Preparation and characterization of 6-O-caffeic acid chitosan

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Abstract. The modified chitosan retains the excellent properties of chitosan and can broaden its application. In this paper, an approach of protection-deprotection was used to synthesize 6-O-caffeic acid chitosan with chitosan as the raw material. Firstly, the free amino group on the 2-position carbon of chitosan was protected by phthaloylation, and then the -OH of 6-position carbon of chitosan reacted with caffeic acid by ester condensation. Finally, in the presence of hydrazine hydrate, the amino group was deprotected to form 6-O-caffeic acid chitosan. Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) characterized the structures of the intermediates and products. The results showed that 6-O-caffeic acid chitosan was successfully obtained. Thermogravimetric analysis indicated that 6-O-caffeic acid chitosan had better thermo-stability than chitosan in nitrogen. This will expand the application of chitosan in various fields.

1. Introduction
Chitosan is a deacetylation product of chitin from shrimp and crab shells. It is the second-largest biological resource next to cellulose in nature and the only natural alkaline cationic polysaccharide found so far [1]. It has a variety of biological activities, such as antibacterial, reducing blood lipids, enhancing immunity, promoting the regeneration and healing of wound surface tissue [2]. Meanwhile, it has excellent properties such as biocompatibility, biodegradability, and non-toxicity. Because of its good physical and chemical properties and many biological activities, chitosan has been widely used in various fields, such as food, agriculture, biomedicine, chemical, textile, cosmetic, animal husbandry and fishery [3,4]. Caffeic acid is a natural catechol compound belonging to hydroxycinnamic acid, which exists in many plants such as fruits, nuts, and coffee beans. It has many physiological activities, such as cardiovascular protection, lipid-lowering, hypoglycemic, anticancer, cholagogic and hemostatic effects [5,6]. Caffeic acid can be used in the clinic as a kind of medicine to raise white blood cells and stop bleeding. Furthermore, it has the functions of contraction, consolidation of microvessels and improvement of coagulation factors. It is often used to prevent bleeding during surgery, as well as hemostasis of hemorrhagic diseases such as internal medicine, obstetrics, and gynecology [7,8].

Mussel secretes mussel mucoprotein through the foot silk, which can adhere firmly to the surface of different substances in the seawater environment [9]. Mussel mucoprotein plays a role through its unique amino acid (dopamine). Catechol in the dopamine side chain is the critical group of adhesion. By simulating the adhesion behavior of mussel mucoprotein, medical products can be developed for tissue adhesion, drug delivery, wound repair and other fields [10,11].
Because there are many amino groups and hydroxyl groups distributed on the macromolecular chain of chitosan, which can form intramolecular and intermolecular hydrogen bonds, chitosan has high crystallinity and poor solubility under neutral or alkaline conditions [12]. It can only be dissolved in dilute acidic aqueous solutions and special organic solvents containing halogen, which significantly limits the application of chitosan [13]. Inspired by the adhesion behavior of mussel mucoprotein, we prepared 6-O-caffeic acid chitosan by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling method using chitosan as a raw material, which laid a foundation for expanding the application field, such as tissue adhesion, hemostasis, wound healing, biomedical coatings, drug delivery and tissue engineering scaffolds.

2. Materials and methods

2.1. Materials

Chitosan (molecular weight 100 kDa and degree of deacetylation >85%), caffeic acid, N,N-dimethylformamide (DMF), phthalic anhydride, EDC·HCl, N-hydroxysuccinimide (NHS), and anhydrous ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Unless otherwise indicated, all the other reagents were analytical grade.

2.2. Synthesis of phthalimide chitosan (P-CS)

Chitosan (1.0 g) was weighed and put into a 150 mL flask, and 90 mL DMF solution (pH=6.6) was used to swell CS. After stirring for 24h at room temperature, 2.9 g phthalic anhydride was added into the above flask. The mixture was heated to 100 °C and kept for 8 hours, and poured into distilled water (300 mL). By centrifuging, the precipitate was collected, washed with anhydrous ethanol three times, and freeze-dried to obtain white samples of phthalimide chitosan.

2.3. Condensation of phthalimide chitosan with caffeic acid

1.00 g phthalimide chitosan was taken into a 100 mL flask, and 40 mL DMF was added. The solution was stirred overnight at room temperature. Subsequently, caffeic acid (0.74 g), EDC·HCl (0.79 g), and NHS (0.23 g) were successively added to the above solution. After stirring for 72 hours in the dark at 25 °C, the reaction mixture was put into 300 mL distilled water. By centrifuging (TG1650-WS centrifuge, Luxiangyi Corporation, Shanghai, China), the precipitate was collected, washed with anhydrous ethanol three times, and freeze-dried (FD-2A-80 vacuum freeze-dryer, Jipu Corporation, Shanghai, China) to obtain off-white samples of phthalimide chitosan-caffeic acid (P-CS-C).

2.4. Synthesis of 6-O-caffeic acid chitosan (CS-C)

The conjugate of P-CS-C (0.5 g) was dissolved in DMF (40 mL) and stirred overnight at 25 °C, and then 75% hydrazine hydrate (5 mL) was added under a nitrogen atmosphere and heated to 80 °C. After stirring for 24 hours, the reaction mixture was cooled to room temperature and put into distilled water. By centrifuging, the precipitate was collected, washed with distilled water and anhydrous ethanol, respectively, and then freeze-dried to obtain off-white samples of 6-O-caffeic acid chitosan (CS-C).

2.5. Structural characterization

2.5.1. FTIR characterization [14]. 1-2 mg powder sample and 200 mg pure KBr were finely ground in an agate mortar and placed in the mold. The sample tablet was then scanned in the wavenumber range of 4000～400 cm⁻¹ by an infrared spectrometer (Spectrum 100, PerkinElmer, Waltham, MA, USA).

2.5.2. XRD characterization [15]. The X-ray diffractometer (XRD-7000, Shimadzu Corporation, Kyoto, Japan) was set as voltage 40 kV, current 40 mA, Cu Kα line, scanning range 5～80°, and scanning speed 15 s/step.
2.5.3. *Thermogravimetric (TG) analysis* [16]. A Perkin-Elmer TG/DTA6300 instrument (PerkinElmer, Waltham, MA, USA) was used to perform thermogravimetric (TG) and differential thermogravimetric (DTG) analysis. The determination conditions were as follows: N$_2$, 50 mL/min; heating rate 10 °C/min and temperature range 20-600 °C.

![Thermogravimetric analysis](image)

Figure 1. Synthesis of 6-O-caffeic acid chitosan (CS-C).

3. Results and discussion

3.1. *Synthesis of 6-O-caffeic acid chitosan (CS-C)*

The synthesis route of chitosan modified by 6-O caffeic acid is shown in Figure 1. Firstly, phthalimide chitosan (P-CS) was prepared by the reaction of phthalic anhydride and chitosan in DMF solution. Then P-CS, caffeic acid, EDC·HCl and NHS were put into distillation flask in a specific order in DMF solution to react, and then centrifugation and drying were carried out to obtain N-phthalimide grafted caffeic acid copolymer (P-CS-C). Finally, P-CS-C was suspended in DMF and a small amount of hydrazine hydrate was added to remove the phthaloyl groups and the target compound of 6-O-caffeic acid chitosan (CS-C) was obtained.

3.2. *Infrared analysis of P-CS*

Figure 2 gives the spectra of CS, P-CS, P-CS-C and CS-C. The characteristic infrared absorption bands are listed in Table 1. From the spectrum of CS, it could be seen that the broad peak at 3415 cm$^{-1}$ was attributed to the overlapping stretching vibration absorption of O-H and N-H on chitosan molecular chain. The absorption peaks at 2915 cm$^{-1}$ and 2862 cm$^{-1}$ belonged to the stretching vibration of C-H on the sugar residue of chitosan. The amide I (C=O stretching), amide II (N-H bending) and amide III (C-N stretching) bands appeared at 1638, 1599 and 1379 cm$^{-1}$, respectively. The absorption peak at 1157 cm$^{-1}$ was ascribed to the stretching vibration of C-O-C in the pyran ring. The characteristic absorption
peak of the primary alcohol hydroxyl group (C₆-OH) was observed at 1078 cm⁻¹. These results were consistent with those reported in the literature [17,18].

![Infrared spectra of chitosan and its derivatives](image)

**Figure 2.** Infrared spectra of chitosan and its derivatives.

**Table 1.** The characteristic FTIR absorption bands of CS and its derivatives.

| Samples | FTIR, cm⁻¹ |
|---------|------------|
| CS      | 3415 (OH, NH), 2915 (C-H), 2862 (C-H), 1638 (C=O), 1599 (NH), 1379 (C-N), 1157 (C-O-C), 1078 (C₆-OH) |
| P-CS    | 1770 (C=O), 1710 (C-N), 1640 (C=O), 1379 (C-N), 1078 (C₆-OH) |
| P-CS-C  | 1770 (C=O), 1728 (COOC), 1709 (C-N) |
| CS-C    | 3402 (OH, NH), 1725 (COOC) |

When compared the infrared spectrum of P-CS with that of CS, new absorption peaks at 1770 and 1710 cm⁻¹ were observed. They were ascribed to the stretching vibration of C=O and C-N, respectively, indicating that the imide group formed after the reaction of phthalic anhydride and chitosan. The absorption peak at 1599 cm⁻¹ resulting from the N-H bending vibration of free amino group in chitosan disappeared almost completely, while the C-N bending vibration absorption peak at 1379 cm⁻¹ increased significantly, which further demonstrated that the free amino groups of CS reacted with phthalic anhydride.

3.3. **Infrared analysis of P-CS-C**

As shown in Figure 2, compared the infrared spectrum of P-CS-C with that of P-CS, The main differences were observed at the bands of 1728 cm⁻¹ and 1078 cm⁻¹. The new characteristic absorption peaks at 1728 cm⁻¹ was attributed to the carbonyl stretching vibration of ester (-COOC), showing the formation of an ester covalent linkage between caffeic acid and chitosan. The characteristic absorption
peak of the primary alcohol hydroxyl group at 1078 cm\(^{-1}\) almost disappeared, indicating that the C6-OH of chitosan had undergone esterification.

### 3.4. Infrared analysis of CS-C

As shown in Figure 2, compared the infrared spectrum of CS-C with that of P-CS-C, the peaks at 1770 and 1710 cm\(^{-1}\) disappeared, indicating that phthalimide group of P-CS-C was deprotected. Compared to CS, CS-C showed an enhanced absorption band around 3402 cm\(^{-1}\), which was due to the introduction of phenol-OH. The characteristic absorption peaks at 1725 cm\(^{-1}\) demonstrated that the ester covalent formed between caffeic acid and chitosan. Moreover, the extreme reduction of absorption peaks of C\(_6\)-OH at 1078 cm\(^{-1}\) was observed, supporting the formation of ester covalent at C\(_6\)-OH of chitosan. Taken together, 6-O-caffeic acid chitosan was successfully prepared.

### 3.5. X-ray diffraction analysis

Figure 3 gives the X-ray diffraction patterns of chitosan and its derivatives. It could be seen that chitosan had two characteristic diffraction peaks at 11° and 20°, respectively, which were ascribed to I (a hydrated crystalline structure) and II (a amorphous structure) crystal bands, respectively [19,20]. However, the intermediates P-CS and P-CS-C did not show any apparent characteristic diffraction peaks, which may be due to the fact that the amino groups on the chitosan molecule were protected by phthalic anhydride and lost the ability to form hydrogen bonds so that the formed macromolecular structures were not regular and had no crystallinity. From the X-ray diffraction pattern of CS-C, a weak diffraction peak at 20° was observed. It could be reasonably explained that when the amino group was deprotected, although the amino group was free, the C\(_6\)-OH of chitosan had been esterified, and the ability of hydrogen bond formation was significantly reduced, resulting in the weak crystallinity of the molecule. In summary, the chemical modification of chitosan decreased the crystallinity and resulted in an amorphous structure.

![Figure 3. X-ray diffraction patterns of chitosan and its derivatives.](image)

### 3.6. TG analysis

TG and DTG curves of CS and CS-C thermal degradation are shown in Figure 4. From Figure 4(a), both CS and CS-C exhibited a degradation pattern of two stage. The two degradation temperature ranges of
CS were 150-300 °C and 450-600 °C. In contrast, the two degradation temperature ranges of CS-C were 200-350 °C and 500-600 °C. It could also be found from the DTG curves in Figure 4(b). The major peaks of CS and CS-C were observed at 238.6 and 272.3 °C. These results indicated that CS-C had better thermal stability than CS in a nitrogen atmosphere, which could be reasonably attributed to intra- and intermolecular interactions. This thermal degradation behavior was similar to that previous reports [21,22].

![Figure 4. TG (a) and DTG (b) curves of CS and CS-C thermal degradation.](image)

4. Conclusions
In this paper, 6-O-caffeic acid chitosan was prepared by the protection of active amino groups in chitosan, selective esterification of C6-OH with caffeic acid, and final deprotection. 6-O-caffeic acid chitosan was characterized by FTIR and XRD. The results showed that 6-O-caffeic acid chitosan was successfully prepared. Thermogravimetric analysis showed that 6-O-caffeic acid chitosan had better thermo-stability than chitosan in nitrogen due to intra- and intermolecular interactions. It will lay a foundation for the deep development and utilization of chitosan in the fields of food, chemical industry, medicine and cosmetics.

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