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Preparation and antimicrobial property of chitosan oligosaccharide derivative/rectorite nanocomposite

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ABSTRACT

Microwave irradiation was used to intercalate quaternized carboxymethyl chitosan oligosaccharide (QCMCO) into the layer of rectorite (REC) to prepare QCMCO/REC (QCOR) nanocomposites in 70 min, which was much faster than conventional heating method of 48 h. The structures and morphology of QCOR nanocomposites were characterized by XRD, TEM, FT-IR and zeta potential analysis, the thermal behavior and antimicrobial activity of QCOR nanocomposites were also discussed. The results revealed that the interlayer distance of QCOR nanocomposites enlarged with the increase of QCMCO content, hydrogen bonding and electrostatic interaction between QCMCO and REC took place. As compared to QCMCO, the crystallinity of QCOR nanocomposites reduced, the thermal stability of QCOR nanocomposites improved, and the inhibitory activity of QCOR nanocomposites against microorganisms was stronger, the lowest minimum inhibition concentration was only 0.025% (w/v), the antimicrobial mechanism was discussed via TEM and SEM micrographs.

1. Introduction

Chitosan is composed of β-(1,4)-2-amino-2-deoxy-D-glucose (GlcN) and β-(1,4)-2-acetamido-2-deoxy-D-glucose (GlcNAc) binary linear copolymer (Kong, Chen, Xing, & Park, 2010; Q. Wang, Zhang, Hu, Yang, & Du, 2008). As a broad-spectrum antibacterial agent, it can inhibit the growth of several bacteria and fungi (Kong et al., 2010; Vinsova & Vavrikova, 2011; Xia, Liu, Zhang, & Chen, 2011). Quaternized carboxymethyl chitosan (QCMC) is the water-soluble amphoteric derivative of chitosan (Liu, Wang, Li, et al., 2012; Liu, Wang, Yang, Sun, 2012; Sun, Du, Fan, Chen, & Yang, 2006). Many researchers have reported the superior antibacterial properties of high-molecular-weight QCMC (Aranaz, Harris, & Heras, 2010; Sun et al., 2006; Vinsova & Vavrikova, 2011), but there are very few reports about the antimicrobial activity of low-molecular-weight QCMC (below 20,000 Da). Since chitosan and its derivatives with low molecular weight have better biological activity (Dutta, Tripathi, & Dutta, 2012), the study on the antibacterial properties of low-molecular-weight quaternized carboxymethyl chitosan oligosaccharide (QCMCO) is of great significance for its further biological application.

In addition, rectorite (REC) is a 2:1 typed layered silicate, the previous study demonstrates that it has not antibacterial activity by itself (Zhou et al., 2010), but it shows dual performance of adsorbing bacteria and killing bacteria when the cationic material with antibacterial activity was intercalated into the interlayer of REC (Deng et al., 2012; Q. Wang, Zhang, Hu, Yang, & Du, 2007; X. Wang, Du, Luo, Lin, & Kennedy, 2007; X. Wang et al., 2009, 2006; X.Y. Wang, Du, Sun, & Lin, 2009; X.Y. Wang et al., 2012). But there has no report about inserting QCMCO into the interlayer of REC in order to combine both antibacterial advantages.

Chitosan-based layered silicate nanocomposite draw 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; X. Wang et al., 2009, 2006; X.Y. Wang et al., 2009), but at present, its preparation mostly adopt solution intercalation technique under traditional heating condition, the reaction time is 6–48 h, and the reproducibility is poor (Liu, Wang, Yang, & Sun, 2011; Liu, Wang, Yang, Wang, et al., 2011; X. Wang et al., 2009; X.Y. Wang et al., 2009). Microwave radiation method, invented by Gerda in 1986 (Gedye, Smith, & Westaway, 1986), is attracting more and more attention for its fast, simple and efficient advantages. Many scholars prepared various chitosan derivatives and low-molecular-weight chitosan by microwave irradiation method (Liu, Wang, Li, et al., 2012; Liu,

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Wang, Yang, et al., 2012; Luo, Wang, Xia, & Wu, 2010; Mecwan, Rapalo, Mishra, Haggard, & Bumgardner, 2011; Tishchenko et al., 2011), Kabiri and other researchers prepared chitosan-based montmorillonite nanocomposite within several minutes (El-Sherif & El-Masry, 2011; Kabiri, Mirzadeh, & Zohuriaan-Mehr, 2007) using microwave irradiation. But so far, there are few reports about chitosan-based REC nanocomposite prepared by microwave irradiation method (Liu, Wang, Li, et al., 2012; Liu, Wang, Yang, et al., 2012; Liu, Wang, Yang, & Sun, 2011; Liu, Wang, Yang, Wang, et al., 2011).

In this paper, QCMCO/REC (QCOR) nanocomposite was prepared rapidly under microwave radiation, their structures were characterized by XRD, TEM, FT-IR, zeta potential analysis and TGA techniques. Moreover, the inhibition ability of QCOR nanocomposites against four bacteria and one fungus was evaluated, and the antibacterial mechanism was discussed.

2. Experimental

2.1. Materials

Chitosan (CS) was purchased from Yuhuan Ocean Biochemical Co. (Zhejiang, China). The degree of deacetylation was 82%, its weight average molecular weight (*Mw*) was 1.5×10^4, 2,3-epoxypropyltrimethyl ammoniumchloride (ETA) was purchased from Dongying Guofeng Fine Chemical Co. Ltd. (Shandong, China), calcium rectorite (Ca^{2+}-REC) with a cation exchange capacity (CEC) value of 45 mmol/100 g refined from the clay minerals was provided by Hubei Mingliu Inc. Co. (Wuhan, China). All other chemicals were of analytical grade.

2.2. Preparation of QCMCO

Quaternized carboxymethyl chitosan oligosaccharide (QCMCO) was prepared by grafting carboxymethyl groups and quaternary ammonium groups on chitosan chain under microwave irradiation according to the previous study (Liu, Wang, Li, et al., 2012; Liu, Wang, Yang, et al., 2012). Briefly, the carboxylmethylation of chitosan was performed by using chloroacetic acid as modification agent at 800 W and 70 °C for 25 min, and then the carboxylmethyl chitosan was quaternized at 800 W and 75 °C for 70 min. The molar ratio of chloroacetic acid and ETA to amino groups of chitosan was 4:1 and 6:1, respectively. The reaction mixture was precipitated by acetone and then washed to neutral pH value. Finally, QCMCO was obtained by dialysis against distilled water and then lyophilization at −50 °C. The *Mw* of the prepared QCMCO was 1.85×10^4 which was determined by gel permeation chromatography method. The degree of substitution of carboxymethyl groups and quaternary ammonium groups were 0.88 and 0.75, respectively, which was determined by deposit-titration method.

2.3. Preparation of QCOR nanocomposites

Quaternized carboxymethyl chitosan oligosaccharide/rectorite (QCOR) nanocomposites were prepared via the intercalation of QCMCO into REC. 0.1 g of REC was dispersed in distilled water, the resulting clay suspension was left for 24 h after vigorous stirring for 30 min and then put into the microwave system. QCMCO solution was obtained in distilled water and dropped slowly into REC suspension and reacted under microwave irradiation for 70 min at 600 W and 80 °C. Finally, QCOR nanocomposites were obtained after being freeze-dried at −40 °C. The nanocomposites with weight ratios of QCMCO to REC of 2:1, 4:1, 8:1 and 20:1 were recorded as QCOR-1, QCOR-2, QCOR-3 and QCOR-4, respectively.

2.4. Characterization of QCOR nanocomposites

X-ray diffraction (XRD) patterns of powder sample were obtained using D8 advance X-ray diffractometer (Bruker, Germany) with a Cu *Kα* radiation (λ = 0.15418 nm) at 40 kV and 50 mA at 25 °C. The relative intensity was recorded in the scattering range of 1–10° at a scanning speed of 1°/min.

The microstructure of REC and QCOR nanocomposite were taken using a JEM-2010 HR transmittance electron microscope (TEM) (JEOL, Japan) at an accelerating voltage of 200 kV. Clay sample for TEM studies was dispersed in 50% ethanol solution and dropped on Cu mesh grids, then dried in an oven at 50 °C for 10 min. Ultrathin films of QCOR nanocomposite were prepared by cutting from the epoxy block with the embedded nanocomposite sheet using an UCL/FC6 ultratome (LEICA, Australia).

Fourier transform infrared (FT-IR) spectra were performed on a Nicolet FT-IR 5700 spectrophotometer (Bruker, Germany) at room temperature by the 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}.

2.5. Crystallization behavior of QCOR nanocomposites

Crystallization behavior was determined by the X-ray diffraction (XRD), the experiment was performed using a diffractometer type D8 advance (Bruker, Germany) with Cu target and *Kα* radiation (λ = 0.15418 nm) at 40 kV and 50 mA. The scanning rate was 2°/min and the scanning scope was 5–45°.

2.6. Zeta potential of QCOR nanocomposites

Zeta potential was determined by 3000HSA typed nanometer particle size and potential analyzer (Malven, England), sample concentration was 0.1% (w/v).

2.7. Thermal stability of QCOR nanocomposites

Thermogravimetric analysis (TGA) was carried out on a SDT-Q500 simultaneous thermal analyzer (TA, USA) under a nitrogen atmosphere from room temperature to 600 °C and at a heating rate of 10 °C/min.

2.8. Antibacterial assay

Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and Fungus *Aspergillus niveus* were provided by Guangdong Institute of Microbiology and incubated on nutrient agar (peptone 1%, beef extract 0.5%, NaCl 0.5%, agar 2%, pH = 7.2).

2.8.1. Determination of the minimum inhibition concentration (MIC)

The minimum inhibition concentration (MIC) was defined as the lowest concentration required inhibiting the growth of bacteria, i.e. the concentration at which no microorganism colony or less than 5 colonies were visible.

The microorganism suspension was adjusted by sterile distilled water to 10^5–10^6 cell/ml. The QCOR nanocomposites, QCMCO and REC suspensions were prepared in PBS. The resulting solutions and the nutrient agar were autoclaved at 121 °C for 20 min. 1 mL of each sample were added to sterile petri-dishes together with 9 mL nutrient agar. A loop of each microorganism suspension was inoculated on cooled nutrient medium by means of drawing a stripe. The microorganisms were cultured at 37 °C. MICs values were read after a 24 h of culture.