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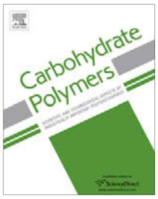

# A novel biopolymer/rectorite nanocomposite with antimicrobial activity

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## ABSTRACT

Rectorite (REC), a type of layered silicate, was used to prepare the intercalated nanocomposites with quaternized chitosan (HTCC). Characterization by XRD and TEM revealed that HTCC were intercalated into the intergallery of REC. Moreover, it was confirmed from FT-IR, XRD and $\zeta$-potential analyses that interaction between HTCC and REC took place. Two *in vitro* antimicrobial assays indicated that all the nanocomposites exhibited strong inhibition against Gram-positive bacteria, Gram-negative bacteria and Fungi under weak acid, water and weak basic condition, particularly against Gram-positive bacteria. Moreover, with increasing the amount or the interlayer distance of organic REC, the antimicrobial activity was stronger. The lowest minimum inhibition concentration values of the nanocomposites against *Staphylococcus aureus* and *Bacillus subtilis* were less than 0.00313% (w/v) in all media tested, and the killing rate on *S. aureus* reached more than 90% in 30 min. The mechanism of the antimicrobial action was briefly discussed.

## 1. Introduction

Antimicrobial agents can be divided into inorganic and organic ones according to their chemical composition. Inorganic antimicrobial agents show long life expectancy and high heat resistance; however, they exhibit weak mould proof activity and large dosage are needed when used, etc., (Guo, Ma, Guo, & Xu, 2005; Zhou, Xia, Ye, & Hu, 2004). While organic antibacterial agents show good inhibition efficiency and a broad spectrum of activity, more importantly they display blending compatibility with organic matrixes such as textile, paints, polymer, etc., however, their relative low stability (e.g., low decomposition temperature and short life expectancy) cannot be ignored (Suci, Vrany, & Mittelman, 1998). As a result, there is urgent need to develop organic–inorganic hybrid materials provided with dual antibacterial advantages of organic antimicrobial agents and inorganic antimicrobial agents as they will become more important in the antimicrobial material market. From this point of view, it is noteworthy to point out the polymer/layered silicate nanocomposites (PLNs) technology, which provide an attractive way to develop new organic–inorganic hybrid materials with properties that are inherent to both types of components.

Rectorite (REC) is a type of layered silicate which has been studied in the PLNs technology in recent years; the structure is similar to montmorillonite (MMT) where most work on PLNs has been concentrated. At the same time, REC also shows adsorption capacity on bacteria and immobilization activity of cell toxin (Zhang, Zhang, & Yu, 2003), which is also similar to MMT (Guo et al., 2005; Herrera, Burghardt, & Phillips, 2000). Modified layered silicates could adsorb both natural and anthropogenic toxin, and exhibited an inhibitory property for the proliferation of bacteria (Guo et al., 2005; Lemke, Grant, & Phillips, 1998). However, there are only several reports about polymer/layered silicate nanocomposites with antibacterial activity, our previous paper reported the good antibacterial activity of chitosan/rectorite nanocomposites (Wang et al., 2006), and Rhim et al. reported the chitosan/clay nanocomposite films with antimicrobial activity (Rhim, Hong, Park, & Ng, 2006).

Chitosan (CS) is the second most abundant polysaccharide in nature, it has been shown to be useful in many different applications; one of them is as a natural antimicrobial agent. CS has advantages over other type of disinfectants because of its higher antibacterial activity, a broader spectrum of activity, a higher killing rate and a lower toxicity towards mammalian cells (Shahidi, Arachchi, & Jeon, 1999). Despite all these advantages, chitosan *per se* lacks good solubility above pH 6.5; its applications in a commercial context are not as wide as expected. Accordingly, CS/REC nanocomposites may be not applied widely (Wang et al., 2006). Therefore, it is very necessary to prepare functional chitosan derivative in attempt to increase its solubility in water and thereby to broaden its applications. N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC) is just the derivative soluble

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in a wide pH range; it can be prepared by a relatively easy chemical reaction of CS and glycidyl-trimethyl-ammonium chloride (GTMAC) (Li, Du, Xu, & Zhan, 2004). There are more advantages in HTCC than in CS. First, it is water-soluble and thus the intercalation between HTCC and REC can proceed in mild condition—water, which does not include organic solvent. In addition, HTCC shows higher positive charge density than pure CS (Qin et al., 2004), so in the intercalation technology, it is easier to intercalate into the interlayer of silicate. And compared to pure CS, HTCC can inhibit the growth of bacteria in acid, water and base solution while CS only show antibacterial activity in acid solution (Qin et al., 2004). Besides, HTCC is potential to be used as an absorption enhancer across intestinal epithelial due to its mucoadhesive and permeability enhancing property; it is very suitable for biomedical application (Kotzle et al., 1999). Therefore, HTCC/layered silicate nanocomposites will have more potential applications than CS/layered silicate nanocomposites.

The focus of this paper is on preparing biopolymer/rectorite nanocomposites based on HTCC; it appears as an improved way of developing a novel PLNs nanocomposite which can inhibit the growth of bacteria in a wide pH range, and it is very favorable to the application of PLNs. First, according to our previous study (Wang et al., 2006), organic rectorite(OREC) was prepared. Then, HTCC/OREC nanocomposites with different mass ratios of HTCC to OREC were prepared. XRD and TEM were used to characterize their structures. FT-IR, XRD and ζ-potential analyses confirmed the interaction between HTCC and REC. At last, this paper detailed antimicrobial activity of HTCC/OREC nanocomposites.

## 2. Experimental

### 2.1. Materials

Chitosan (CS) from a shrimp shell was purchased from Yuhuan Ocean Biochemical Co. (Taizhou, China). The degree of deacetylation was 92% (determined by elemental analysis) (Xu, McCarthy, & Gross, 1996) and its weight average molecular weight ($M_w$) was $2.1 \times 10^5$ (determined by GPC method) (Qin, Du, & Xiao, 2002). Calcium rectorite ($Ca^{2+}$-REC) refined from the clay minerals was provided by Hubei Mingliu Inc., Co. (Wuhan, China). Cetyltrimethyl ammonium bromide (CTAB) was supplied by Xinrui Science and Technology Inc., Co. (Wuhan, China). All other chemicals were of analytical grade.

### 2.2. Synthesis of quaternized chitosan

N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC) was prepared as shown in Scheme 1 according to reference (Li et al., 2004). About 0.5 mol of concentrated hydrochloric acid was dropped into the solution of trimethylamine (0.5 mol) at 4 °C. After stirring for about 10 min, 0.42 mol of epoxy chloropropane were added into the resulting solution at 35 °C. After homogenization, the mixing solution was heated to 54 °C, and was then trickled slowly by aqueous NaOH solution (50 ml 40 wt %). The addition of NaOH was performed within 1–1.5 h, after another 2 h of stirring, the reaction mixture was refined by vacuum distillation at 40 °C for 20 min, then intermediate for the preparation of HTCC———2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC) was obtained. Subsequently, 12 g of chitosan was dispersed in EPTMAC solution after which was adjusted to pH 9 and was stirred at 75 °C for 6 h. Then the reaction mixture was dialyzed, precipitated by acetone, and finally vacuum-dried to obtain HTCC. Its $M_w$ was $1.08 \times 10^5$ (determined by GPC method) and the degree of substitution (DS) was 82% (determined by potentiometry) (Li et al., 2004).

### 2.3. Preparation of HTCC/OREC nanocomposites

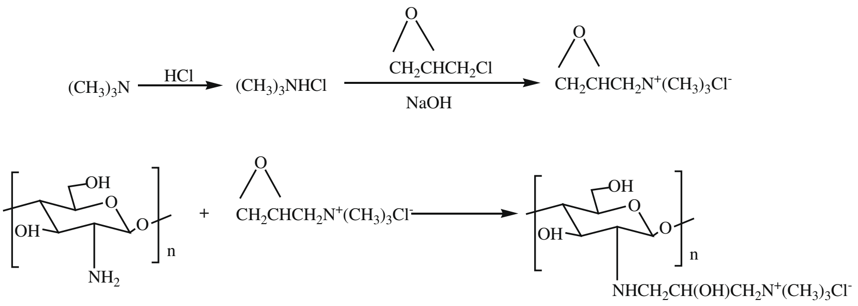
Organic rectorite (OREC) was prepared according to our previous work (Wang et al., 2006). eparation of HTCC/OREC nanocomposites was as follows: HTCC was dissolved in water to obtain the 0.5% (w/v) solution. The resulting solution was added slowly into the pretreated OREC suspension under stirring at 80 °C to obtain the nanocomposites with initial HTCC/OREC weight ratios of 1:1, 2:1, 4:1. The resulting mixture was agitated for 2 days. Finally the nanocomposites were freeze-dried at −50 °C and ground to powder. A conceptual illustration of the intercalation of HTCC into REC is shown in Scheme 2. Firstly, quaternary alkylammonium (CTAB) cations enter into the interlayer of rectorite by ion-exchange reactions, the alkyl chains arrange orderly between the interlayer because of cation strong interaction (Vaia, Teukolsky, & Giannelis, 1994). In this way, the normally hydrophilic silicate surface is converted to an organophilic one, making the intercalation of polymers easier. Therefore, after that, quternized chitosan (HTCC) can intercalate into the clay gallery in the help of agitation action and the interaction between HTCC and CTAB chains (Ray & Okamoto, 2003).

### 2.4. Characterization

The X-ray diffraction (XRD) experiment was performed using a D8 Advance diffractometer (Bruker, USA) with Cu target and Kα radiation ($\lambda = 0.154$ nm) at 40 KV and 50 mA. The scanning rate was 0.5°/min and the scanning scope of $2\theta$ was 1°–10° and 5°–45° in a fixed time mode with a step interval of 0.02° at room temperature.

Ultrathin films for transmission electron microscopy (TEM) were prepared by cutting from the epoxy block with the embedded nanocomposites sheet at room temperature using an LKB-8800 ultratome. The TEM micrographs were taken using a transmittance electron microscope [JEM-2010 FEF (UHR), JEOL, Japan] at an accelerating voltage of 200 KV.

FT-IR spectra were recorded in KBr pellets on a Nicolet FT-IR 5700 spectrophotometer (Madison, USA) by the method of transmission.



**Scheme 1.** Preparation of HTCC.