Molecular Crystals and Liquid Crystals

ISSN: 1542-1406 (Print) 1563-5287 (Online) Journal homepage: http://www.tandfonline.com/loi/gmcl20

The Novel Route to Prepare Immobilized Macrocyclic Compound on 6-OH of Chitosan with Good Solubility and Prime Study on Its Applications

Chen Yu, Li Wei-Ping, Ye Yan-Chun, Guo Yan-Wen, Wang Tian-Wei & Xing Yi-Fan

To cite this article: Chen Yu, Li Wei-Ping, Ye Yan-Chun, Guo Yan-Wen, Wang Tian-Wei & Xing Yi-Fan (2014) The Novel Route to Prepare Immobilized Macrocyclic Compound on 6-OH of Chitosan with Good Solubility and Prime Study on Its Applications, Molecular Crystals and Liquid Crystals, 604:1, 202-212, DOI: 10.1080/15421406.2014.968499

To link to this article: http://dx.doi.org/10.1080/15421406.2014.968499

Published online: 15 Dec 2014.

Mol. Cryst. Liq. Cryst., Vol. 604: pp. 202–212, 2014

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421406.2014.968499

Taylor & Francis
Taylor & Francis Group

The Novel Route to Prepare Immobilized Macrocyclic Compound on 6-OH of Chitosan with Good Solubility and Prime Study on Its Applications

CHEN YU,[1,*] LI WEI-PING,[2] YE YAN-CHUN,[2]
GUO YAN-WEN,[2] WANG TIAN-WEI,[1] AND XING YI-FAN[3]

[1]School of Material Science and Engineering, Beijing Institute of Technology, Beijing, P. R. China
[2]School of Chemistry, Beijing Institute of Technology, Beijing, P. R. China
[3]Department of Chemical and Materials Engineering, The University of Auckland, Auckland, New Zealand

*β-cyclodextrin (β-CD) immobilization of a chitosan derivate on C_6-OH (CTS-6-CD) with good solubility was prepared through a novel method. During the process the amino group of the chitosan was protected by phthalic anhydride and deprotected in the hydrazine hydrate solution. β-CD was immobilized onto the C_6 position of chitosan by a nucleophilic displacement reaction. The prepared derivate can be dissolved in hydrazine hydrate (*v:v *= 1:1), ethylenediamine/ethanol (*v:v *= 1:1), 1% aqueous acetic acid, DMSO and DMAc. The application of the 6-OH cyclodextrin-immobilized chitosan derivatives in an electrochemical biosensor was examined in a preliminarily manner.*

Keywords: Chitosan; β-cyclodextrin; solubility; nucleophilic displacement reaction; immobilization

1. Introduction

Macrocyclic compounds, such as cyclodextrin, calixarene, crown ether and cucurbituril, etc. have attracted more and more attentions in recent years. There specific cavity structures could stimulate the important models for the complex structures and processes in the organism; by the way, they could be used as the multi-functional carriers in the fields of materials or medicine [1–3]. Chitosan, as its great physiological activities, has become a new type of highly studied functional polymers [4–6]. Immobilization of macrocyclic compounds, especially cyclodextrin on chitosan have been well studied in the literature [7–8], but in most cases the modification was made on the 2-NH2 of chitosan, which is not conducive to perform further amino group modification and manipulate its unique properties. If cyclodextrin can be immobilized on chitosan at the 6-OH position, and at the same time reserve the 2-NH2 group, this can greatly expand the applications of such derivatives.

*Address correspondence to Chen Yu, School of Material Science and Engineering, Beijing Institute of Technology, Beijing 100081, P. R. China. E-mail: cylsy@163.com

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/gmcl.

There are also some studies has been explored on this field [9–12]. Since the 6-OH has a lower reactivity than the 2-NH2, preparation of 6-OH grafted cyclodextrin derivatives is more difficult and thus the loading capacities were low so far (less than 70 μmol·g^{-1}), much lower than the loading capacity of the 2-NH2 immobilized CD on chitosan.

In our previous study, the preparation routes for 6-OH cyclodextrin-immobilized chitosan derivatives were explored in depth [13–15]. The reaction routes via nucleophilic substitutions or click reactions were established. The loading capacity of the prepared derivates could reach more than 200 μmol·g^{-1}. Applications of such chitosan derivates in the sensitive film of a biosensor or in other fields become possible with a significant improvement of the immobilized loading.

However, as there are strong intramolecular and intermolecular hydrogen bonds between chitosan and cyclodextrin, the 6-OH cyclodextrin-immobilized chitosan derivatives prepared through above methods are insoluble. The poor solubility restricts the wide use of the derivative, and thus the excellent properties of 2-NH_2 of chitosan and the hydrophobic cavity of cyclodextrin are not available for use.

In this study, a novel process for protecting the 2-NH_2 of the chitosan skeleton with phthalic anhydride, and deprotection with hydrazine hydrate solution, was developed. By combining with a nucleophilic displacement reaction of the 6-OH activated chitosan derivate, a new route for the preparation of CTS-6-CD with good solubility was explored. The application of the derivate with good solubility in the field of electrochemical biosensors was then studied in a preliminarily manner. The overall reaction route is shown in Figure 1.

2. Materials and Experiment

2.1. Materials

Chitosan (CTS) with a deacetylation degree of 95.5% of chemical grade and supplied by Zhejiang Yuhuan Biochemical Co., Ltd. (China). β-cyclodextrin (β-CD, $M_w \approx 1135.0$ *m.p.*, = 290–300°C) was of analytical grade and was purchased from Tianjin Bodi Chemical Co., Ltd. (China). Mono-[6-(2-aminoethyl)-amino-6-deoxy]-β-cyclodextrin (β-CDen) was prepared according to the literature [13]. Phthalic anhydride was of analytical grade and was supplied by Tianjin Kemiou Chemical Reagent Co., Ltd. (China). *p*-toluenesulfonyl chloride was of analytical grade and was supplied by Tianjin Guangfu Fine Chemical Research Institute (China). Other analytical grade reagents were obtained from the Beijing Chemical Reagents Company (China) and were used as received.

2.2. Protection of the 2-NH_2 of Chitosan by Phthalic Anhydride

To a 3-neck round bottom flask equipped with condenser and nitrogen protection, 5.0 g CTS and 15 g phthalic anhydride was dissolved in DMF (100 mL). After stirring at 130°C for 6 h, the product was precipitated by an excess amount of ethanol and filtered. Then the product was dried under vacuum to afford 2-phthaloyl chitosan (PA-CTS).

2.3. Synthesis of PA-CTS-6-p-sulfonyl Ester (PA-CTS-6-OTs)

To the mixture of pyridine and DMF (100 mL, 1:1, w/w) was added 3.0 g of PA-CTS. The mixture was stirred at 30°C for 2 h to let PA-CTS fully swell, then pyridine solution of *p*-toluenesulfonyl chloride (50 mL, $n_{\text{PA-CTS}}$:$n_{\text{TsCl}} = 1$:5) was added dropwise into the flask.

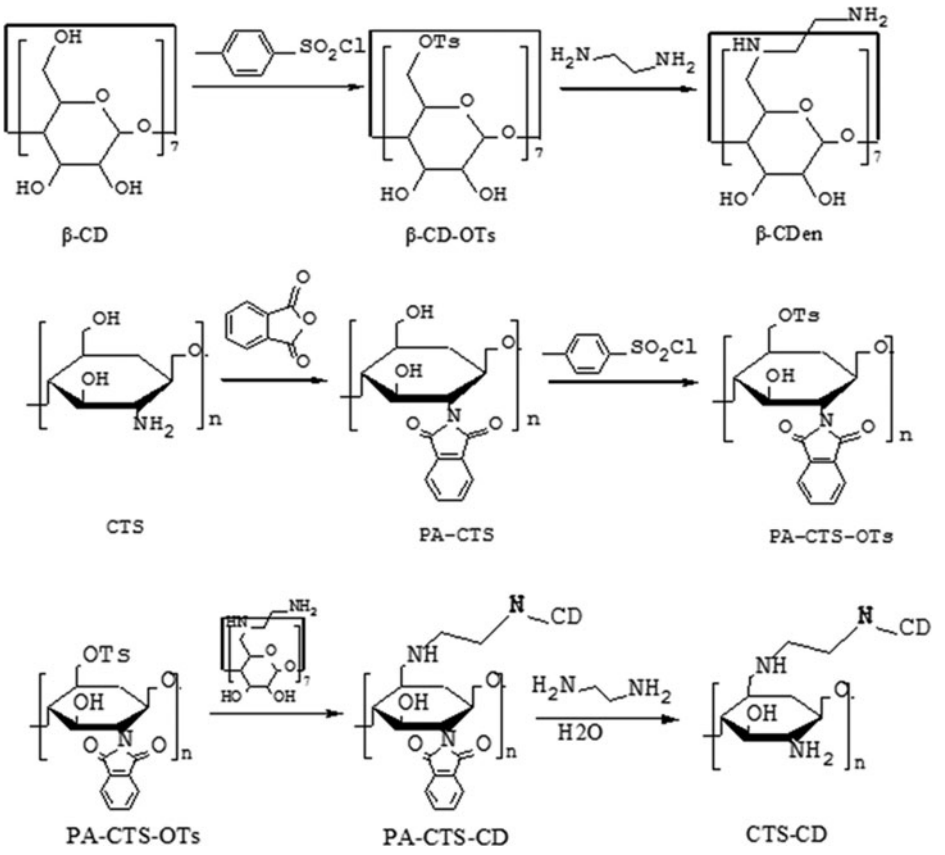

Figure 1. The novel reaction route for the preparation of CTS-6-CD.

After stirring at 30°C for 24h, the reaction mixture was precipitated by ethanol and filtered, then washed with ethanol and ether subsequently, and dried under vacuum to give PA-CTS -6-OTs.

2.4. Synthesis of Phthaloyl Protected Chitosan C_6 Immobilized Cyclodextrin Derivate (PA-CTS-6-CD) via Nucleophilic Substitution

To a three-necked flask equipped with a condenser was added CDen and PA-CTS-6-OTs. A small amount of DMF was added to swell the solids. The mixture was stirred at room temperature to ensure even mixing. Then the reaction was heated to 80°C and reacted for 8 h. The product was cooled and dialyzed (molecular weight cut-off at 3,500) in water for a period of 5 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 PA-CTS-6-CD as a yellow powder.

2.5. Deprotection of PA-CTS-6-CD

PA-CTS-6-CD was soaked in hydrazine hydrate solution (V_{H2NNH2}:V_{H2O} = 1:1) in 1% (w/v) concentration and stirred for 4 hours at 80°C. After the phthaloyl protecting group

was hydrolyzed, ethanol was added into the reaction mixture and the formed precipitate was filtered, washed with water to neutral, and then washed with ethanol and aceton subsequently, dried under vacuum to give the amino deprotected immobilized product, chitosan 6-OH immobilized cyclodextrin derivate (CTS-6-CD). The loading capacity of CTS-6-CD prepared in this study was 212.16 μmoL $\cdot g^{-1}$, which was tested according to the method from literature [15].

2.6. Study on the Solubility of CTS-6-CD

The solubility of CTS-6-CD prepared through the above method and other chitosan 6-OH immobilized cyclodextrin derivates prepared through a method from literature [13∼15] were tested with hydrazine hydrate (*v:v* = 1:1), ethylenediamine/ethanol solution (*v:v* = 1:1), 1% acetic acid aqueous, DMSO, DMF, DMAc as the solvent. The test molar concentration for CTS-6-CD was 0.05 mol/L and the product was soaked in the solvent for 1h to observe the dissolving situation.

2.7. Characterization

FTIR spectra were obtained on a NEXUS-470 series FTIR spectrometer (Nicolet Co., USA). KBr pellets of the samples were used.

Thermogravimetric analyses (TGA) of the samples were made on a Q5000IR thermogravimetric analyzer (TA Instruments, USA) at a heating rate of 20°C/min with nitrogen used as the purge gas.

Wide angle X-ray diffraction (XRD) of samples was recorded with an ALC-100.4 type X-ray diffractometer (Beijing Sartorius Instrument Co., Ltd., China).

The electrochemical properties of electrode modified by product were studied by using CHI660 electrochemical workstation and three-electrode test system, composed of glassy carbon working electrode, platinum counter electrode and calomel reference electrode.

3. Results and Discussion

3.1. FTIR Characterization

Structures of synthesized products in the reactions were identified and confirmed by FTIR spectroscopy (Figure 2).

Comparing the characteristic peaks in FTIR spectrum between β-CD-OTs and β-CD, a series of characteristic absorption peaks of the benzene ring could be found in the spectrum of β-CD-OTs. These included the stretching vibration absorption peak of the benzene ring at 1600 cm^{-1}, the deformation vibration absorption peak of the benzene ring at 840 cm^{-1}, the absorption peak attributed to the para orientation of the benzene ring at 810 cm^{-1}. These results indicated that the benzene ring structure with the para-orienting group was linked with β-CD. The absorption peak of the sulfonic acid ester around 1365 cm^{-1} and 1175 cm^{-1} were seen, which demonstrates that the hydroxyl group of β-CD has reacted with *p*-toluensulfonyl chloride to afford the structure of the sulfonic acid ester.

Comparing the characteristic peaks in FTIR spectra between β-CD-OTs and β-CDen, a stretching vibration absorption peak of the amino group at 1590 cm^{-1} was found in the spectrum of β-CDen. On the other hand, the benzene ring skeleton stretching vibration absorption peak at 1600 cm^{-1} and the bending vibration absorption peaks at 840 cm^{-1} and 810 cm^{-1} in the spectrum of β-CD-OTs also became weak for the corresponding peaks

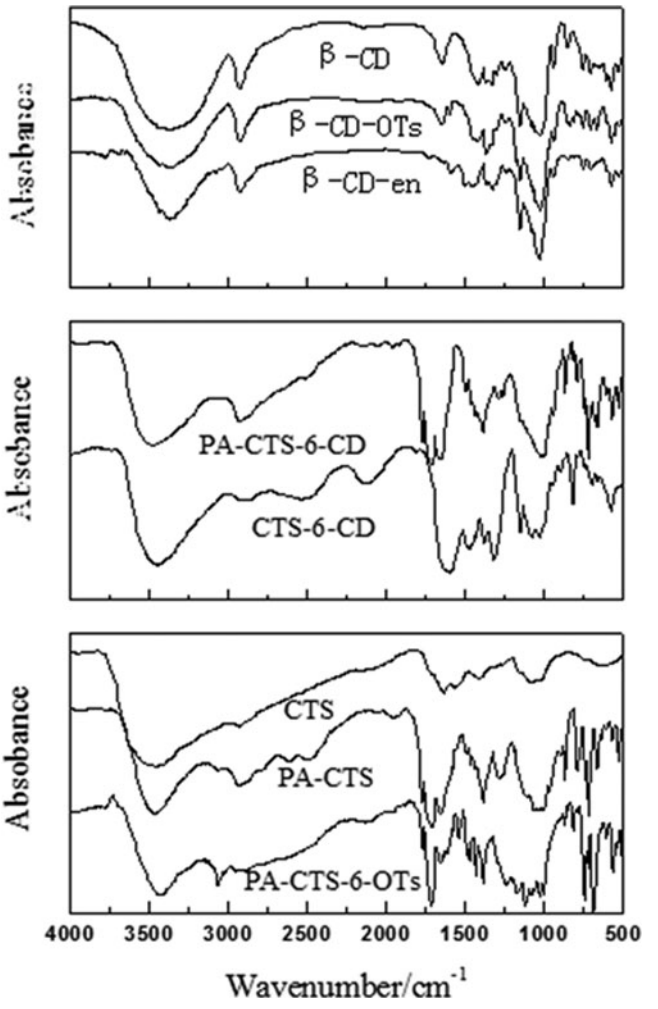

Figure 2. FTIR spectra of the synthesized products.

in the spectrum of β-CDen. In addition, the asymmetric stretching vibration absorption peak at 1369 cm^{-1} and symmetric stretching vibration absorption peak at 1175 cm^{-1} of the *p*-toluenesulfonate group in the spectrum of β-CD-OTs became weak for the corresponding peaks in the spectrum of β-CDen. These results indicated that the *p*-toluenesulfonate group of β-CD-OTs was successfully nucleophilic substituted by an amino group.

In the FTIR spectrum of the PA-CTS generated from CTS, the stretching vibration absorption peaks of the C=O group appeared at 1776 cm^{-1} and 1716 cm^{-1}, the deformation vibration absorption peak of O=C-N group was seen at 571 cm^{-1}, the out-plane deformation vibration absorption peak of C=O in tertiary amine was at 529 cm^{-1}, and the absorption peaks at around 721 cm^{-1}, 743 cm^{-1}, 794 cm^{-1} were attributed to the out-plane deformation vibration of the ortho-substituted Ar-H. All of the above results illustrate that the phthalic anhydride has reacted with the C_2-NH_2 structure of chitosan to afford the amide structure.

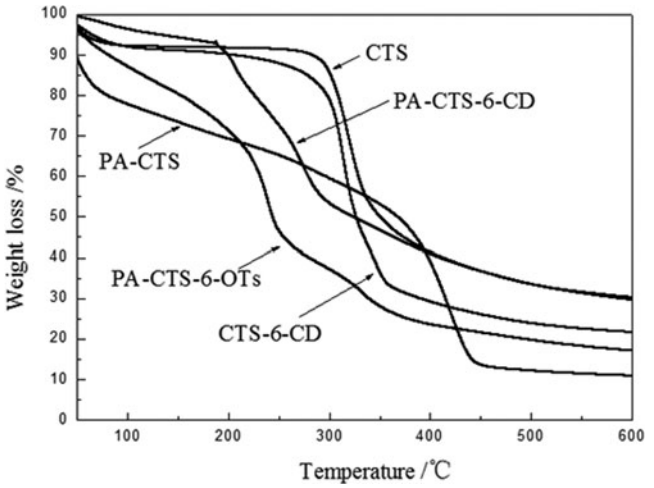

Figure 3. TGA spectrums of synthesized products.

Comparing the characteristic peaks in FTIR spectra between PA-CTS-6-OTs and PA-CTS, the absorption peak attributed to the para orientation of the benzene ring appeared at 817 cm^{-1}, and the absorption peak of the sulfonic acid ester around 1172 cm^{-1} could also be found, which demonstrates that the *p*-toluensulfonyl chloride has reacted with the primary hydroxyl group of C_6 position of chitosan to afford the structure of the *p*-toluensulfonic acid ester.

In the FTIR spectrum of the PA-CTS-6-CD generated from the nucleophilic substitution of PA-CTS-6-OTS and CDen, the absorption peak of sulfonic acid ester around 1172 cm^{-1} had disappeared. On the other hand, the absorption peak attributed to the para orientation of the benzene ring at 817 cm^{-1} was also clearly weakened. The absorption peak around 3400 cm^{-1} attributed to the hydrogen bond of the polymer was obviously widened. So the results of the nucleophilic substitution could be demonstrated.

After deprotecting the phthalic structure from PA-CTS-6-CD, the deformation vibration absorption peak of O═C-N group at 571 cm^{-1} disappeared, the out-plane deformation vibration absorption peak of C═O in tertiary amine at 529 cm^{-1} weakened, and the absorption peaks around 721 cm^{-1}, 743 cm^{-1}, 794 cm^{-1} attributed to the out-plane deformation vibration absorption peak of the ortho-substituted Ar-H disappeared. These results indicated that the phthalic structure was removed under the effect of the hydrazine hydrate and the 2-NH_2 chitosan 6-OH immobilized cyclodextrin derivate was obtained as the final product.

3.2. TGA Characterization

TGA was used to study the thermal properties of the products and the results are shown in Figure 3. Comparing with the TAG curve of CTS, the temperature onset for decomposition for PA-CTS was lower and the end temperature was delayed. The weight loss ratio for PA-CTS was 72.9%, which is obviously higher than the 30.8% for CTS. The above results indicate that the thermal stability of PA-CTS was obviously lower than that of CTS after introduction of the phthalic structure.

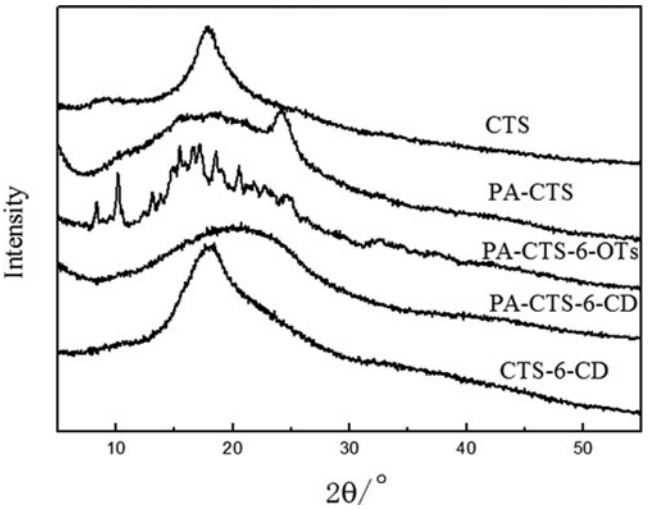

Figure 4. XRD patterns of synthesized products.

After 6-OH of PA-CTS was tosylated, there are two stages of decomposition in the TGA curve of PA-CTS-6-OTs. The first stage occurred in the range of 141.5 to 289.1°C, corresponding to the decomposing of the unstable tosylate structure. The second stage was occurred in the range of 289.1 to 413.4°C, due refer to decomposition of the PA-CTS structure.

It can be seen from Figure 3 that after the cyclodextrin was immobilized, the temperature for the thermal decomposition of PA-CTS-6-CD was obviously hysteretic compared to that of PA-CTS-6-OTs. After substitution of the tosyl group, formation of the more stable linkage for cyclodextrin, and the hydrogen bond between cyclodextrin and chitosan, the thermal stability of PA-CTS-6-CD was obviously higher than that of PA-CTS-6-OTs. The above results can confirm that the cyclodextrin structure has been immobilized on the chain of chitosan.

After the removal of phthaloyl groups to form CTS-6-CD, the temperature interval for decomposing (209.6 to 423.6°C) and the temperature for the highest weight loss rate (314.6°C) were all higher than that of PA-CTS-6-CD, which was 183.8 to 322.5°C for the decomposing interval and 273.7°C for the highest weight loss rate. The above results were attributed to the removal of the unstable phthaloyl groups and the intramolecular and intermolecular hydrogen bonds that were enhanced.

3.3. XRD Characterization

Structural analysis of CTS, PA-CTS, PA-CTS-6-OTs, PA-CTS-6-CD, CTS-6-CD were performed by XRD. As can be seen in the XRD patterns (Figure 4), CTS had strong diffraction peaks at around $2\theta = 8^\circ$ and $2\theta = 18^\circ$. After reacting phthalic anhydride with the 2-NH_2 of chitosan, the distance between the chitosan molecular chains increased and the crystallinity of polymer was decreased. As a result, the sharp diffraction peaks of chitosan around 8° and 18° were dispersed. In addition, after introducing the new substituent group, new diffraction peaks around $2\theta = 5^\circ$ and $2\theta = 25^\circ$ emerged in the XRD spectra of PA-CTS. As a result of the introduction of the large sized tosyl group, new peaks at 8°, 10°, 16° and 20° appeared in the XRD curve for PA-CTS-6-OTs. Combined with the results

Table 1. Solubility of CTS-6-CD prepared through different methods

Solvent product	Hydrazine hydrate	Ethylenediamine/ ethanol solution	1% Acetic acid aqueous	DMSO	DMF	DMAc
CTS-6-CD*	√	√	√	√	×	√
CTS-6-CD**	×	×	×	×	×	×
CTS-6-CD***	×	×	×	√	×	×
CTS-6-CD****	×	×	×	×	×	×

Note: CTS-6-CD* was prepared according to the method of this work. CTS-6-CD** was prepared according to literature [13]. CTS-6-CD** was prepared according to literature [14]. CTS-6-CD** was prepared according to literature [15].

of FTIR, substitution of tosyl on the 6-OH of PA-CTS can be confirmed further. In XRD curve of PA-CTS-6-CD, all of the peaks related to tosylate disappeared after substitution with CDen; there was only a dispersion of the diffraction peak at 20°. After the removal of phthaloyl groups from the skeleton, strong diffraction peak could be observed around $2\theta = 20^\circ$. The result confirmed that after removing the phthaloyl groups from the chitosan skeleton, the intramolecular and intermolecular hydrogen bonds formed between chitosan and cyclodextrin were enhanced. XRD characterization combined with infrared and thermal analysis data provided further evidence of the step-by-step reaction.

3.4. Study on the Solubility of CTS-6-CD

The solubility of CTS-6-CD prepared through the above method and other methods proposed by our research group were tested. The results are listed in Table 1.

In literature [13], CTS-6-CD was prepared through the nucleophilic displacement reaction of the 6-OH activated chitosan derivate. The obtained immobilization was 170.81 μmol·g^{-1}. In the literature [14], β-CD was immobilized via the nucleophilic displacement reaction of the 6-OH activated chitin, by further removing acetyl groups on the chitin main chain, which yielded CTS-6-CD. The substitution capacity of CTS-6-CD was 128.68 mol·g^{-1}. In the literature [15], CTS-6-CD was prepared via click chemistry of the 6-OH activated chitosan derivate. The obtained degree of immobilization was 211.98 μmol·g^{-1}.

It can be seen from Table 1 that although β-CD immobilization derivates of chitosan on C_6-OH were all prepared successfully through the above methods, the solubility for CTS-6-CD prepared from the method reported by the previous literature was very poor. The poor solubility restricts the wide application of the derivates, and thus the above derivatives were not used yet. However, for CTS-6-CD prepared through the current method, it can dissolve in many solvents, such as hydrazine hydrate (v:v = 1:1), ethylenediamine/ethanol solution (v:v = 1:1), 1% aqueous acetic acid aqueous, DMSO and DMAc etc. This maybe because that the reaction activity of phthalic anhydride to 2-NH_2 of chitosan is higher than glutaraldehyde or other protection reagent. The substitution degree of 2-NH_2 was high and then the solubility of PA-CTS was good. This was beneficial to immobilize of cyclodextrin on the 6-OH of chitosan skeleton. The immobilization degree of CTS-6-CD prepared through the current method reached up to 212.16 μmol·g^{-1}. After deprotection of PA-CTS-6-CD, the intermolecular interaction for the skeleton of chitosan would be destoried severely. So the solubility of CTS-6-CD prepared from the current method could

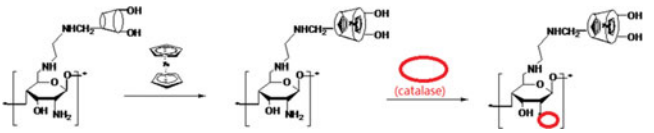

Figure 5. Reactions of the CTS-6-CD in the electrochemical biosensor.

be improved greatly. Thus the application of CTS-6-CD can be considered and the excellent properties of 2-NH_2 on the chitosan skeleton and the hydrophobic cavity can be made use of.

3.5. Initial Study on the Application of CTS-6-CD in an Electrochemical Biosensor

The chemical immobilization of cyclodextrin on the chitosan skeleton can be a feasible means to generate supramolecular host material within a sensing membrane. The approach combines the excellent supramolecular inclusion properties of cyclodextrin with the remarkable advantages of chitosan in membrane formation, immobilization, functionalization and biochemical properties, which allows biosensing membranes to be prepared with outstanding performance. Owing to the good solubility of CTS-6-CD prepared from the above method, application of CTS-6-CD in the electrochemical biosensor became feasible.

Firstly, ferrocene was included within the product. The ferrocene solution in anhydrous ethanol was added into the solution of CTS-6-CD (0.05 mol/L) in ethylenediamine/ethanol (v/v = 1:1), the stoichiometric ratio of CTS-6-CD to ferrocene was 1:4, and the mixture was heated in an 80°C oil bath at reflux for 6 h. After the reaction was completed, the precipitate was washed with doubly deionized water, then washed with tetrahydrofuran to remove unreacted ferrocene, and finally dried to give the ferrocene inclusion product of CTS-6-CD. The inclusion ratio of ferrocene in cyclodextrin can reach 40.16%.

Catalase was then immobilized on the 2-NH_2 of the ferrocene inclusion product of CTS-6-CD. A solution of ferrocene inclusion product of CTS-6-CD (0.02 mol/L in 1% aqueous acetic acid) was adjusted to neutral by addition of aqueous sodium hydroxide, and the catalase solution (30.0 mg/mL in 0.1 mol/L PBS buffer, pH = 7) was added. The immobilization solution was left to react at 30°C for 1 h. Afterwards, a certain amount of glutaraldehyde solution was added to the reaction system and the crosslinking reaction was carried out at a certain temperature for 1 h. Then the mixture was centrifuged, filtered, washed with deionized water to remove unreacted glutaraldehyde, and with 0.1 mol/L PBS buffer to remove unreacted catalase. This produced the catalase immobilization product of the ferroence inclusion product of CTS-6-CD. The activity of catalase immobilized for the product prepared under these conditions reached 411.3 U/g.

The reaction patterns of the CTS-6-CD in the electrochemical biosensor are shown in Figure 5.

A glassy carbon electrode was modified by 2-NH_2 catalase immobilized and ferroence inclusion products of CTS-6-CD, and then used to detect the concentration of H_2O_2. The glassy carbon electrode was mirror polished with 0.3 μm Al_2O_3 and ultrasonicated sequentially in deionized water, absolute ethanol and deionized water for 1 min at each step. The electrode was then hung to dry at 25°C. The solution of 2-NH_2 catalase immobilized and ferrocene inclusion product of CTS-6-CD (0.005 mol/L, in 1% aqueous acetic acid) was oscillated for 30 min and an aliquot of 6 μL was added drop-wise onto the glassy carbon electrode. The electrode was hung to dry at 25°C to furnish the modified electrode. An

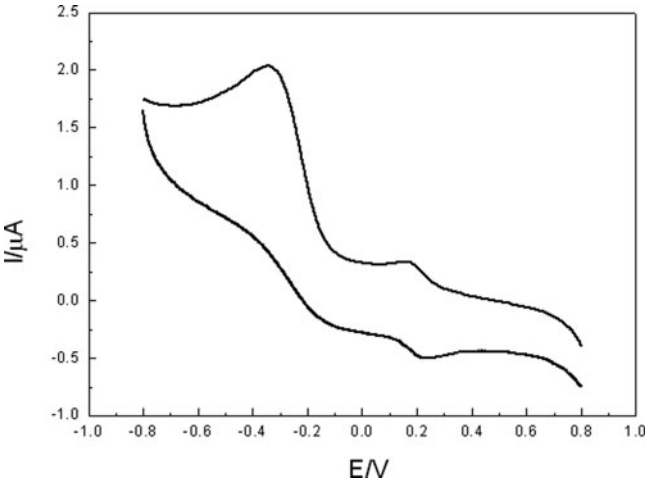

Figure 6. The cyclic voltammetry curve of the modified electrode (scan rate, 100 mV/s).

electrochemical workstation was used with a three-electrode system, *i.e.*, the modified electrode as the working electrode, a saturated calomel electrode as the reference electrode, and a platinum electrode as the counter electrode, The cyclic voltammograms of the modified electrode in test solutions were recorded.

The cyclic voltammetry curves of the modified electrode from −0.80 V to 0.80 V were measured in PBS buffer solution (5.00 mL, 0.1 mol/L, pH = 7.0) containing 5 μL 0.1 mol/L H_2O_2 solution (Figure 6). It can be seen that the modified electrode gave two pairs of peaks in 0.1 mol/L blank PBS buffer, which are the redox peaks of ferrocene and the the redox peaks of catalase, respectively. This proves the catalysis of catalase and signal amplification of ferrocene. The above effect is contributed to improve the sensitivity and limit of detection to H_2O_2.

The optimal working pH of the 2-NH_2 catalase immobilized and ferroence inclusion product of CTS-6-CD modified electrode was 7.0. The peak current has a linear relationship with H_2O_2 concentration in the range of 1.0×10^{-4} to 1.0×10^{-3} mol/L. The linear regression equation was $I = 0.00379C_{H_2O_2} - 0.05987$. The limit of detection was 5×10^{-6} mol/L. From the above study, the new application of 6-OH cyclodextrin-immobilized chitosan derivatives in an electrochemical biosensor was established, and the results showed great promise for the application of the above derivatives.

4. Conclusions

Novel C_6 immobilized cyclodextrin derivatives on chitosan (CTS-6-CD) with good solubility were prepared via nucleophilic substitution of CDen on the 6-OH activated chitosan derivate. The 2-NH_2 of chitosan was phthaloyl protected and the 6-OH was tosylated, and later the phthaloyl was removed in the hydrazine hydrate solution. The achievement of the step-by-step reactions was confirmed by FTIR characterization. The thermal stabilities and crystallization properties of each product were studied using TGA and XRD characterization. The loading capacity of CTS-6-CD prepared in this study was 212.16 μmoL·g^{-1}. The CTS-6-CD prepared through the current method could dissolve in many solvents, such as hydrazine hydrate (*v:v* = 1:1), ethylenediamine/ethanol (*v:v* = 1:1), 1% aqueous

acetic acid, DMSO and DMAc etc. The reaction route was an effective method to prepare the immobilized cyclodextrin derivatives on the 6-OH position of chitosan with high loading capacity and good solubility. By including ferrocene as the electron mediator in the cyclodextrin hydrophobic cavity, and crosslinking catalase to the 2-amino groups of CTS-6-CD, the 6-OH cyclodextrin-immobilized chitosan derivatives can be applied in an electrochemical biosensor.

Funding

This work was supported by the Natural Science Foundation of China (No. 50903008) and the basic study program of Beijing Institute of Technology (No. 20080942002, No. 20090942004).

References

[1] Yang, J. S., & Yang, L. (2013). *J. Mater. Chem. B*, *1*, 909.
[2] Díez, P., Piuleac, C., Martínez-Ruiz, P., Romano, S., Gamella, M., Villalonga, R., Pingarrón, J. M. (2013). *Anal. Bioanal. Chem.*, *405*, 3773.
[3] Wang, W., Deng, Y., Li, S., Liu, H. N., Lu, Z. X., Zhang, L. M., Lin, L., & Xu, L. J. (2013). *J. Biomed. Nanotechnol.*, *9*, 736.
[4] Upadhyaya, L., Singh, J., Agarwal, V., & Tewari, R. P. (2013). *Carbohydr. Polym.*, *91*, 452.
[5] Suginta, W., Khunkaewla, P., & Schulte, A. (2013). *Chem. Rev.*, *113*, 5458.
[6] Susanto, H., Samsudin, A. M., Rokhati, N., & Widiasa, I. N. (2013). *Enzyme Microb. Tech.*, *52*, 386.
[7] Hitoshi, S., & Sei-ichi, A. (2004). *Prog. Polym. Sci.*, *29*, 887.
[8] Prabaharan, M., & Mano, J F. (2006). *Carbohydr. Polym.*, *63*, 153.
[9] Ma, Z. W., Zhang, B. L., He, W., Wu, Z. F., Sun, J. Y., & Wang, Q. T. (2007). *Chin. J. Synth. Chem.*, *15*, 605.
[10] Sreenivasan, K. (1998). *J. Appl. Polym. Sci.*, *69*, 1051.
[11] Zhang, X., Wang, Y. T., & Yi, Y. (2004). *J. Appl. Polym. Sci.*, *94*, 860.
[12] Zha, F., Lu, R. H., & Chang, Y. (2007). *J. Macromol. Sci., Part A: Pure Appl.Chem.*, *44*, 413.
[13] Chen, Y., Yang, K., Ye, Y. C., & Tan, H. M. (2009). *Acta Polym. Sin.*, (7), 712.
[14] Chen, Y., Ye, Y. C., Wang, L. Y., Guo, Y. W., & Tan, H. M. (2012). *J. Appl. Polym. Sci.*, *125*, E378.
[15] Chen, Y., Ye, Y. C., Li, R. R., Guo, Y. W., & Tan, H. M. (2013). *Fiber. Polym.*, *14*, 1058.