with a deacetylation degree of 18.9% was chemical grade and supplied by Zhejiang Yuhuan Biochemical (China). β-cyclodextrin (β-CD, $M_w \approx$ 1135.0, m.p. = 290–300°C) was analytical grade and purchased from Tianjin Bodi Chemical (China). *p*-Toluenesulfonyl chloride was analytical grade and supplied by Tianjin Guangfu Fine Chemical Research Institute (China). Mono-[6-(2-aminoethyl)-amino-6-deoxy]-β-cyclodextrin (CDen) was synthesized according to the method by Chen et al.[18] Other analytical grade reagents were obtained from Beijing Chemical Reagents Company (China) and used as received.

Synthesis of 6-OTs-chitin

To a 5% solution of lithium chloride in dimethylacetamide (DMAc) was added 1.0 g of chitin. The mixture was stirred at 20°C for 12 h to let chitin fully swell, then added with triethylamine and 1.5 g of *p*-toluenesulfonyl chloride in DMAc. After stirring at 40°C for 24 h, the reaction mixture was quenched by pouring into a large excess of water with stirring to precipitate the brown crude product. The solid was filtered and washed thoroughly with water and ethanol, then dried under vacuum to give 6-OTs-Chitin.

Synthesis of cyclodextrin substituted (at 6-OH) Chitin derivative (Chitin-en-CD)

To a three-necked flask equipped with a condenser was added CDen and 6-OTs-Chitin. A small amount of organic solvent was added to swell the solids. The mixture was stirred and heated at 50°C to ensure even mixing. At the end of the reaction, a yellow viscous liquid was obtained. The liquid was cooled and dialyzed (molecular weight cut-off at 3500) in water for a period of 7 days by changing water twice per day. During the dialysis, yellow precipitate was formed in the bag. The product was filtered and dried under vacuum to yield Chitin-en-CD as a yellow powder.

Synthesis of C6-immobilized cyclodextrin derivatives on chitosan (CTS-en-CD)

A mixture of 1.0 g of Chitin-en-CD and 25 mL of 45% NaOH solution was stirred and gradually heated to 100°C for 10 h. The crude product was precipitated by the addition of water and allowed to stand overnight. The supernatant was removed and the precipitate was repeatedly washed with water to a neutral pH, and then dried under vacuum to obtain a brown powder (CTS-en-CD).

Characterization

FTIR spectra were obtained on a NEXUS-470 series FTIR spectrometer (Nicolet Co., USA). Tested

samples were prepared with KBr pellets and scanned with a resolution 3 cm^{-1} (200 scans).

Wide angle X-ray diffraction (XRD) of samples was recorded on a X'pert Pro MPD type X-ray diffractometer (PaNalystical Co., Holand) with Cu Kα characteristic radiation (wavelength $\lambda = 0.154$ nm) at a voltage of 40 kV and a current of 50 mA. The scanning rate was 5°/min and the scanning scope of 2θ was from 5° to 50° at room temperature.

Thermogravimetric analyses (TGA) were carried out on a TA 2000 thermogravimetric analyzer (Dupont Co., USA) from 25 to 550 with heat rate of 20 °C/min under nitrogen.

UV–vis spectra were obtained on a Pgeneral TU-1810 UV–vis spectrophotometer (Beijing Purkinje General Instrument, China).

RESULTS AND DISCUSSION

FTIR analysis

The FTIR spectra of chitin, 6-OTs-Chitin, Chitin-en-CD ,and CTS-en-CD are shown in Figure 2. By comparing the FTIR spectra of Chitin with 6-OTs-Chitin, the absorption peak at 1602 cm^{-1} was attributed to the stretching vibration of the benzene ring, while the C—H bending vibration of the benzene ring were assigned at 811 cm^{-1} and 677 cm^{-1}. For the S=O bond of the tosyl group, the peak at 1347 cm^{-1} was attributed to the asymmetrical stretching vibration and the peak at 1175 cm^{-1} corresponded to the symmetrical stretching vibration. The results showed that the tosyl groups were immobilized on the C6 position of the pyranose ring.

After nucleophilic substitution with CDen, the absorption peaks at 1347 cm^{-1} and 1175 cm^{-1} from the tosyl group disappeared. The absorption peak at 1602 cm^{-1} due to the stretching vibration of the benzene ring disappeared and the adsorption at 811 cm^{-1} and 677 cm^{-1} from the bending vibration of the benzene ring were significantly decreased. The stretching vibration at 3420 cm^{-1} assigned to —OH was enhanced and broadened. The peak at 1640 cm^{-1} was broadened for the bending vibration of water absorbed by cyclodextrin. The peak at 1430 cm^{-1} assigned to the bending vibration of O—H and the peak at 1156 cm^{-1} from the stretching vibration of C—O—C were both increased. All of these indicate that *p*-toluenesulfonyl ester on the C6 position of chitin has been displaced by nucleophilic substitution with CDen.

After the acetyl groups of Chitin-en-CD were removed, the absorption peak at 1596 cm^{-1} corresponding to the bending vibration of the amide bond was observed. The peak at 1664 cm^{-1} assigned to C=O was decreased and shifted to a lower wavenumber. The characteristic absorption peaks of the cyclodextrin remained unchanged. Therefore, the FTIR results clearly showed that the desired product (CTS-en-CD) was obtained after deacetylation of Chitin-en-CD.

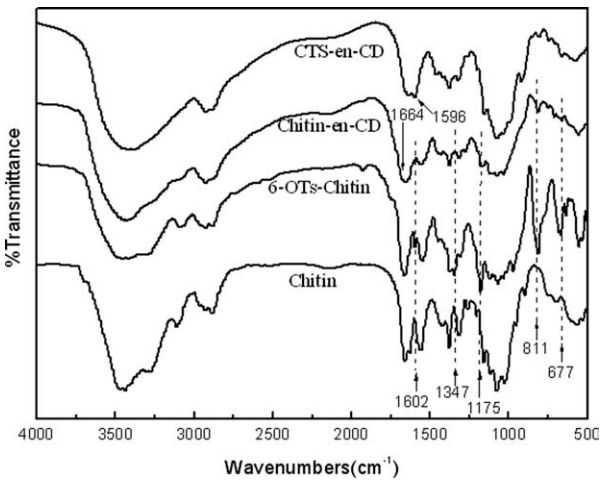

Figure 2 The FTIR spectra of chitin, 6-OTs-Chitin, Chitin-en-CD, and CTS-en-CD.

XRD analysis

Structural analyses of chitin, 6-OTs-Chitin, Chitin-en-CD, and CTS-en-CD were performed by XRD. As can be seen in the XRD patterns (Fig. 3), chitin has strong absorption peaks near 7° and 20°. As a result of the introduction of the large size tosyl group, the diffraction peak around 7° disappeared in 6-OTs-chitin, and the 20° diffraction peak showed diffusion with the emergence of new peaks at 12°, 15°, 16°, and 28°. In chitin-en-CD, all the peaks related to tosylate disappeared or became weaker after substitution with CD; there is only a dispersion of the diffraction peak at 20°. After the removal of acetyl groups from the skeleton, further diffusion of the weak diffraction peak in CTS-en-CD was observed at 20°. The decrease in crystallinity of the product, along with further increases in amorphous structure was confirmed by the disappearance of other peaks. XRD characterization combined with infrared data provided further evidence of the step-by-step reaction.

TG analysis

TGA was used to study the thermal properties of the product. As shown in Figure 4, the temperature for the highest weight loss rate of 6-OTs-chitin was significantly lower than that for chitin, which can be explained by the instability of *p*-toluenesulfonyl ester at high temperatures. When the ester groups of 6-OTs-chitin were replaced by CDen to form a more stable C—N bond, the temperature for the highest

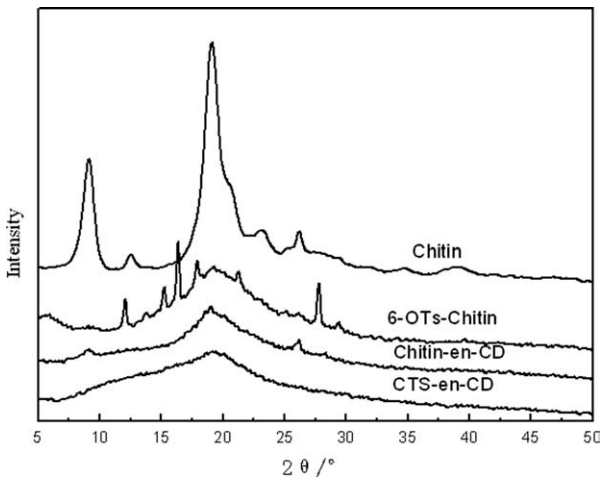

Figure 3 The XRD patterns of chitin, 6-OTs-Chitin, Chitin-en-CD, and CTS-en-CD.

weight loss rate increased, because chitin-en-CD has superior thermal stability than 6-OTs-chitin. After the removal of acetyl groups to form CTS-en-CD, the temperature for the highest weight loss rate increased due to further improvement in thermal stability.

Determining the substitution degree of cyclodextrin

In the presence of concentrated sulfuric acid, cyclodextrins will hydrolyze into monosaccharides and subsequently the carbohydrate will form uronic derivatives by rapid dehydration, which could condense with phenol to afford orange compounds with stable color. The absorbance of cyclodextrin can be measured at 490 nm and the concentration of cyclodextrin in the sample can be determined by using a standard curve.[19] This method is widely used to determine the substitution level of cyclodextrin on chitosan.[20]

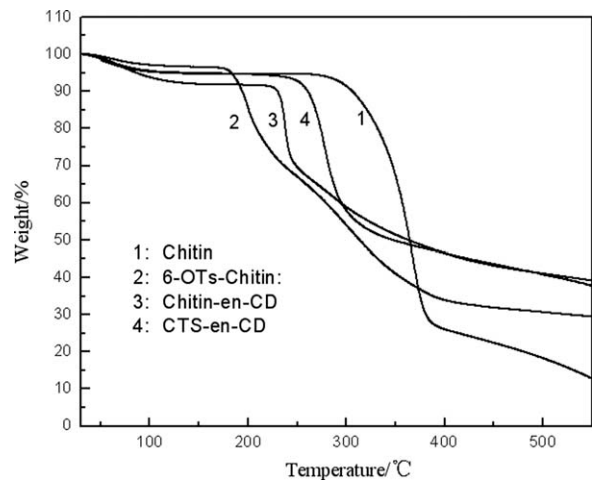

Figure 4 The TG curves of chitin, 6-OTs-Chitin, Chitin-en-CD, and CTS-en-CD.

According to Shen et al.'s method,[19] a cyclodextrin stock solution (80% wt) was prepared by dissolving cyclodextrin in distilled water (2 mL) and phenol/water co-solvent (100 mL), and then further diluted to make different concentration standard solutions: 0.01, 0.02, 0.03, 0.04, 0.05, 0.07, and 0.10 g/L. Concentrated sulfuric acid (5 mL) was slowly added into the standard solution and reacted for 30 min at the room temperature. The UV–vis absorbance of the hydrolysis product was measured for each standard to obtain the equation of the standard curve as:

$$Y'_{CD} = 0.04858 + 13.94142X'_{CD} \quad (1)$$

where Y'_{CD} is the UV absorbance of the product after CD hydrolysis and X'_{CD} is the CD concentration (g/L).

By comparing the UV absorption curves of chitin-en-CD and chitin (Fig. 5), it was found that under concentrated sulfuric acid hydrolysis conditions in the presence of phenol in an aqueous solution, the hydrolyzed pyranose ring from chitin has a certain degree of UV absorption at 490 nm. The standard working curve based on the hydrolysis of chitin in concentrated sulfuric acid is expressed as follows:

$$Y'_{CTN} = 0.04098 + 0.08141X'_{CTN} \quad (2)$$

where Y'_{CTN} is the absorbance of the products from chitin hydrolysis under the test conditions, X'_{CTN} is the concentration of chitin in the solution (g/L).

If X_i represents the concentration of the total substituted products, and X_{CD} is the concentration of substituted CD and X_{CTN} is the concentration of chitin, then the following equation can be derived:

$$X_i = X_{CTN} + X_{CD} \quad (3)$$

Assuming Y_i as the total absorbance of the hydrolyzed product, Y_{CD} as the absorbance at 490 nm

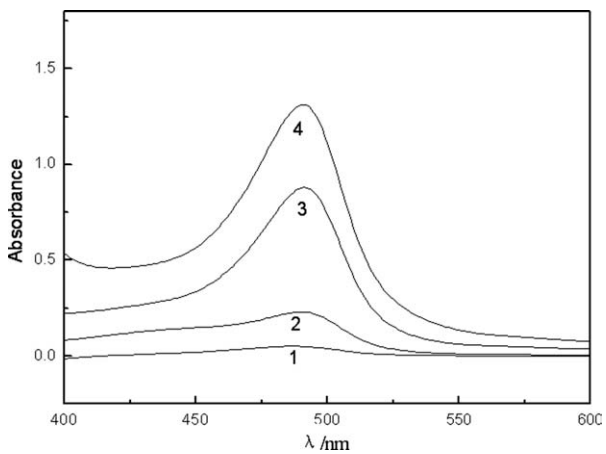

Figure 5 The UV absorption spectra of the hydrolysis products.

from the CD hydrolysis product, and Y_{CTS} as the absorbance from pyranose absorbance from chitin hydrolysis, the following equations can be obtained:

$$Y_i = Y_{CTN} + Y_{CD} \quad (4)$$

$$Y_{CD} = 0.04858 + 13.94142X_{CD} \quad (5)$$

$$Y_{CTN} = 0.04098 + 0.08141X_{CTN} \quad (6)$$

Equations (3), (5), and (6) can be combined into eq. (4) to give the concentration of substituted CD in the solution as

$$X_{CD} = \frac{Y_i - 0.08956 - 0.08141X_i}{13.816101} \quad (7)$$

The substitution degree of CD on chitin is:

$$Q = \frac{X_{CD} \times 1000}{X_i \times 1135} \quad (8)$$

where Q is the substitution capacity (mol/g) of CD with M_w of 1135.

After the deacetylation of chitin-en-CD to CTS-en-CD, the UV absorption curves of the corresponding hydrolysis products of CTS-en-CD and chitosan were measured in an aqueous phenol solution. Whereas the acid hydrolysis product from CTS-en-CD has a strong UV absorption peak at 490 nm, hydrolyzed chitosan showed no UV absorption at 490 nm. This rules out the possibility of interference of chitosan backbone for determining the CD substitution from concentrated sulfuric acid hydrolysis. The substitution capacity of CD on chitosan can also be calculated by eq. (8), where X_i represents the concentration of CTS-en-CD.

Effects of the reaction conditions on the substitution degree of chitin-en-CD

The efficiency of nucleophilic substitution of CDen to 6-OTs-Chitin was evaluated to obtain an idea of the reaction parameters that influence the substitution capacities. The substitution capacities could be affected by four parameters: the organic solvent used, the mass ratio of nucleophile to tosyl-chitin ($m_{CDen}/m_{6\text{-OTs-Chitin}}$), the reaction time, and the reaction temperature.

6-OTs-Chitin was allowed to swell in different organic solvents and then reacted with CDen. As shown in Table I, although the LiCl/DMAc solvent system could swell 6-OTs-Chitin well, the substitution capacity was low, because the solubility of CDen in this solvent system was low. The other three organic solvents tested were DMAc, DMF, and DMSO, which are all strong polar solvents. Their polarities increase in the sequence: DMAc < DMF < DMSO. The substitution capacities of Chitin-en-CD prepared in these solvents also increased in the same sequence. Since the tosyl group is a good leaving group, the C^+ ion could be formed readily on the C6 position of 6-OTs-Chitin. Thus, nucleophilic substitution of the sterically hindered CDen to 6-OTs-Chitin follows the S_N1 nucleophilic substitution mechanism. For S_N1 nucleophilic substitution, ion-pair separation and formation of C^+ ion were enhanced with increasing solvent polarity, which would also promote the degree of substitution. Therefore, DMSO was selected as the solvent for the nucleophilic substitution reaction in order to achieve the highest substitution capacity.

TABLE I
Effects of the Reaction Conditions on the Loading Capacities of Chitin-en-CD

Organic solvents	m_{CDen} : $m_{6\text{-OTs-Chitin}}$	Reaction time (h)	Reaction temperature (°C)	Loading capacities (μmol g^{-1})
5% LiCl/ DMAc	5 : 1	9	65	30.36
DMAC				69.59
DMF				69.84
DMSO				80.15
DMSO	1 : 1	9	65	57.01
	2 : 1			60.18
	3 : 1			69.85
	4 : 1			78.51
	5 : 1			80.15
	6 : 1			83.01
DMSO	5 : 1	5	65	50.26
		6		61.33
		7		68.64
		8		74.12
		9		80.15
		10		80.21
DMSO	5 : 1	9	55	66.26
			60	68.34
			65	80.15
			70	83.57
			75	88.59
			−80	87.62

The results in Table I clearly indicate that the higher the mass ratio of nucleophile to tosyl-chitin, the higher the corresponding substitution capacity. Similarly, extending the reaction time resulted in a higher substitution capacity. It was found that 75°C was the best temperature for higher substitution capacity.

By comparing the substitution capacity of Chitin-en-CD synthesized at different conditions, we found that the optimal conditions to synthesize Chitin-en-CD with the highest substitution level were as follows: dimethyl sulfoxide as the solvent, the mass ratio of nucleophile to tosyl-chitin of 6 : 1, and 9-h reaction time at 75°C. Chitin-en-CD synthesized at these optimal conditions have a substitution capacity of 93.97 μmol/g based on the above calculation method.

After chitin-en-CD was deacetylated, the deacetylation degree of chitosan of the corresponding CTS-en-CD was 89.5%, as determined by the alkalimetry method. Moreover, the experimental CD substitution capacity of CTS-en-CD was found to be 128.68 mol/g, which is much higher than those from literature.[9–12]

CONCLUSION

Chitosan derivatives immobilized with C6 substituted cyclodextrin (CTS-en-CD) were prepared via nucleophilic substitution of CDen on 6-OTs-Chitin, followed by the removal of acetyl groups on the chitin main chain. The success of each reaction step was confirmed by FTIR and XRD analysis. The thermal stability of the product was studied by TGA analysis. The substitution capacity of Chitin-en-CD and CTS-en-CD were 93.97 and 128.68 mol/g, respectively, which are much higher than those previously reported. This method has demonstrated to be effective for the preparation of 6-OH substituted cyclodextrin derivatives of chitosan with high substitution levels.

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