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Preparation and characterization of a quaternized chitosan

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Abstract A novel method was developed to obtain *N*-(2-hydroxy)-propyl-3-trimethylammonium chitosan chloride (HTCC) using glycidyl trimethyl ammonium chloride (GTMAC) and chitosan in a homogeneous system. The factors affecting the degree of substitution (DS) of HTCC, including the amount of perchloric acid, the reaction temperature, and the reaction time, were especially investigated. Under optimal conditions (i.e., with 2.0, 1.9, and 15 g of chitosan, perchloric acid, and GTMAC, respectively), chitosan was pre-reacted with GTMAC at 60 °C, and then the reaction was continued at 80 °C for 8 h. The as-produced HTCC had a DS of 86.9%. Its structure was characterized by Fourier transform infrared spectroscopy and nuclear magnetic resonance. Moreover, it had a good solubility in water within a wide pH range. Besides, the composite was formed from HTCC loaded on bentonite surface.

Introduction

Chitosan is derived from chitin through deacetylation. Chitin exists in fungal walls and exoskeletons of crustaceans and insects and is the second most abundant polysaccharide in nature [1]; therefore, the source of chitosan is abundant. Chitosan has been widely applied in various fields, including pharmaceutics [2–4], food science [5–7], wastewater treatment [8–10], and cosmetics due to its unique properties, such as bacterial resistance, nontoxic nature, and biodegradability. Nevertheless, these activities are limited to acidic conditions due to its poor solubility in water [11, 12]; hence, it is essential to improve its water solubility to expand its range of applications. Therefore, many researchers have been focusing on the preparation of chitosan derivatives. Quaternation is one of the various methods that can enhance water solubility. Quaternized chitosan has a broad application in environmental field such as waster treatment due to its unique properties, such as biocompatibility, biodegradation, biological activity, low toxicity, and so on. As one of quaternized chitosans, chitosan-*N*-2-hydroxypropyl trimethyl ammonium chloride (HTCC), is itself a good absorbent and flocculent agent. Viviane et al. [13] found that HTCC was an effective adsorbent for the removal of Cr(VI) from aqueous solution. Huang et al. [14] prepared a positively charged composite nanofiltration membrane using HTCC as membrane-forming reagent, and would use it to treat waste-water drained from the production of sodium alginate. Besides, HTCC was also applied in the pharmaceutical field. For example, Xu et al. [15] prepared a HTCC nanoparticles based on ionic gelation process of HTCC and sodium tripolyphosphate (TPP), and would use for the carrier of Bovine serum albumin (BSA).

At present, HTCC is generally synthesized by the heterogeneous reaction between chitosan and glycidyl trimethylammoniumchloride (GTMAC) in water, alcohol, or alkaline alcohol. For example, Wu et al. [16] reported that HTCC was obtained by the reaction between chitosan and GTMAC in water, and an innovative hydrogel system composed of HTCC/glycerophosphate with sensitivity to temperature and pH was prepared and used as an intelligent

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drug carrier. Peng et al. [17] synthesized HTCC with differing degrees of substitution (6, 18, and 44%) of quaternary ammonium by reacting chitosan with GTMAC.

However, under neutral or alkaline conditions, GTMAC is easily hydrolyzed and transformed into 2,3-dihydroxypropyl trimethyl ammonium chloride, thus weakening its binding with chitosan. Moreover, GTMAC also undergoes intermolecular polymerization in ethanol or water, thus reducing the grafting efficiency of chitosan; hence, the HTCC obtained by the heterogeneous reaction often shows a relatively low degree of substitution (DS).

This article presents a new method to prepare HTCC under acidic conditions (i.e., in the presence of perchloric acid). Chitosan dissolves in perchloric acid, and thus the reaction mixture containing GTMAC and chitosan forms a homogeneous system. Furthermore, perchloric acid does not react with GTMAC due to the perchloric acid's low nucleophilicity. Thus, quaternization can be conducted more easily. The factors affecting the DS (such as the amount of perchloric acid, the reaction temperature, and the reaction time) are thereafter studied. Moreover, the characteristics of both HTCC and the composite formed between HTCC and bentonite are evaluated.

Experiments

Materials

Chitosan (MW 5.0×10^5, degree of deacetylation (DD) was 90%) was purchased from by the Sinopharm Group Chemical Reagent Limited Company (China). GTMAC was obtained from Dongying Guofeng Fine Chemical Co. Ltd. (Shandong, China). Bentonite powder with a particle size of 200 meshes was acquired from the chemical factory of Shentai, Xinyang, Henan, China. All other chemicals were of analytical grade and were used as received.

Instruments/apparatus

Fourier-transform infrared spectroscopy (FTIR) of the samples were taken using an Avatar-360 IR spectrometer from Nicolet in the wave number range of 400–4000 cm^{-1}. Thermogravimetric (TG) analysis was done using a Simultaneous DTA-TG apparatus (DTG-60AH, SHIMADZU). XRD patterns of the samples were done using an X-ray diffraction with Cu anode, running at 40 kV and 40 mA. Diffraction measurements were conducted within the 2θ angle of 0°–50°, at the scanning rate of 4°/min. The pH of the solution was measured using a laboratory scale pH meter (LiDa instrument, Shanghai, China). A temperature controlled water bath flask shaker (Kanghua, Jiangsu, China) was used for shaking the solutions. The determination of (VI) concentration in solution was done on a UV–visible spectrophotometer (model 754 N) made in Shanghai, China.

Preparation of HTCC

HTCC was prepared in a homogeneous system, and Fig. 1 illustrates the reaction scheme. Chitosan (2.0 g, 12.3 mmol) was dispersed in deionized water (30.0 mL) and completely dissolved in perchloric acid (1.9 g) at room temperature. An aqueous solution of GTMAC (15 g, 98.8 mmol) was added to chitosan suspension at 60 °C in three aliquots at intervals of 0.5 h to enable prereaction with chitosan, and then the reaction was continued for 8 h at 80 °C. Finally, the turbid, yellowish reaction solution was poured into acetone, thus precipitating the product. After washing with acetone several times, the white precipitated product was collected by filtration. To obtain HTCC of greater purity, the product was dissolved in deionized water and precipitated with acetone again; subsequently, it was filtered and dried in an oven at 60 °C.

Characteristics of HTCC

Fourier-transform infrared spectroscopy and ^{13}C-nuclear magnetic resonance

The Fourier-transform infrared (FTIR) spectra of chitosan and HTCC in the range of 500–4000 cm^{-1} were obtained to confirm the existence of various functional groups on

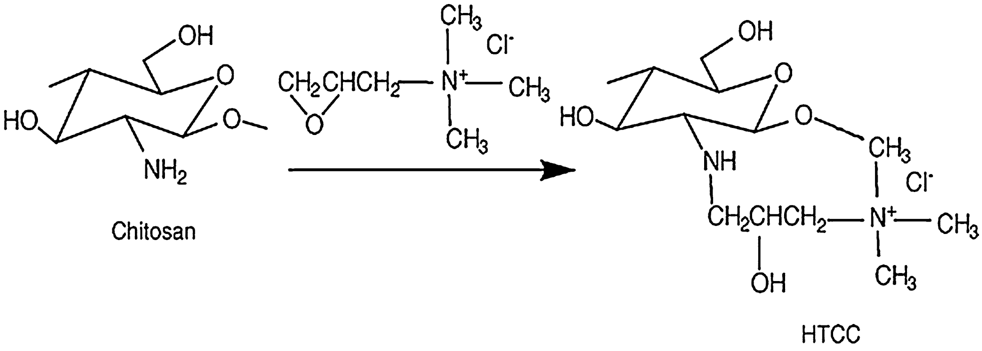

Fig. 1 Reaction scheme of quaternized chitosan (HTCC)

HTCC at room temperature with KBr pellets. The ^{13}C-nuclear magnetic resonance (NMR) spectrum of HTCC was recorded on a Varian mercury VX-300 spectrometer, and the chemical shifts were measured with reference to methanol in D_2O.

Determination of degree of substitution

The degree of substitution (DS) was determined by titrating the amount of Cl^- ions on the HTCC with aq$AgNO_3$ solution. DS is calculated according to the following equation:

$$\mathrm{DS} = \frac{CV/1000}{(CV/1000) + (W - (CVM_2/1000))/M_1}$$

where C (mol L^{-1}) is the concentration of $AgNO_3$ solution, V (mL) is the volume of $AgNO_3$ solution, W (g) is the weight of quaternary chitosan, M_1 (mol g^{-1}) is the molar mass of glucosamine, and M_2 (mol g^{-1}) is the molar mass of quaternary chitosan.

Estimation of water volubility over a wide pH Range

HTCC (0.2 g) was suspended in 10 mL deionized water at different values of pH, which were adjusted using 0.1 M HCl or NaOH solution. The solubility of HTCC in water was evaluated after stirring at room temperature. In addition, the pH values of the solution before and after dissolution were measured to observe any changes of pH.

Composite formation between HTCC and bentonite

As mentioned in introduction, HTCC was an effective adsorbent for the removal of Cr(VI) from aqueous solution. However, HTCC is a relatively high-cost biopolymer, and it is apt to loss during this adsorption process, so it is expected that a new novel adsorbent was prepared by HTCC loaded onto clay for purpose of reducing the amount of HTCC and avoiding the loss of HTCC. Bentonite is one of the clays commonly used in fabrication of adsorbents due to its high cation-exchange capacity and low cost. A kind of nanocomposite in this study was prepared by modifying bentonite with a negatively charged surface with HTCC containing a distribution of positively charged groups, $–N^+ (CH_3)_4$, that is, a modified bentonite was obtained. The general procedure adopted for the preparation of the HTCC–bentonite composite is as follows. Bentonite (5 g) was soaked in 30 mL of water and taken in a three-necked round-bottom flask, and HTCC (1.0 g) in 30 mL of distilled water was added to it. The contents were maintained at 80 °C for 2 h. The composite obtained was then washed with distilled water until it was free from HTCC. The product was dried in an oven at 105 °C for 2 h and then ground to obtain particles of 200-mesh size. The nanocomposite was named HTCC–bentonite, which would be characterized by X-ray diffraction (XRD) and Thermogravimetric (TG) analysis.

Results and discussion

Conditions for dissolution of HTCC

Theoretically, HTCC may have a substitution degree of 200%, because two –Hs are substituted for $–NH_2$ in chitosan molar. However, in practice, such theoretical values are not obtained due to the different methods or conditions of preparation. In general, the better the solubility in water is, the higher is the DS. Thus, the solubility in water was mainly investigated by measuring the DS.

Effect of the amount of perchloric acid on water solubility

For this test, the amount of perchloric acid used was between 0.7 and 1.9 g. Chitosan (2.0 g) was dispersed in deionized water (30.0 mL), and chitosan was completely dissolved by adding perchloric acid in drops. Subsequently, GTMAC (10 g) was dissolved in deionized water (20 mL) and added to the above chitosan solution at room temperature, and the reaction was continued for 8 h at 80 °C. The product was treated according to the method described in "Instruments/apparatus" section. The water solubility was observed by adding the resultant products (0.2 g) to deionized water (10 mL) and stirring at room temperature. The results suggest that the water solubility improves gradually with an increase in the quantity of perchloric acid used. However, the product obtained was completely dissolved in water when the amount of perchloric acid reached 1.9 g. Therefore, a weight of 1.9 g of perchloric acid was chosen for further experiments.

Effect of prereaction on DS

Prereaction, different from the reaction mode mentioned in "Effect of the amount of perchloric acid on water solubility" section, was carried out as follows. Chitosan (2.0 g) was completely dissolved by adding 1.9 g perchloric acid. Subsequently, GTMAC (10 g) was dissolved in deionized water (20 mL) and added to the above chitosan solution in three aliquots at intervals of 0.5 h while simultaneously increasing the temperature to 60 °C. Subsequently, the reaction was further continued for 8 h at 80 °C. As shown in Table 1, once the prereaction was carried out, the DS of HTCC reached 72%, and it increased by 19.6% relative to the solution without prereaction. This is mainly because the reaction between chitosan and GTMAC is an exothermic reaction,

Table 1 Effect of pre-reaction on DS

The method of pre-reaction	Without pre-reaction	With pre-reaction
DS	52.4	72.0

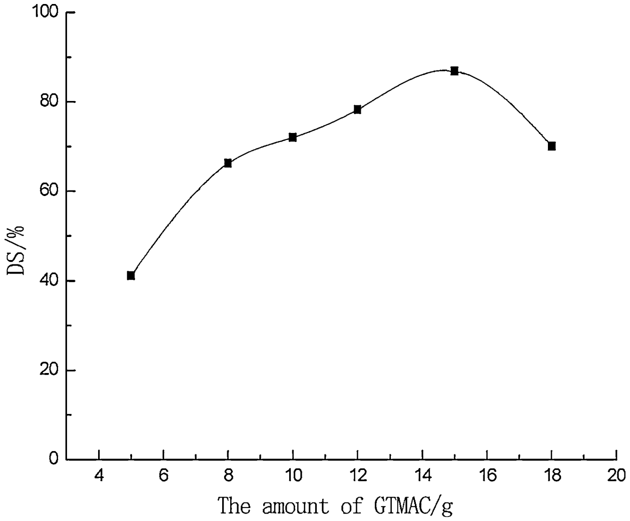

Fig. 2 Effect of the amount of GTMAC on DS

releasing a large quantity of heat during the reaction process. If GTMAC is added all at once, the reaction would become fiercer and be difficult to be control. Thus, the prereaction was helpful in the reaction between chitosan and GTMAC and for the improvement of the DS. Therefore, prereaction has been applied in all the following experiments.

Effect of the amount of GTMAC on DS

The effect of the amount of GTMAC on the DS is shown in Fig. 2. The DS increased by 45.7% when the amount varied between 5 and 15 g. GTMAC continuously enters the inside of chitosan with an increase in GTMAC concentration; thus, the reaction between chitosan and GTMAC becomes complete, leading to improvement in the DS. However, further addition of GTMAC to the chitosan solution resulted in a reduction in the DS, which is probably due to the intermolecular polymerization of GTMAC. Therefore, 15 g GTMAC was adopted as the optimum level.

Effect of the reaction temperature on the DS

The reaction between GTMAC and chitosan is often carried out at a relatively high temperature [16, 18]. Thus, reaction temperatures in the range of 60–90 °C were investigated. As listed in Table 2, elevated temperature improves the activity of chitosan and facilitates the nucleophilic addition reaction between GTMAC and chitosan, thereby increasing the DS with an increase in the temperature until 80 °C. However, chitosan is prone to decomposition under high temperatures, and hence the DS began to reduce when the temperature crossed 80 °C. Thus, the apt temperature for the reaction was concluded to be 80 °C.

Table 2 Effect of the reaction temperature on DS

Reaction temperature (°C)	DS
60	65.2
70	68.7
80	86.9
90	68.5

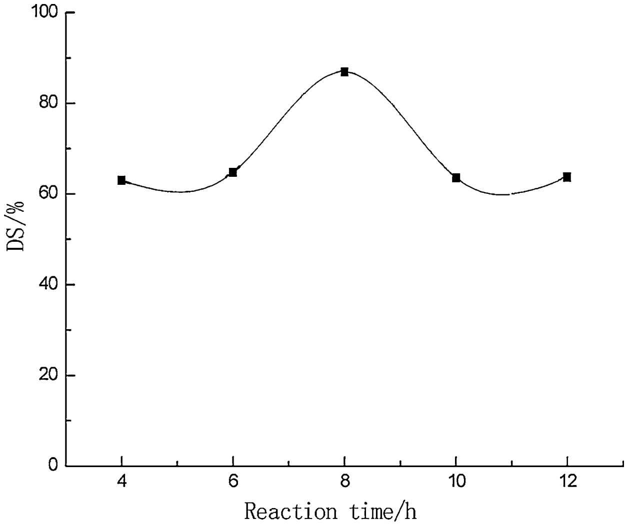

Fig. 3 Effect of the reaction time on DS

Effect of the reaction time on DS

The effect of the reaction time on the DS is shown in Fig. 3. The DS increased from 62.9 to 86.9% when the reaction time was increased from 4 to 8 h. This is because the longer the reaction time, the more complete is the reaction, resulting in an increasing DS. However, the solution in the reaction system became increasingly viscous with the progress of the reaction; thus, the mass transfer between chitosan and GTMAC becomes difficult and the availability of GTMAC decreases, leading to a reduction in the DS, as shown in latter experiments. Therefore, 10-h duration was chosen as the optimum reaction time.

Based on the above monofactorial experiments, the preparation conditions for HTCC with a high DS are as follows: chitosan 2.0 g, perchloric acid and GTMAC: 1.9 and 15 g, respectively. The pre-reaction was carried out at 60 °C, and then the reaction was continued for 8 h at 80 °C. The product, HTCC, was obtained as a white powder and had a DS of 86.9%. Its characteristics will be studied and reported in further studies.

Characteristics of the as-synthesized HTCC

FITR of chitosan and HTCC

The IR spectra of both chitosan and HTCC were studied to identify the existence of quaternary amino groups on the HTCC chains (Fig. 4). In the spectrum of HTCC, the characteristic peak (1590 cm^{-1}) representing the $–NH_2$ group was weakened and a new peak appeared at 1477 cm^{-1}, which was attributed to the methyl groups of the ammonium groups. Compared with chitosan, the peaks corresponding to the vibrations of the skeletal saccharide oxygen bridges involving the C–O stretching appearing between 1154 and 1079 cm^{-1} did not change in HTCC, proving the introduction of the quaternary amino groups at the $–NH_2$ sites on the chitosan chains.

^{13}C-NMR of HTCC

Currently, NMR is the most effective technique to determine the structure of chitosan derivatives. The ^{13}C-NMR spectrum of HTCC is shown in Fig. 5. The peak at $\delta = 54.7$ ppm was attributed to the carbons of the trimethylammonium group (C-4*). The peaks at $\delta = 103.0$, 65.3, 73.6, 75.2, 78.5, and 60.8 ppm were attributed to the C-1, C-2, C-3, C-4, C-5, and C-6, respectively. The peaks at $\delta = 53.4$ and 65.9 ppm were attributed to C-1* and C-3*, respectively. The peak appearing at $\delta = 68.8$ ppm was attributed to C-2*. The peak at $\delta = 22.8$ ppm was attributed to the carbons of the residual CH_3–acetyl. The results further prove the introduction of quaternary amino groups at the $–NH_2$ sites on the chitosan chains.

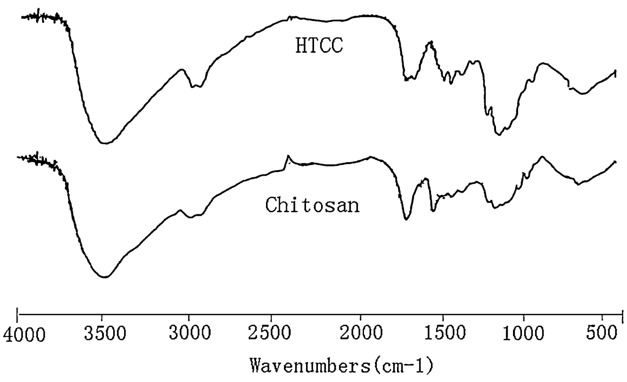

Fig. 4 FTIR spectra of chitosan and HTCC

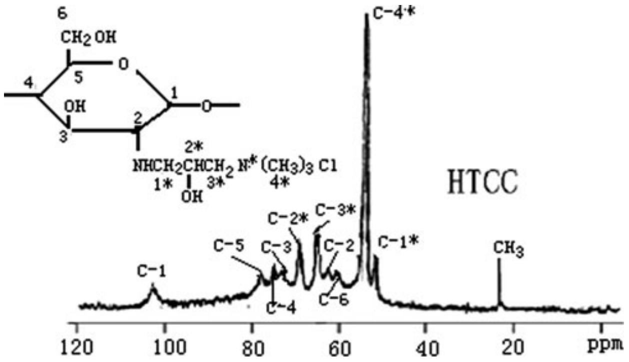

Fig. 5 The ^{13}C NMR spectrum of HTCC

Estimation of water solubility over a wide pH range

The water solubility of HTCC was investigated by dissolving HTCC in water at pH values of 2.99, 4.54, 6.46, 9.58, and 11.46. HTCC was completely dissolved in water at acidic, neutral, or basic pHs because of the presence of positively charged quaternary ammonium groups. It was obvious that HTCC is soluble in water over a wide pH range. In addition, the change in pH before and after dissolution was observed. As shown in Table 3, after the dissolution of HTCC, the pH of the solution increased at pH <7, decreased at pH >7, but did not change at pH 7. It is suggested that the pH of the solution changes when HTCC is dissolved under acidic or basic conditions.

HTCC–bentonite composite

X-ray diffraction analysis of the composite X-ray diffraction (XRD) is an effective method for investigating the intercalation status of bentonite. The XRD patterns of the raw and HTCC–bentonite samples are shown in Fig. 6. Similar to raw bentonite, HTCC–bentonite shows a diffraction peak at 6.06°, showing that HTCC covers only the surface of bentonite and does not penetrate into the interlayer space. Ma et al. [19] also observed a similar phenomenon , that is, chitosan was reported to cover only the surface of bentonite when chitosan was used for the modification of bentonite. However, compared with the intensity of the diffraction peak at 6.06° in bentonite was

Table 3 The change of pH in water with different pH

Order number	1	2	3	4	5
pH before dissolution	2.99	4.54	6.46	9.58	11.46
pH after dissolution	4.35	6.91	6.98	7.31	10.40

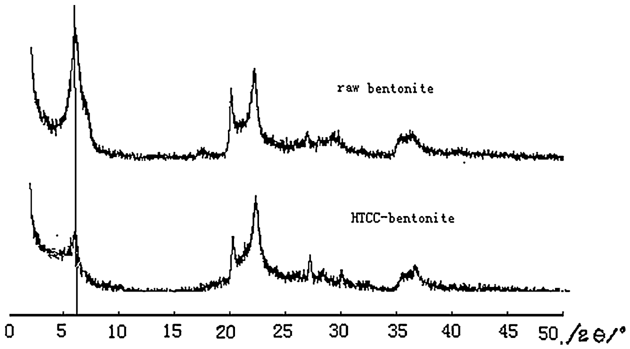

Fig. 6 XRD patterns of raw bentonite and HTCC–bentonite

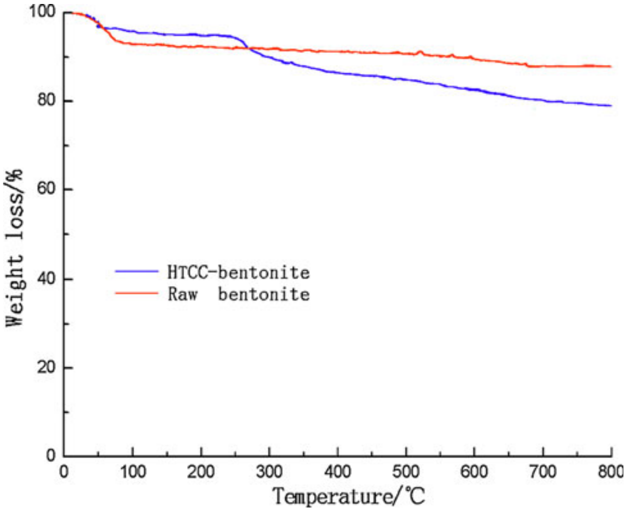

Fig. 7 TG plots of raw bentonite and HTCC–bentonite

much weaker than the one in HACC–bentonite. The results reveal the interaction between HACC and bentonite.

Thermogravimetric analysis The thermogravimetric (TG) plots of raw bentonite and HTCC–bentonite are shown in Fig. 7. For raw bentonite, the TG diagram showed a mass loss of 7.5% below 100 °C, which could be attributed to the desorption of physically adsorbed water on the surface layer; 2.5% loss in the temperature range of 100–800 °C, which could be attributed to the dehydroxylation of the aluminosilicate layer. However, the mass loss of the HTCC–bentonite sample was 2.5% below 250 °C due to the part replacement of the physically adsorbed water by HTCC and 16.0% in the range of 250–800 °C due to both the decomposition of the HTCC loaded on the bentonite and the dehydroxylation of the clay layers. Compared with the mass loss of the raw bentonite, the loss of HTCC–bentonite was more by 8.5%. Therefore, it can be concluded that HTCC was loaded onto the surface of bentonite and that the composite between HTCC and bentonite was formed.

Adsorption of Cr(VI) on HTCC–bentonite composite Adsorption experiments were carried out with 57 mg/L Cr(VI) solution at pH 1 using 1.0 g HTCC–bentonite with temperature controlled water bath flask shaker (200 rpm) at 30 ± 1 °C for 40 min to attain equilibrium. Removal of Cr(VI) reached 88.7%, with an adsorption content of 2.65 mg/g. Moreover, the HTCC–bentonite shows a markedly higher adsorption capacity than HTCC or bentonite alone. Therefore, HTCC–bentonite has promising potential for application in the removal of Cr(VI). Its adsorption content would attempt to be increased and the characteristic of its adsorption for Cr(VI) would be studied later.

Conclusions

Quaternized chitosan, *N*-(2-hydroxyl)-propyl-3-trimethylammonium chitosan chloride (HTCC), was synthesized using GTMAC and chitosan in a homogeneous system. Effect of the amount of GTMAC, the reaction time, and the reaction temperature, etc., on the DS of HTCC was investigated. FTIR spectroscopy and ^{13}C-NMR confirmed the existence of quaternary amino groups on the HTCC chains. The DS of HTCC obtained was 86.9% under optimal preparation conditions. Moreover, HTCC showed good water solubility over a wide range of pH. In addition, HTCC may be used for the modification of betonite. The HTCC–bentonite composite had a great potential for application in the removal of Cr(VI) from aqueous solutions.

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