Synthesis of Carboxymethyl Chitosan for the fabrication of Chitosan/Montmorillonite Nanocomposites

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ABSTRACT

Chitosan/montmorillonite nanocomposites can impart water vapor and oxygen barrier and antimicrobial properties to paper. However, this application is limited by the low water-solubility of chitosan. Carboxymethylation can increase the water-solubility of chitosan. In this work, carboxymethyl chitosan was synthesized by grafting with chloroacetic acid, and intercalated montmorillonite nanocomposites were prepared by intercalation of carboxymethyl chitosan with sodium-based montmorillonite. The process conditions were optimized for the fabrication of nanocomposites. Characterizations were conducted by FT-IR, 1H-NMR and XRD. FT-IR and 1H-NMR results showed that carboxyl groups were introduced into the chitosan, and carboxymethyl chitosan was generated. XRD results confirmed the insertion of CM-CTS into interlayers of Na+-MMT. As indicated from XRD results, chemical modification of chitosan resulted in enhanced intercalation. Carboxymethyl chitosan resulted in formation of exfoliated chitosan/montmorillonite nanocomposites.

Keywords: Chitosan; Carboxymethyl chitosan (CM-CTS); Montmorillonite; Nanocomposites

1. INTRODUCTION

With the increasing attention to limited natural resources and the environmental issues, a variety of renewable and biodegradable biopolymers such as polysaccharides, proteins, lipids, and their composites, derived from plant and animal resources, have been investigated and widely used in all walks of life.1-3

Chitosan is a polysaccharide obtained from partial deacetylation of the second abundant natural polymer, chitin. Besides, chitosan is the only alkaline natural amino polysaccharide which is composed of β-(1,4)-2-acetamino-2-deoxy-D-glucose binary linear copolymer.4 Due to its non-toxicity, biodegradability, biocompatibility, antimicrobial activity, p and bioactivity, chitosan can be applied in diversified fields. However, the poor solubility in water and most common organic solvents limits its widespread application.5 As a result, numerous soluble derivatives have been studied.6-8

Carboxymethyl chitosan (CM-CTS) with good solubility both in water and organic solvents is one of the most important chitosan derivatives. It can be synthesized by introduction of carboxymethyl groups to chitosan chain.9,10

Carboxymethyl chitosan (CM-CTS) is a water-soluble chitosan derivative with excellent antibacterial property.11 At the same time, carboxymethyl chitosan also has the following excellent features: (1) moisture-resistance, (2) film-forming property, (3) high molecular weight, and (4) non-toxicity. Therefore, carboxymethyl chitosan has been widely used in agriculture, medicine, chemical industry, food industry, and cosmetics industry.

MMT is a clay mineral consisting of stacked silicate sheets with a high aspect ratio and a plate-like morphology. This high aspect ratio plays an important role in the enhancement of mechanical properties of composite materials. Chemically, MMT consists of two fused silicate tetrahedral sheets sandwiching an edge-shared octahedral sheet of either magnesium or aluminum hydroxide.12 Montmorillonite can be modified by cationic exchange reactions between the sodium ions in the montmorillonite layers and cations such as Ni, Co, Zn,13 cationic surfactants,14,15 salts of fatty acids,16 and polymers.17-19 However, some cationic substances are difficult to degrade in nature and may be toxic to humans and the environment. Therefore, the use of natural cationic polymers such as chitosan has generated increasing interest.10,20,21

There are a lot of research reports on chitosan-intercalated montmorillonite. To improve the solubility of Chitosan, carboxymethyl chitosan was prepared. At the same time, chitosan was chemically modified under varying conditions. CM-CTS samples were intercalated into the interlayer of Na+-MMT, and the impact of intercalation of on the resulting nanocomposites was investigated by XRD.

2. EXPERIMENTAL

2.1 Materials

Chitosan was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Its degree of
deacetylation (DD) was controlled at 80.0–95.0%. Sodium based montmorillonite (Na⁺-MMT) was purchased from Nanocor Co. USA, and its cation exchange capacity was 120 meq/100g. Chloroacetic acid was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Other reagents were of all analytical grade and used without further purification.

2.2 Determination of Degree of Deacetylation of Chitosan

The degree of deacetylation (DD) of chitosan was determined by using acid-base titration. 0.3g of chitosan was dissolved in 30mL HCl (0.1M), and then 2-3 drops of methyl orange-aniline blue (indicator) was added, followed by titration with 0.1 M NaOH solution. The consumed volume was recorded. DD was calculated as follows:

\[
\text{DD\%} = \frac{(\text{NH}_2\%)}{9.94\%} \times 100%
\]

Where, 0.016 (g) is the equivalent amount of amino with 1mL 1mol/L hydrochloric acid solution; 9.94% stands for the theoretical content of amino groups in chitosan; \(C_1\) (mol/L) and \(V_1\) (mL) represent the molarity and volume of HCl standard solution, respectively; \(C_2\) (mol/L) and \(V_2\) (mL) represent the molarity and volume of NaOH standard solution, respectively; \(G\) (g) is the weight of sample; \(W\) is the moisture of sample.

2.3 Preparation of Carboxymethyl Chitosan (CM-CTS)

Carboxymethyl chitosan (CM-CTS) was prepared according to the previously reported procedures with slight modification. Briefly, 5g of CTS was swollen in 75 mL of isopropanol at 30 °C for 30 min. Then, 40% (w/w) NaOH was added to the solution to alkalize at 45°C for 1.5 h. After the completion of alkalization, a solution of monochloroacetic acid in isopropanol was added dropwise into the mixture. The reaction was continued under stirring. After the completion of the reaction, the pH of the mixture was adjusted to 7 with 10% HCl. Finally, the product was filtered and washed with 80% methyl alcohol and absolute ethyl alcohol, and the precipitate was dried at 60°C.

The carboxymethyl reaction of chitosan is as follows:

2.4 Preparation of CM-CTS/Na⁺-MMT Nanocomposite

CM-CTS/Na⁺-MMT nanocomposite was prepared via an ion exchange reaction using a modified version of the method described by Huang et al. 24 A 2% CM-CTS solution was slowly added to a 4% sodium montmorillonite colloid suspension. The mixture was continuously stirred at 1000 rpm for 30 min followed by dewatering. The resulting mixture was washed with distilled water twice to remove excess CM-CTS, and then dried at 70 °C followed by powdering.

2.5 Characterization

2.5.1 Determination of Degree of Deacetylation of Chitosan

The degree of deacetylation (DD) of chitosan was measured by using acid-base titration, and the DD of the chitosan was calculated as 91%.

2.5.2 Infrared Spectroscopic Analysis

CM-CTS was analyzed by Fourier transform infrared spectrometry (FT-IR, model Spectrum 650, China) using a KBr disc. The determination was carried out under a transmittance mode at 4000-400 cm⁻¹, resolution of 4cm⁻¹, and scan number of 16 times/sample. The infrared spectral analysis was conducted to determine the functional groups. For comparison purpose, infrared spectrum analysis was also carried out on neat chitosan.

2.5.3 Nuclear Magnetic Resonance Analysis

\(^1\)H-NMR spectra of chitosan and CM-CTS were obtained with a Nuclear magnetic resonance spectrometer (AVANCE III 600MHz, Bruker Biospin, Switzerland). D₂O was used as solvent. Due to the fact that the solubility of chitosan in pure D₂O is very poor, a drop of concentrated hydrochloric acid was added into D₂O.

2.5.4 X-ray Diffraction Analysis

XRD spectra of Na⁺-MMT,CTS/Na⁺-MMT and CM-CTS/Na⁺-MMT in the powder form were measured by a Ultima IV multipurpose X-ray diffractometer with Cu target and Kα radiation (λ= 1.54) at 40 KV and 30 mA. The scanning rate was 5°/min, and the scanning scope of 20 was 5-15° in a fixed time mode at room temperature. The interlayer of Na⁺-MMT and CM-CTS/Na⁺-MMT can be calculated by using Bragg’s equation:

\[2d\sin\theta = n\lambda\]
Where \( n \) is an integer; \( \lambda \) is the wavelength of incident wave; \( d \) is the spacing between the planes in the atomic lattice, and \( \theta \) is the angle between the incident ray and the scattering planes.

3. RESULTS AND DISCUSSION

3.1 Process optimization of carboxylation

According to previous publications, the factors governing the carboxymethylation of chitosan with monochloroacetic include \( \text{m(CICH}_2\text{COOH)}/\text{m(CTS)} \), \( \text{m(NaOH)}/\text{m(CTS)} \), temperature, and time. Therefore, the orthogonal experiment with four factors and three levels was carried out on the basis of four factors. The effect of CM-CTS intercalated Na\(^+\)-MMT was used as a benchmark for differential analysis. Factors and levels of orthogonal experiments and the results of differential analysis are shown in Tables 1 and 2.

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Table 1. Levels and factors of orthogonal experiment

As seen from the results shown in Table 2, the effect of CM-CTS on the intercalation of montmorillonite was dependent upon the process conditions. The layer spacing of montmorillonite was 0.99 nm, while the distance between the 1-7 groups and the 9 group was higher than that of montmorillonite itself. This indicates that CM-CTS was successfully intercalated into montmorillonite layer. Furthermore, the 8 group formed the exfoliated nanocomposite. In an exfoliated nanocomposite, the silicate layers are completely delaminated from each other and are well-dispersed. This is the most ideal effect. The optimum conditions was: reaction time of 6 h, temperature of 60 \(^\circ\)C, \( \text{m(CICH}_2\text{COOH)}/\text{m(CTS)} \) of 3/1, and \( \text{m(NaOH)}/\text{m(CTS)} \) of 4.5/1.

3.2 FT-IR Analysis of Chitosan and CM-CTS

To confirm the carboxymethylation of chitosan, FT-IR of chitosan and carboxymethyl chitosan were analyzed. Fig. 1 shows FT-IR spectra of CTS and CM-CTS.

![FT-IR spectra of CTS and CM-CTS](image)

Fig. 1. FT-IR spectra of (a) CTS and (b) CM-CTS

As shown in Figs. 2 and 3, the \(^1\)H-NMR spectrum of CM-CTS was different from that of chitosan. In the \(^1\)H-NMR spectra of chitosan, the peak at 1.98 ppm represents three protons of N-acetyl glucosamine (GlcNAc), and the peak at 3.0 ppm represents H-2 proton of glucosamine (GlcN) residues. The protons of H-1 [GlcN (H-1D) and GlcNAc (H-1A)] resonate at 4.6 and 4.8 ppm, respectively. The signals of H5, H6, and H7 protons were

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observed at between 3.5 and 3.8 ppm. In the $^1$H-NMR spectra of CM-CTS, the resonance of 6-substituted and 3-substituted carboxymethyl-protons (-OCH$_2$COOD) appeared in the spectral region of 4.2-4.5 ppm. Meanwhile, the resonance of 2-substituted carboxymethyl-protons (-NCH$_2$COOD) occurred in the spectral region of 3.2-3.4 ppm. This indicates that the product was N$_2$O-carboxymethyl chitosan, which is consistent with the results of FT-IR spectra. In addition, compared with chitosan, the area of the absorption peak of CM-CTS at about 1.98 ppm was obviously smaller, which indicates that in the carboxymethylation reaction, deacetylation of chitosan occurred.

3.4 XRD Analysis

XRD patterns of Na$^+$-MMT, CTS/Na$^+$-MMT and CM-CTS/Na$^+$-MMT nanocomposites are shown in Fig. 4. The peak at 20=8.9° was observed for pure Na$^+$-MMT. As compared to Na$^+$-MMT, the $d_{001}$ diffraction peak of CTS/Na$^+$-MMT nanocomposites shifted toward lower angle (20=5.74°). From the Bragg equation, it is known that the interlayer spacing of montmorillonite increased from 0.98 nm to 1.54 nm, indicating that CTS entered into the interlayer of Na$^+$-MMT. For CM-CTS/Na$^+$-MMT, the diffraction peak disappeared. This disappearance of diffraction inside montmorillonite layer was considered as the random dispersion and complete exfoliation of montmorillonite plate inside CM-CTS matrix. Carboxymethylation modification of chitosan can not only increase the water-solubility of chitosan, but also enhance the intercalation-related interaction.

Fig.4. XRD patterns of (a) Na$^+$-MMT, (b) CTS/Na$^+$-MMT, and (c) CM-CTS/Na$^+$-MMT.

4. CONCLUSIONS

Carboxymethyl chitosan was successfully synthesized for nanocomposite fabrication. With NaOH as the catalyst, carboxymethylation of chitosan with chloroacetic acid resulted in formation of N$_2$O-carboxymethyl chitosan. It was found that this chemically modified chitosan inserted into the montmorillonite interlayer and formed exfoliated nanocomposites which have potential applications in functional packaging materials.

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REFERENCES