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Preparation of Amphoteric *N,O*-Carboxymethyl Hydroxypropyl Chitosan by a Two-Step Reaction

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ABSTRACT: A novel *N,O*-carboxymethyl hydroxypropyl chitosan (HPCMS) derivative was prepared by a two-step reaction. Water-soluble hydroxypropyl chitosan (HPCS) with a degree of substitution of hydroxypropyl higher than 0.8 was first synthesized by the reaction of chitosan (CS) with propylene oxide (PO) with alkali as a catalyst. Then, amphoteric chitosan derivatives (HPCMS) with a degree of substitution of carboxymethyl ranging from 0.42 to 1.38 were prepared by the reaction of HPCS with chloroacetic acid in an aqueous solution with alkali as a catalyst. The structures of the polymers were characterized by Fourier transform infrared spectroscopy and NMR; this showed that the hydroxypropylation mainly occurred on the —OH groups at the C-6 of CS in the reaction of CS with PO. In the reaction of HPCS with chloroacetic acid, both the —OH and —NH_2 groups of HPCS were susceptible to the carboxymethylation. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40460.

KEYWORDS: functionalization of polymers; polysaccharides; synthesis and processing

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INTRODUCTION

Chitosan (CS) is a cationic polysaccharide obtained by the partial deacetylation of chitin, which originates from the shells of crustaceans and has been the second most abundant natural polysaccharide after cellulose.[1–4] CS has many advantages, such as abundant resources, nontoxicity, biodegradability, biocompatibility, and antibacterial properties; thus, it has been widely used in the fields of agriculture, pharmaceuticals, cosmetics, food industries, environmental protection, and biotechnology.[5–7] Although CS is soluble in aqueous dilute acids below pH 6.5, it is insoluble in neutral water and most organic solvents.[8] This greatly limits its applications. Special attention has been paid to its chemical modification to obtain water-soluble CS derivatives.[9–11]

Among various methods to improve the solubility of CS, hydroxypropylation and carboxymethylation are the most attractive. Both the hydroxypropyl derivatives and carboxymethyl derivatives of CS are water-soluble and exhibit huge potential application.[12,13] In addition, hydroxypropyl chitosan (HPCS) and carboxymethyl chitosan as reaction intermediates can be further modified. Peng et al.[8] reported the preparation of water-soluble HPCS derivatives with different degrees of substitution and weight-average molecular weights from CS and propylene epoxide under basic conditions and investigated their antimicrobial activities. In a study by Chen et al.,[14] *N,O*-carboxymethyl chitosan was prepared from CS with chloroacetic acid as the carboxymethylating agent in an alkaline medium. Anitha et al.[15] described and reported the synthesis, characterization, cytotoxicity, and antibacterial studies of CS, *O*-carboxymethyl chitosan, and *N,O*-carboxymethyl chitosan nanoparticles.[15] There have been many reports on the hydroxypropyl and carboxymethyl modification of CS,[8,14–19] whereas there have been no reports on the hydroxypropyl modification of CS followed by carboxymethyl modification to obtain amphoteric derivatives with more substituents. The preparation of a novel CS derivative by a two-step reaction is a promising approach because it can be easily carried out and it provides a wide variety of molecular designs. Meanwhile, compared with the traditional carboxymethyl modification of CS, which always progresses in concentrated alkali conditions, [16–19] this two-step reaction can be used to prepare water-soluble CS derivatives under moderate conditions. In other words, water-soluble amphoteric derivatives can be prepared under milder conditions. This is of great benefit for preventing side reaction and for preparing products with good color.

Compared with CS and carboxymethyl chitosan, these new derivatives enhance their performances and make them more biocompatible, homogeneous, hydrophilic, biodegradable, and

amenable to various physical forms. Amphoteric CS derivatives are quite significant in view of the preparation of polysaccharide-based materials, such as hydrogels, which have potential applications in medicine and cosmetics. Multifunctional CS derivatives can also be used to make new kinds of polysaccharide drugs, such as antibacterials, antioxidants, and antitumor drugs. In addition, *N,O*-carboxymethyl hydroxypropyl chitosan (HPCMS) may show promise in wound healing, drug-delivery systems, tissue engineering, antimicrobial agents, and antitumor applications. It was reported that HPCS grafted with maleic acid showed better inhibition effects against *Staphylococcus aureus* and *Escherichia coli* than HPCS.[20] In addition, the *N*-lauryl carboxymethyl chitosan derivative is a good carrier of taxol in the targeting of tumors.[21]

In this study, a series of novel amphoteric CS HPCMS derivatives were prepared by the hydroxypropyl modification of CS followed by carboxymethyl modification. ^{1}H-NMR, ^{13}C-NMR, and Fourier transform infrared (FTIR) spectra were used to characterize the structures and the degrees of substitution (DSs) of the products.

EXPERIMENTAL

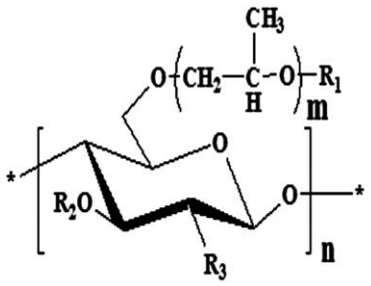
Materials

CS was purchased from Chengdu Kelon Chemical Reagent Factory (China); it had a degree of deacetylation of 95% and a molecular weight of 1×10^5 to 3×10^5. All of the other reagents were analytical grade and were used without further purification.

Synthesis of HPCMS

HPCS. HPCSs with different degrees of substitution of hydroxypropyl (DS_{HP}'s) were prepared from CS and propylene oxide (PO) under alkaline conditions. A typical example was described as follows: 2.0 g of CS, 20 mL of isopropyl alcohol, and 5 mL 15% NaOH were mixed in a three-necked bottle and stirred for 1 h at room temperature. Then, 5 mL of 9% tetramethyl ammonium hydroxide and 20 mL of PO were added. The suspension was stirred for 30 min at room temperature and refluxed 8 h at 60°C.[16] The reaction mixture was neutralized by the addition of 1:1 v/v HCl. Then, the crude product was isolated from the reaction solution by the addition of abundant acetone. After centrifugation, the crude product was washed with 95% ethanol three times and anhydrous ethanol one time and was then dried *in vacuo* at 60°C for 24 h.

HPCMS. HPCMSs with different degrees of substitution of carboxymethyl (DS_{CM}'s) were prepared from the reaction of HPCS and chloroacetic acid in an alkaline solution. A typical example is described as follows: 3.0 g of HPCS and 40 mL of deionized water were added to a three-necked bottle with stirring at room temperature for 0.5 h. Then, 3.15 g of NaOH dissolved in 10 mL of H_2O was slowly added to the reactor. After it was stirred for 1 h at room temperature, 4.96 g of chloroacetic acid dissolved in 10 mL of H_2O was added dropwise into the reactor. The reaction mixture was then stirred continuously for 6 h at a temperature of 60°C. A uniform and transparent solution was obtained at last. The solution was adjusted to pH 7.0 by the addition of acetic acid. Then, the crude product was precipitated by acetone. After centrifugation, the crude product was dissolved in a suitable quantity of deionized water and separated by the addition of abundant ethanol to the solution. In accordance with this method, the product was purified three times. Finally, the product was dried at 60°C on vacuum for 24 h.



$R_1, R_2 = H$ or CH_2COOH

$R_3 = NHCOCH_3, NH_2, NHCH_2COOH$, or $N(CH_2COOH)_2$

Scheme 1. Chemical structure of HPCMS.

Characterization of the CS Derivatives

The IR spectra were obtained from samples in KBr pellets with a Bruker Tensor 27 FTIR spectrophotometer. NMR spectra were recorded at 298 K on a Bruker Avance III 600-MHz NMR spectrometer. All of the products were hydrolyzed by 10% DCl for ^{1}H-NMR and ^{13}C-NMR spectral analysis. The DS value of each sample was calculated with the ^{1}H-NMR spectra.

RESULTS AND DISCUSSION

The chemical structure of HPCMS is shown in Scheme 1. The functional groups, including —NH_2 and —OH, were all susceptible to the alkylation reaction. In acid and neutral media, C_2—NH_2 had a higher reaction activity, whereas in alkaline medium, C_6—OH had a higher reaction activity.[3] In the process of the hydroxypropylation of CS, the substitution mainly occurred at C_6—OH with alkali as a catalyst.[20] The newly formed secondary hydroxyl groups of hydroxyl propyl had less steric hindrance than C_3—OH, which could also react with PO to form short side chains when the C_6—OH was substituted completely.[8,22] In the reaction of HPCS with chloroacetic acid, both O substitution and N substitution took a place at higher temperature in an alkaline medium.[17,23,24]

Characterization

FTIR. Figure 1 shows the FTIR spectra of CS, HPCS, and HPCMS. In the IR spectrum of HPCS, the new peaks at 2970 and 1380 cm^{-1} were assigned to the C—H stretching and bending vibrations of —CH_3; this indicated that hydroxypropyl was introduced into the CS.[25] The absorption peak of primary alcohol at 1030 cm^{-1} disappeared; this indicated that the substitution mainly occurred at the C_6 position.[20] In the IR spectrum of HPCMS, the new absorption peaks at 1600 and 1420 cm^{-1} corresponded to the absorption peaks of the asymmetric stretching vibrations and symmetric stretching vibrations, respectively, of —COO^-. This indicated that —CH_2COOH was introduced into the HPCS.[26]

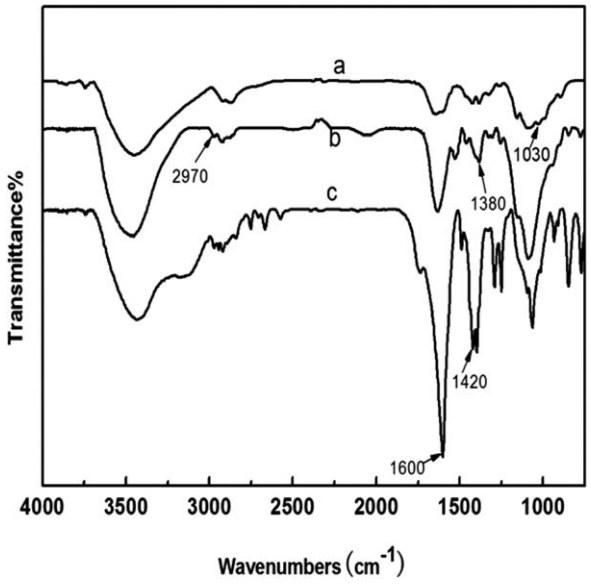


Figure 1. FTIR spectra of (a) CS, (b) HPCS, and (c) HPCMS.

NMR. Figure 2 shows the ^{1}H-NMR spectrum of HPCS. The signals between 4.9 and 5.2 ppm were the resonances of H1, the signals between 3.2 and 4.2 ppm were the resonances of H2–H8. The signal at 2.12 ppm was the resonance of three protons from *N*-acetyl, which was the residual acetyl group of CS.[27,28] In addition, the signals between 1.1 and 1.4 ppm were assigned to the resonance of three protons from —CH_3 of HPCS; this indicated that a hydroxypropyl group was introduced into the chain of CS.[8,16]

Figure 3 shows the ^{1}H-NMR spectrum of HPCMS. Typical signals at 4.30 and 4.64 ppm were assigned to resonances of the methene protons of —$NHCH_2COOH$ and —OCH_2COOH, respectively. The weak signal at 4.51 ppm was assigned to the methene protons of —$N(CH_2COOH)_2$. The signals at 5.0–5.4 ppm were the resonances of H1.[17]

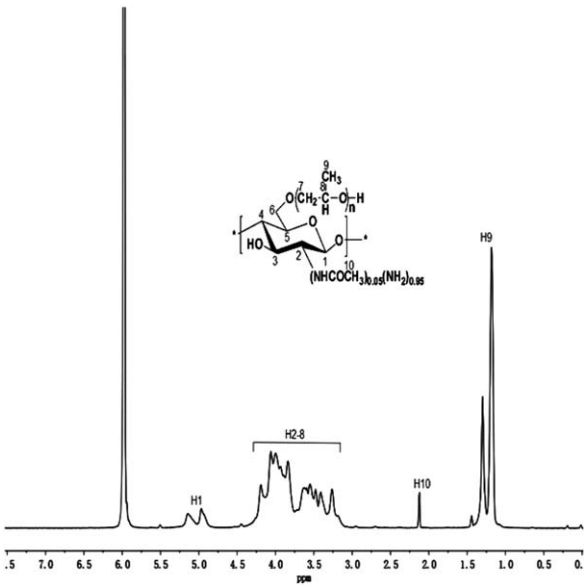


Figure 2. ^{1}H-NMR of HPCS.

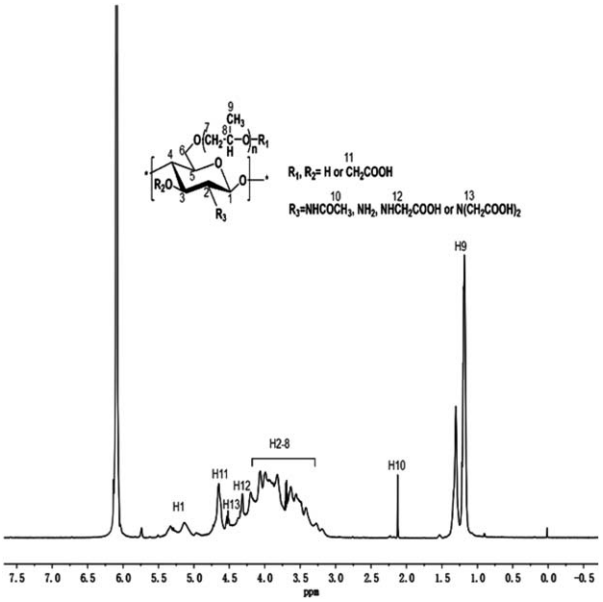


Figure 3. ^{1}H-NMR of HPCMS.

Figure 4 shows the ^{13}C-NMR spectrum of HPCMS. The signals for —CH_2COOH substituted on —OH and —NH_2 were at 175.76 and 169.75 ppm. The three signals at 68.83, 67.30, and 46.70 ppm were assigned to —CH_2COOH groups substituted on O-3, *O*-hydroxypropyl, and N-2 positions.[17,29] The signal at 54.08 ppm was assigned to —CH_2— of —$N(CH_2COOH)_2$.[27] The signals at 19.27 and 17.74 ppm were assigned to the —CH_3 of the hydroxypropyl of HPCMS. The signals at 65.77 and 61.18 ppm were caused by the methylene and methine carbons in the hydroxypropyl unit.[22] Other signals at 95.63, 75.66, 74.12, 72.87, 62.36, 59.64, and 20.17 ppm were assigned to the carbons of C1, C4, C5, C3, C6, C2, and —$NHCOCH_3$, respectively.[17,30]

DS_{HP} and DS_{CM} were calculated from the corresponding peak area in the ^{1}H-NMR spectra of HPCS and HPCMS, respectively. The specific formulas are as follows:

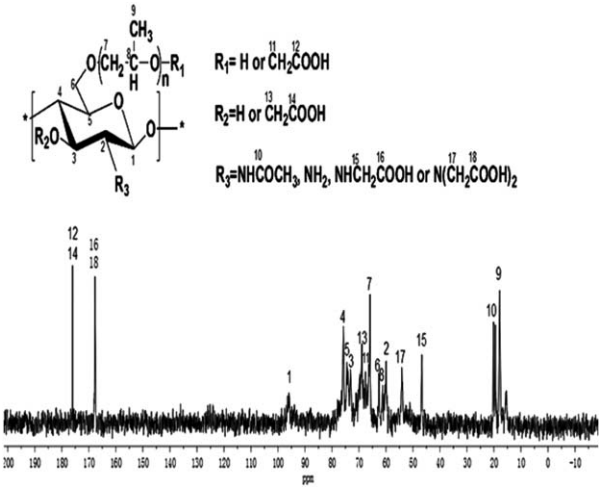


Figure 4. ^{13}C-NMR of HPCMS.

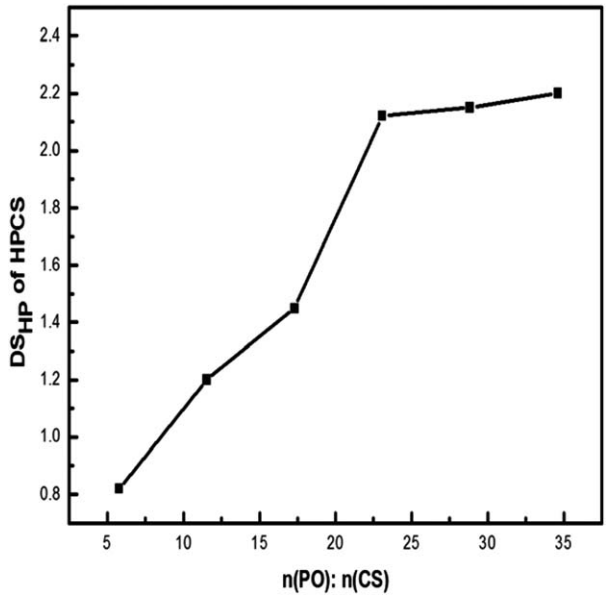


Figure 5. Effect of the dosage of PO on DS_{HP} (2 g of CS, 5 mL of 20% sodium hydroxide, 20 mL of isopropyl alcohol, and reaction at 60°C for 8 h).

$$DS_{HP} = \frac{1}{3}\frac{I_{H9}}{I_{H1}} \quad (1)$$

$$DS_{CM} = \frac{1}{2}\left(I_{H11} + I_{H12} + \frac{1}{2}I_{H13}\right)/I_{H1} \quad (2)$$

where I_{H1} is the peak area of H1; I_{H9} is the peak area of the proton peak of —CH_3 at the hydroxypropyl; and I_{H11}, I_{H12}, and I_{H13} are the peak areas of the proton peaks of the methylenes at —OCH_2COOH, —$NHCH_2COOH$, and —$N(CH_2COOH)_2$, respectively.

Effect of the Reaction Conditions on DS_{HP}

Effect of the Dosage of PO on DS_{HP}. Figure 5 shows the effect of the dosage of PO on DS_{HP} when the concentration of sodium hydroxide was 20%. The reaction temperature was kept at 60°C because PO volatilized rapidly, and it was very difficult to maintain the PO in the reaction mixture at higher temperatures. DS_{HP} increased fast when the molar ratio of PO to CS increased from 5 to 23. When the molar ratio of PO to CS reached 23, DS_{HP} was 2.12 and almost did not change further. This was due to the fact that C_6—OH with a higher reaction activity was almost completely substituted when the molar ratio of PO to CS reached 23, and the steric hindrance of the reaction groups increased with increasing DS_{HP}.

Effect of the Alkalinity on DS_{HP}. A series of reactions at different alkalinities were carried out to investigate the effect of the alkalinity on DS_{HP}. As shown in Figure 6, DS_{HP} of the product increased first and then decreased with increasing alkalinity. DS_{HP} reached a maximum of 2.41 when the concentration of NaOH solution was 15%. A further increase in the concentration of NaOH had two side effects: the hydrolysis of PO and the degradation of CS.

Effect of the Reaction Conditions on DS_{CM}

Table I shows the effect of the reaction conditions on DS_{CM}. HPCS with a DS_{HP} of 2.12 was selected as the raw material for its good solubility in neutral water. Through changes in the reaction temperature, reaction time, molar ratio of chloroacetic acid to HPCS, and molar ratio of NaOH to chloroacetic acid, the effects of different reaction conditions on DS_{CM} were studied and analyzed.

Effects of the Reaction Temperature and Reaction Time on DS_{CM}. The effects of the reaction temperature and reaction time on DS_{CM} are shown in Table I from HPCMS1 to HPCMS9. The DS_{CM} of the product first increased with increasing temperature and then decreased. Even though a higher temperature accelerated the carboxymethylation reaction of HPCS, chloroacetic acid hydrolyzed rapidly at higher temperatures. Then, the chloroacetic acid's utilization rate and DS_{CM} decreased. As a result, 60°C was a suitable temperature for the carboxymethylation reaction of HPCS.

When we changed the reaction time, we found that when the reaction time was greater than 6 h, the value of DS_{CM} remained almost unchanged. A further extension of the reaction time may have aggravated the side reaction.

Effect of the Molar Ratio of Chloroacetic Acid to HPCS on DS_{CM}. Because the C_6—OH groups were almost completely substituted in the hydroxypropylation of CS, the remaining reaction groups of HPCS were C_3—OH, C_2—NH_2, and hydroxyl groups on hydroxypropyl. In alkaline aqueous solution, all of the three groups were susceptible to the carboxymethylation reaction; this was confirmed in the ^{1}H-NMR and ^{13}C-NMR spectra of HPCMS. As shown in Table I (HPCMS10–HPCMS13 and HPCMS3), DS_{CM} increased rapidly when the molar ratio of chloroacetic acid to HPCS increased from 1:1 to 5:1 and leveled off after the molar ratio of chloroacetic acid to HPCS reached 5:1. This indicated that the carboxymethylation of HPCS reached equilibrium when the molar ratio of chloroacetic acid to HPCS was 5:1. As the molar ratio of chloroacetic acid to HPCS continued to increase, this

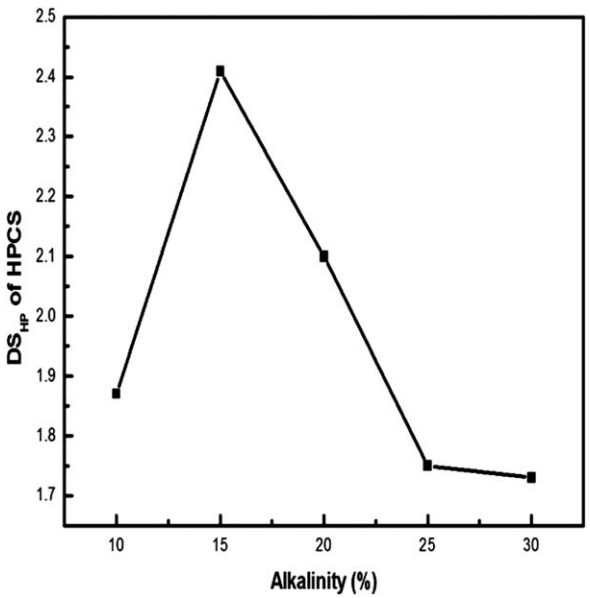


Figure 6. Effect of the alkalinity on DS_{HP} (2 g of CS, 20 mL of PO, 20 mL of isopropyl alcohol, and reaction at 60°C for 8 h).

Table I. Effects of the Reaction Conditions on DS_{CM}

Sample	$n_{ClCH2CH2COOH}/n_{HPCS}$	$n_{NaOH}/n_{ClCH2CH2COOH}$	Temperature (°C)	Time (h)	DS_{CM}
HPCMS1	5:1	1.5:1	40	6	0.68
HPCMS2	5:1	1.5:1	50	6	1.05
HPCMS3	5:1	1.5:1	60	6	1.25
HPCMS4	5:1	1.5:1	70	6	1.02
HPCMS5	5:1	1.5:1	80	6	0.98
HPCMS6	5:1	1.5:1	60	4	0.72
HPCMS7	5:1	1.5:1	60	5	1.07
HPCMS8	5:1	1.5:1	60	7	1.30
HPCMS9	5:1	1.5:1	60	8	1.38
HPCMS10	1:1	1.5:1	60	6	0.42
HPCMS11	2:1	1.5:1	60	6	0.50
HPCMS12	3:1	1.5:1	60	6	0.82
HPCMS13	6:1	1.5:1	60	6	1.30
HPCMS14	5:1	1.2:1	60	6	1.05
HPCMS15	5:1	2:1	60	6	1.18

may have aggravated the side reaction (the hydrolysis of chloroacetic acid), decreased the alkalinity of the reaction system, and wasted the raw materials. So the proper molar ratio of chloroacetic acid to HPCS was 5:1.

Effect of the Alkalinity of the Reaction Medium on DS_{CM}. The effect of the alkalinity of the reaction medium on DS_{CM} is shown in Table I for HPCMS14, HPCMS15, and HPCMS3. DS_{CM} increased first and then decreased with increasing alkalinity. The value of DS_{CM} reached a maximum when $n_{NaOH}/n_{ClCH2CH2COOH} = 1.5:1$ (where n amount of substance). The materials were difficult to alkalize, and the active center of HPCS was insufficient when the reaction medium had a low alkalinity. An increase in the alkalinity increased the active center of HPCS; this was beneficial for the reaction but also accelerated the hydrolysis reaction of chloroacetic acid and led to a decrease in DS_{CM}. The optimal molar ratio of NaOH to chloroacetic acid was found to be 1.5:1.

CONCLUSIONS

A novel water-soluble amphoteric CS derivative was prepared through the hydroxypropylation of CS followed by carboxymethylation. The DS for hydroxypropyl and carboxymethyl groups could be easily controlled through changes in the reaction conditions. The introduction of hydroxypropyl destroyed the crystal structure of CS and afforded the product with good water solubility. The carboxymethylation reaction of HPCS was performed in the aqueous phase and proceeded smoothly. Both the —NH_2 and —OH groups reacted with chloroacetic acid. This process for the preparation of water-soluble amphoteric CS is of great interest in applications such as wound-dressing materials, intelligent hydrogels, and water engineering.

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