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Carboxymethyl chitosan/clay nanocomposites and their copper complexes: Fabrication and property

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ABSTRACT

To obtain environmentally friendly antifouling agent, an effort was made to intercalate carboxymethyl chitosan into the interlayer of organic montmorillonite to prepare carboxymethyl chitosan/organic montmorillonite nanocomposites and their copper complexes. In comparison, carboxymethyl chitosan–copper complexes were also obtained. Their structures were characterized by X-ray diffaraction, transmittance electron microscopy and Fourier transform infrared, and their thermal behavior and antimicrobial activity were discussed. The results revealed that the interlayer distance of carboxymethyl chitosan/organic montmorillonite nanocomposites enlarged with the increasing mass ratio of carboxymethyl chitosan to organic montmorillonite, when the mass ratio was at 20:1, the layer spacing of carboxymethyl chitosan/organic montmorillonite nanocomposites reached the maximum of 3.68 nm. As compared to other samples, carboxymethyl chitosan/organic montmorillonite–copper nanocomposites showed much higher thermal stability and inhibitory activity against *Escherichia coli*, the lowest minimum inhibition concentration was only 0.0003125% (w/v). The study provides a new method to find novel antifouling agent.

1. Introduction

In the process of the development of marine antifouling paints, the self-polishing antifouling paint with the function group of organotin (TBT) acrylic resin was praised as special weapons of marine antifouling. However, TBT caused serious damage to marine ecological environment (Antizar-Ladislao, 2008), therefore it is urgent to find a non-toxicity antifouling paint.

Chitosan is natural, biocompatible, biodegradable, non-toxic and multifunctional resource, which is regarded as environmentally friendly antifoulant by chemists (He, Davis, & Illum, 1999; Khan, Badshah, & Airoldi, 2015; Kurmaev et al., 2002; Rhazi et al., 2002). Chitosan is the only kind of alkaline natural amino polysaccharide which is composed of β-(1,4)-2-acetamino-2-deoxy-D-glucose binary linear copolymer (Ling, Li, Zhou, Wang, & Sun, 2015; Li, Liu, Ye, Wang & Sun, 2015). Carboxymethyl chitosan (CMC) is water-soluble chitosan derivative with remaining excellent antibacterial properties (Liu, Wang, Li et al., 2012; Liu, Wang, Yang & Sun, 2012; Sun, Du, Fan, Chen, & Yang, 2006), which can chelate effectively with metal salts (Paradossi, Chiessi, Venanzi, Pispisa, & Palleschi, 1992), and the self-polishing antifouling paint can be simulated by chelating CMC with metal ion such as copper ion that is widely considered as a broad antibacterial material and used to kill algae (Heuser, Rivera, Nunez, & Cardenas, 2009; Muzzarelli & Tubertini, 1970). But the thermal stability and the antimicrobial activity of CMC–Cu complex are still not satisfactory.

From this point, it is noted that chitosan-based layered silicate nanocomposites have drawn people's attention for coupling of numerous merits of chitosan and layered silicate (Deng et al., 2012; Liu, Wang, Yang & Sun, 2011; Liu, Wang, Yang, Wang & Sun, 2011; Wang et al., 2009; Wang et al., 2006). Montmorillonite (MMT) is a 2:1 typed layered silicate with high thermal stability, and more interestingly, the previous study demonstrated that MMT had no antibacterial activity itself, but it showed dual perfor-

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mance of adsorbing bacteria and killing bacteria when the cationic material with antibacterial activity was intercalated into the interlayer of MMT (Guo, Ma, Guo, & Xu, 2005; Yao-Zong, Shi-Rong, & Delvaux, 2004). OMMT is modified MMT with surfactant, compared with pure MMT, OMMT owns a higher interlayer spacing and a larger specific surface area, and it even shows stronger antimicrobial activity. So the addition of OMMT may improve the possibility for CMC–Cu complex as the antifouling agent. However, there is still no report about preparing the composite of CMC, MMT and copper ions in order to combine their thermostability and antibacterial advantages.

In this work, CMC and CMC–Cu complexes were firstly obtained, and organic montmorillonite (OMMT) was prepared to make the insertion of CMC into the interlayer of MMT easier. Afterwards, CMC/OMMT and CMC/OMMT–Cu nanocomposites were prepared. Their structures were characterized by XRD, TEM and FT-IR, and TGA was used to investigate the thermal stability. Furthermore, the inhibition ability against *Escherichia coli* was evaluated.

2. Experimental

2.1. Materials

Chitosan (CS) was purchased from Haidebei Ocean Biochemical Co., Ltd. (Jinan, China). Its degree of deacetylation was 85%, and its weight average molecular weight (Mw) was 2.0×10^5. Chloroacetic acid was purchased from Kelong Chemical Reagent Factory (Chengdu, China). Sodium based montmorillonite (Na–MMT) was purchased from Josiah reagent factory, its cation exchange capacity was 87 mmol/100 g, Cetyltrimethyl Ammonium Bromide (CTAB) was purchased by Henan Titaning Chemical Technology Co., Ltd. (Henan, China). All other chemicals were of analytical grade.

2.2. Preparation of CMC with three different degree of substitution (DS)

Carboxymethyl chitosan (CMC) was prepared by grafting carboxymethyl groups on primary hydroxyl group of chitosan using microwave irradiation according to the previous study (Liu, Wang, Li, Zeng, Sun & Kennedy, 2012). Briefly, the carboxymethylation of chitosan was performed in the microwave system by using chloroacetic acid as modification agent at 800 W and 70 °C for 25 min. The reaction mixture was precipitated by isopropanol, centrifuged and washed to neutral pH value. Afterwards, CMC was obtained by dialysis with distilled water and lyophilization. The obtained CMCs with three different mass ratios of chitosan to chloroacetic acid were designated as CMC1, CMC2 and CMC3, respectively. The reaction is shown in Scheme 1.

2.3. Preparation of CMC–Cu complexes

Carboxymethyl chitosan–Cu (CMC–Cu) was prepared via the complexation with copper ion, as shown in Scheme 1. 0.1 g of anhydrous cupric sulfate and 0.33 g CMC were dispersed in 5 mL distilled water to obtain copper sulfate aqueous solution and CMC aqueous solution, respectively. Blue flocky precipitate was obtained after those two resulting solutions were dropped into distilled water with vigorous stirring at 60 °C for 6 min. The precipitate was filtered out after keeping warm and stirring for 20 min, and then washed by alcohol until no copper ion was tested by NaOH solution. Finally, CMC–Cu complex was obtained after vacuum drying. The CMC–Cu samples prepared by three CMCs with different DS were recorded as CMC1–Cu, CMC2–Cu and CMC3–Cu, respectively.

2.4. Preparation of CMC/OMMT and CMC/OMMT–Cu nanocomposites

OMMT was prepared according to the previous study (Liu et al., 2011). 1% OMMT suspension was put in flask, stirring for several hours with high speed until OMMT was dispersed completely in the water. CMC solution was obtained in distilled water and dropped slowly into OMMT suspension and reacted under microwave irradiation for 90 min at 800 W and 80 °C. Finally, CMC/OMMT nanocomposites were obtained after being freeze-dried. The nanocomposites with weight ratios of CMC to OMMT of 2:1, 4:1, 8:1 and 20:1 were recorded as CMC/OMMT-1, CMC/OMMT-2, CMC/OMMT-3 and CMC/OMMT-4, respectively.

CMC/OMMT–Cu nanocomposites were prepared according to the method mentioned in Section 2.3. Four different weight ratios of CMC/OMMT–Cu were prepared and designated as CMC/OMMT–Cu-1, CMC/OMMT–Cu-2, CMC/OMMT–Cu-3 and CMC/OMMT–Cu-4, respectively. The experiments were done as illustrated in Scheme 2.

2.5. Characterization

The weight-average molecular weight (Mw) of CMC was measured by GPC-LLS. Efficient liquid chromatography pump 515 (Waters, U.S.A.), 79911GF-084 PL gel permeation chromatography column (Agilent, U.S.A.), column temperature was 30 °C, moving phase were the mixture of 0.2 mol/L CH_3COOH, 0.1 moL/L CH_3COONa, 0.2 mol/L NaCl, flow speed was 1.0 mL/min. RI-150 differential refractive index detector (Wyatt, U.S.A.); operating system was DAWN HELEOS-II Optilab (Wyatt, U.S.A.), concentration of sample was 0.4 mg/mL.

The degree of substitution (DS) of CMC was measured by using the potentiometric titration. 0.2 g CMC was dissolved in 15 mL HCl (0.1 M), and titrated with a concentration of about 0.1 M calibrated NaOH solution, meanwhile the pH was recorded. The alkalimetric curve was recorded on 888 Titrino Plus (Metrohm, Switzerland). DS_{CM} was calculated as follows:

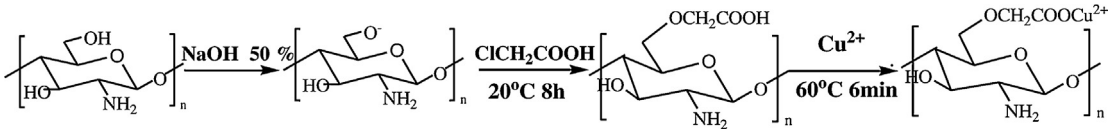
$$DS_{CM} = \frac{0.203 \times A}{1 - 0.080A} \quad (1)$$

$$A = \frac{V_{NaOH} \times C_{NaOH}}{W} \quad (2)$$

where 0.203 is the (milli–molar) molecular weights of acetylglucosamine residues (chitosan skeleton unit); 0.080 stands for the weight of sodium carboxymethyl per milligram-equivalent; V_{NaOH} (mL) and C_{NaOH} (mol/L) represent the volume and molarity of NaOH standard solution, respectively; W (g) is the weight of sample.

FT-IR spectra were measured by Nicolet FT-IR 5700 spectrophotometer (Madison, America) by a KBr pellets method. The spectra were collected for each measurement over the spectral range 4000–400 cm^{-1} with a resolution of 4 cm^{-1}.

XRD of powder samples were measured by a D8 advance X-ray diffractometer (Bruker, Germany) with Cu target and Kα radiation



Scheme 1. The synthesis course of CMC and CMC–Cu complexes.