

High Strength Cellulose Composite Films Reinforced with Clay for Applications as Antibacterial Materials*

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Abstract High strength cellulose composite films with antibacterial activities were prepared by dispersing montmorillonites (MMT) into cellulose solution in LiOH/urea aqueous solvent followed by regeneration in ethanol coagulation bath, and then by soaking in 5 wt% hexadecylpyridine bromide ethanol solutions to induce the antibacterial action. The cellulose/MMT composite films were characterized by field emission scanning electron microscopy, transmission electron microscopy, thermogravimetric analysis, FTIR, UV-spectra, wide angle X-ray diffraction and mechanical test. The results revealed that MMT was dispersed well in the cellulose matrix to form layer structure with a thickness of approximately 3 nm. The mechanical properties of the cellulose/MMT composite films were significantly improved to achieve 132 MP for tensile strength as a result of the MMT delamination. The hexadecylpyridine bromide was fixed well in the cellulose/MMT matrix through cation exchange, leading to the excellent antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*, which is important in their practical applications.

Keywords: Cellulose/MMT composite films; Antibacterial activities; High strength; Hexadecylpyridine bromide.

INTRODUCTION

Various inorganic and organic antimicrobial materials have been intensively investigated to resolve the problem of microbial contamination^[1–4]. As the most abundant renewable source, cellulose has been widely used in fields such as textiles, packaging and medical supplies^[5, 6]. However, similar to most polysaccharides, cellulose tends to be adhesive to microbes and lacks antibacterial action, limiting its wide applications^[7, 8]. Hence, many methods have been developed for the preparation of antibacterial textiles for the prevention of disease transmission through clothing materials, such as physical or chemical incorporation of biocides such as silver, copper and quaternary ammonium into cellulose film, fiber and microspheres^[9–11]. However, the use of metal nanoparticles on textiles can lead to concerns for human safety because the nanoparticles may detach from the fiber surface and penetrate into the human body^[12]. Pyridinium salts are a new class of efficient, broad-spectrum organic antiseptics. Due to their advantages such as low foam, strong capability of sludge stripping, and suitable usage in the wide range of pH values, pyridinium salts have been extensively utilized in the preparation of antibacterial materials^[13]. Nevertheless, direct use of pyridinium salts for industrial and domestic applications is not safe because of its antibiotic resistance in bacteria^[14]. So, it is necessary to develop some new techniques to overcome such shortcomings. It is noted that montmorillonites (MMT) are layered aluminum silicate materials with high specific surface area, absorption ability, cation exchange capacity, non-toxicity and environmental

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friendly characteristics^[15], and which can easily intercalate with selected organic or inorganic substances with antimicrobial properties^[16, 17]. Additionally, it has been demonstrated that MMT has high security and a synergistic effect with other antimicrobial materials^[18]. Thus, MMT composite materials have broad applications in the biomedical field, especially as antibacterial materials because of their antibacterial activity and adsorption of bacteria such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*)^[19]. Recently, polymer-clay nanocomposites have attracted much attention^[20] because these layered silicates can greatly improve the mechanical and thermal properties of the polymer matrix with a very low amount of incorporation^[21–23]. However, to the best of our knowledge, the antibacterial application of MMT in cellulose has seldom been reported.

Thus, a worthwhile endeavor would be to develop antibacterial cellulose/MMT composite films from cellulose. In the present work, the composite films were prepared by dispersing MMT into the cellulose solution in aq. alkaline/urea, and then antibacterial films were fabricated *via* the adsorption of hexadecyl pyridine bromide (HB) through cation exchange. The structure and properties of the films were characterized, and their antibacterial activities were evaluated against *E. coli* and *S. aureus* bacteria. The major goal of this work was to prepare new cellulose based antibacterial composite films by using MMT and HB, broadening the applications of cellulose.

EXPERIMENTAL

Materials

A cellulose sample (cotton linter pulp, Hubei Chemical Fiber Co. Ltd., China) with an α -cellulose content of more than 95% was used in this study. Its viscosity-average molecular weight (M_η) was measured at $(25 \pm 0.05)^\circ\text{C}$ by viscometry in LiOH/urea aqueous solvent and calculated by $[\eta] = 3.72 \times 10^{-2} M_\eta^{0.77}$ to be 9.3×10^4 ^[24]. Montmorillonite (MMT) with 110 meq/100 g of cation-exchange capacity (CEC) was purchased from Fenghong New Material Co, Ltd. (Zhejiang, China). Urea, lithium hydroxide, sodium montmorillonite, hexadecyl pyridine bromide, ethanol and other reagents were purchased from Shanghai Chemical Reagent Co. Ltd., China.

Preparation of the Cellulose/MMT Composite Films

Cellulose was dissolved in 4.6 wt% LiOH/15.0 wt% urea solution pre-cooled to -12°C according to previously reported method^[25] to form a 4 wt% transparent cellulose solutions within 3 min. A 5 wt% montmorillonite aqueous solution pre-cooled to 0°C was immediately dropped into the cellulose solution under vigorous stirring for 3 min to form a milky cellulose/MMT mixture solution at the ambient temperature, then centrifuged at 7200 r/min for 10 min at 5°C to remove the air bubbles. The mixture solution was immediately cast on a glass plate to give a 0.5 mm thick layer and then immersed into ethanol to give cellulose/MMT gel sheets. The resulting gel sheets were washed with deionized water to remove the excess urea and LiOH and were then dried at room temperature to obtain the cellulose/MMT composite films. The composite films with 3 wt%, 5 wt%, 10 wt% and 20 wt% MMT were labeled as RCM3, RCM5, RCM10 and RCM20, respectively. The pure regenerated cellulose film was denoted as RC.

Hexadecylpyridine bromide (HB) was dissolved in ethanol to prepare 5 wt% hexadecylpyridine bromide solutions, and then the RC and RCM5 gel sheets were soaked in the above solutions to prepare the RC-HB and RCM5-HB composite hydrogels, respectively. The resultant hydrogels were subsequently washed with anhydrous ethanol and water and then dried in air at room temperature to obtain the RC-HB and RCM5-HB composite films.

Antibacterial Activity Tests

E. coli and *S. aureus* were selected as indicators of experimental bacteria. Luria Bertani (LB) broth was used as a growing medium for the microorganisms of *E. coli* and *S. aureus*. The RC, RCM5, RC-HB and RCM5-HB composite films were cut into disc shapes with a diameter of 6.85 mm. All culture mediums and materials were sterilized in an autoclave at 121°C for 30 min prior to the experiments. The discs were subsequently placed on

E. coli and *S. aureus*. The culture plates were incubated at 37 °C for 24 h. The antibacterial activity of the RCM5-HB was quantified by the size of the inhibitory zone.

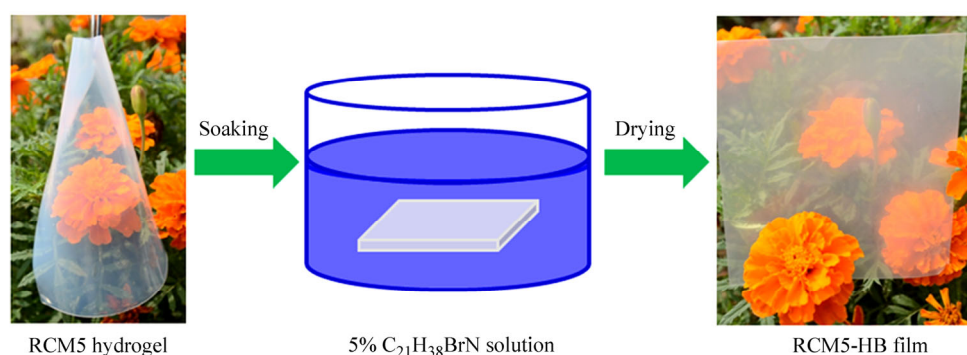
Characterization

Fourier transform infrared (FTIR) spectra were recorded using an FTIR spectrometer (1600, Perkin-Elmer Co., MA) in a wavelength range of 4000 cm^{-1} to 400 cm^{-1} . Powdered and vacuum-dried samples were obtained, and the test specimens were prepared by the KBr disk method. Wide angle X-ray diffraction measurements were recorded on a WAXD diffractometer (D8-Advance, Bruker, USA). The patterns with Cu $K\alpha$ radiation ($\lambda = 0.15405\text{ nm}$) at 40 kV and 30 mA were recorded in the region of 2θ from 4° to 40° with a scanning rate of $2^\circ/\text{min}$. The test samples were cut into a powder and dried in a vacuum oven for 24 h at 60 °C before testing. Scanning electron microscope (SEM) observation was carried out using a field emission scanning electron microscope (FESEM, Zeiss, SIGMA) at an accelerating voltage of 5 kV. The samples were coated with Au for SEM observation. Transmission electron microscopy (TEM) images were obtained with a JEOL JEM-2010 (HT) electron microscope using an accelerating voltage of 200 kV. A copper grid coated with a holey carbon support film was used to prepare the samples for TEM observation. Optical transmittance (Tr) of the films was measured using a UV-Vis spectrometer (UV-160A, Shimadzu, Japan) at wavelengths from 200 nm to 800 nm. Thermo gravimetric analysis (TGA) was carried out using a Netzsch thermogravimetric analyzer (STA449C, 31MFC, G Jupiter, German). The dried powdered samples were heated in a crucible from 40 °C to 600 °C at a heating rate of 10 K/min under the nitrogen atmosphere. The mechanical properties of the films were measured using a universal tensile tester (CMT 6503, Shenzhen SANS Test machine Co. Ltd., Shenzhen, China) according to ISO527-3-1995 (E) at a cross-head speed of 2 mm/min and were expressed as an average of five measurements.

RESULTS AND DISCUSSION

Structure and Interface Interaction Between MMT and Cellulose

On the basis of the strong cationic exchange capacity of MMT (CEC = 110 meq/100 g), a typical organic cationic antimicrobial agent-hexadecylpyridine bromide (HB) was used as the antimicrobial layer of the composite films. Scheme 1 depicts the preparation process of the antimicrobial RCM5-HB composite films and their photographs. The FTIR spectra of RC, RCM5, RC-HB and RCM-HB are shown in Fig. 1. The vibrational band at $3300\text{--}3500\text{ cm}^{-1}$ corresponded to the stretching for the cellulose hydroxyl groups, which became weaker and shifted to higher frequency with an increase of MMT content in the composite films. This indicated that the original hydrogen bonding in cellulose was broken by the incorporation of MMT. The bands at approximately 993 and 664 cm^{-1} were attributed to the bending vibrations of the Si—O bond in MMT. In the FTIR spectrum of RCM-HB, the peaks at 2932 and 2853 cm^{-1} were ascribed to the asymmetric and symmetric vibration of methylene groups $(\text{CH}_2)_n$, respectively, in the aliphatic chain of HB, confirming the existence of HB in the composite films. These results supported the schematic depiction in the Scheme 1, indicating that the RCM-HB composite films were developed successfully, and there was a hydrogen bonding interaction between MMT and cellulose.



Scheme 1 Preparation process and photographs of the RCM5-HB composite film

Figure 2 shows the curves of light transmittance for the RC, RCM5, RCM-HB and RCM5-HB films. The RC and RCM-HB films exhibited good optical transparency. The optical transmittance for the RC and RCM-HB films at 600 nm was approximately 90%, and decreased to 80% for the composite films with MMT content of 5 wt%. Moreover, the optical transmittance of the RCM5-HB composite film decreased with the addition of HB. On the other hand, the RCM5-HB peak at 240 nm also confirmed the presence of HB in the composite film. These results further demonstrated the fixation of HB on MMT.

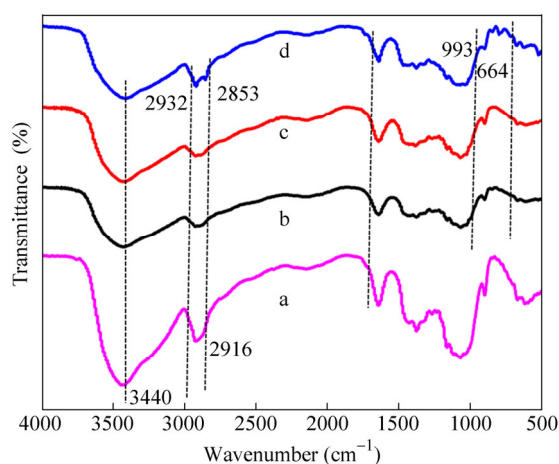


Fig. 1 FTIR spectra of the regenerated cellulose (a), RCM5 (b), RC-HB (c) and RCM5-HB (d)

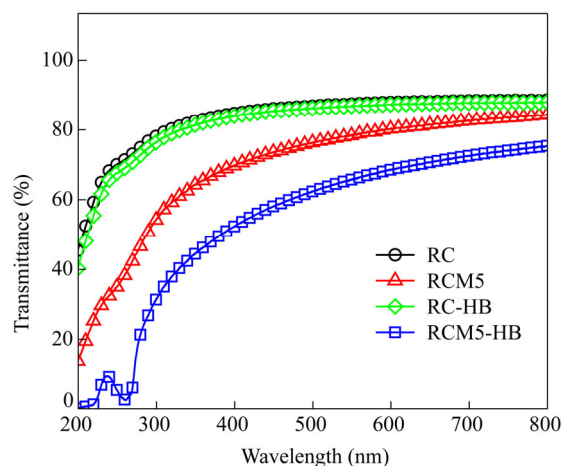


Fig. 2 Transmittance of the regenerated cellulose, and the RCM5, RC-HB and RCM5-HB composite films

Figure 3 shows the XRD patterns of the RC, MMT and RCM composite films with different MMT contents. Three peaks were observed for RC at $2\theta = 12.2^\circ$, 20.2° and 22.2° , corresponding to the (1 $\bar{1}$ 0), (110) and (200) crystal planes of cellulose II. The d -spacing of the (001) plane for MMT was calculated to be 1.2 nm from the diffraction peak at $2\theta = 6.6^\circ$ by using the Bragg equation. The diffraction peak at $2\theta = 6.6^\circ$ for the composite films increased gradually with an increase of the MMT content in the RCM5, RCM10 and RCM20, indicating the extensive clay separation between the layers. Namely, the MMT silicate layers were delaminated in the regenerated cellulose matrix. Therefore, the RCM composite films were composed of the delaminated clay and the cellulose with a strong adhesion action between MMT and cellulose.

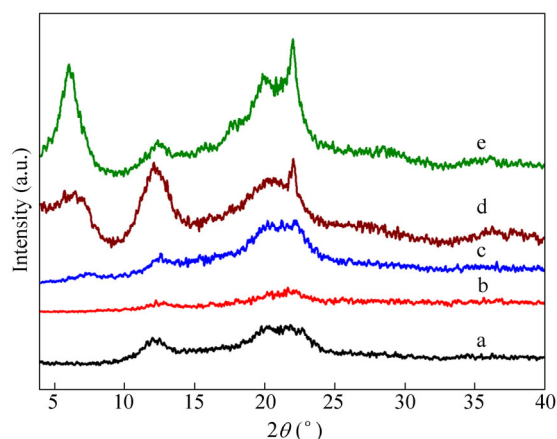


Fig. 3 WAXD spectra of the regenerated cellulose film (a), RCM3 (b), RCM5 (c), RCM10 (d) and RCM20 (e)

Figure 4 shows the SEM images of the cross-section for the RC and RCM composite films obtained by the freeze-drying treatment. Clearly, the cellulose film in the wet state exhibited a homogenous submicron-porous structure, whereas a layered structure appeared in the RCM composite films. As shown in SEM images, MMT was well-dispersed in the cellulose matrix. Microfibril strands consisting of MMT and cellulose were arranged in parallel form in the composite films, similar to the “orientation” morphology. Moreover, with increasing MMT content, the layered structure of the clay became rough, suggesting certain phase separation.

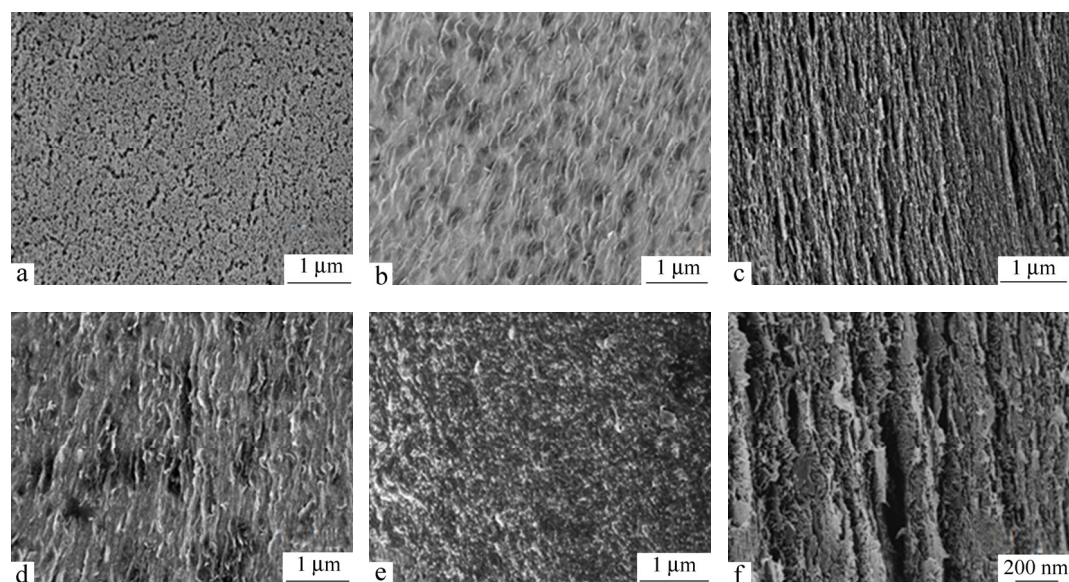


Fig. 4 SEM images of cross-section for the regenerated cellulose film (a), RCM3 (b), RCM5 (c, f), RCM10 (d) and RCM20 (e) composite films by freeze-dried method

TEM observations are necessary to further verify the extent of delamination and exfoliation between MMT and the polymer. The TEM images of the RCM5 hydrogels and films are shown in Figs. 5(a) and 5(b). Obviously, MMT was homogeneously and randomly dispersed in the cellulose hydrogel matrix. Moreover, the morphology of the most delaminated MMT could be observed with a mean thickness of approximately 3 nm. The clay layer had greater regularity and dispersed uniformly in the cellulose films, indicating the occurrence of orientation during the drying process^[26]. The MMT tactoids were highly delaminated into thin lamellas by the cellulose in the RCM5 composite films. Based on the evidence from XRD, SEM and TEM results, the RCM5 composite films with a highly delaminated or intercalated structure have been successfully fabricated. The results obviously supported a high affinity between the cellulose and MMT, which could be assigned to some type of physical interaction, such as electrostatic interactions and/or hydrogen bonding. This could be explained by the fact that cellulose exhibits a very high affinity to the MMT layers and can enter the galleries of mineral clays and widen the interlamellar spaces. Basing on the above results, a proposed schematic illustration of the architecture of the RCM5 composite hydrogels and films with highly intercalated structures is presented in Scheme 2. The highly delaminated MMT layers were covered by cellulose chains. This could be explained by the maximization of the contact area between MMT and cellulose chains through hydrogen bonds, leading to the formation of microfibril strands arranged in parallel in the composite films. Here, the hydrogen bonding sites are associated with the oxygen atoms on the silicate layer surface and the hydrogen atoms in the cellulose OH groups. The XRD, SEM and TEM results supported the proposed structure illustrated in the schematic. The intercalated and layered exfoliation platelet structure of the MMT formed in the regenerated cellulose matrix led to the development of organic/inorganic hybrid films.

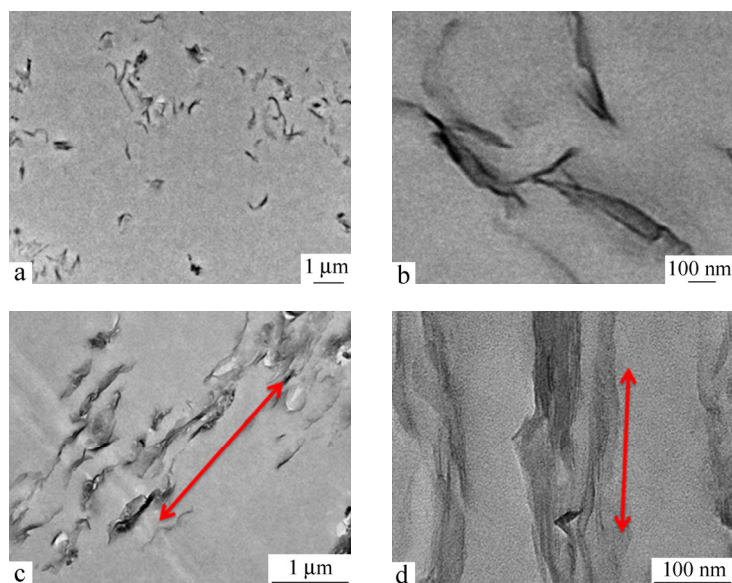
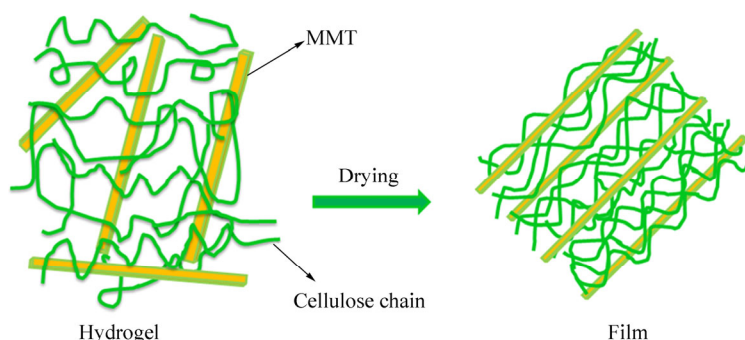


Fig. 5 TEM images of the RCM5 composite hydrogel (a, b) and film (c, d)



Scheme 2 Schematic depiction of the MMT dispersed in the cellulose hydrogel and film

Thermal and Mechanical Properties of the RCM Biocomposite Films

To evaluate the thermal stability of the composite films for their practical applications, thermal decomposition was examined by TGA under N_2 atmosphere. The TGA curves of cellulose/MMT composite films are shown in Fig. 6. Thermal degradation of the cellulose film was divided into two stages: free and bound water loss (40–200 °C) and carbonized phase of cellulose (400–600 °C). The maximum thermal degradation temperature of the cellulose/MMT composite films gradually decreased with an increase of the MMT content, as a result of the catalytic properties of the incorporated inorganic particles such as Al_2O_3 ^[27]. However, the cellulose/MMT composite films still exhibited excellent thermal stability. The stress-strain curves of RC and MMT/cellulose composite films in the dry state are shown in Fig. 7. Compared to RC, the mechanical strength of the composite films increased with MMT incorporation. The tensile strength of the composite films was higher than that of RC, and increased from 119 MPa to 132 MPa with increasing the MMT content for the RCM3 and RCM5 films. This, further confirmed the presence of a strong inter-facial interaction between the cellulose and MMT. The improvement of the mechanical properties supported the above conclusion that the MMT platelets were well-dispersed in the cellulose matrix and could therefore combine tightly with the cellulose. This was consistent with the results obtained from XRD, SEM and TEM. The TGA curves of HB and RCM5-HB films in air are shown in Fig. 8. The small weight loss observed in the temperature range of 70–200 °C was attributed to the release of moisture from the samples. Thermal degradation of hexadecylpyridine bromide occurred in the 200–300 °C region. The decomposition temperature of the RCM5-HB sample may be conveniently divided into two

regions: (1) evolution of the HB between 200 and 300 °C and (2) the cellulose decomposition between 300 and 600 °C. Inspection of the TG data suggested that the RCM5-HB composite film had good thermal stability, and the HB content in the composite film was calculated to be 4.6 wt%.

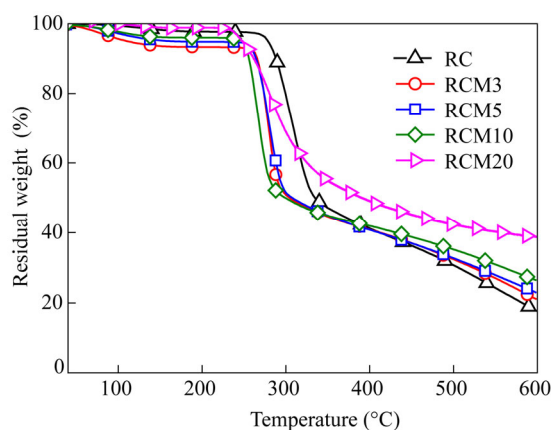


Fig. 6 TGA curves of regenerate cellulose and MMT/cellulose composite films (RCM3, RCM5, RCM10 and RCM20) under nitrogen atmosphere

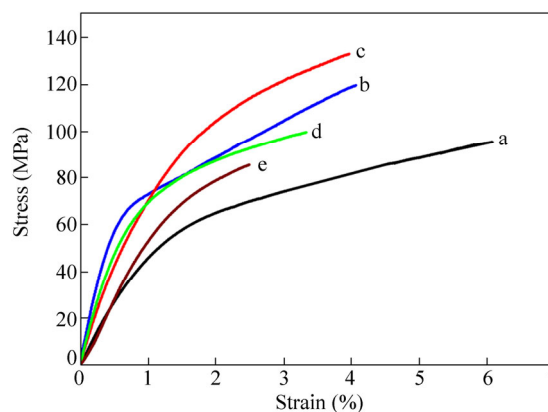


Fig. 7 Stress-strain (σ - ϵ) curves of the regenerated cellulose film (a), and the RCM3 (b), RCM5 (c), RCM10 (d) and RCM20 (e) composite films

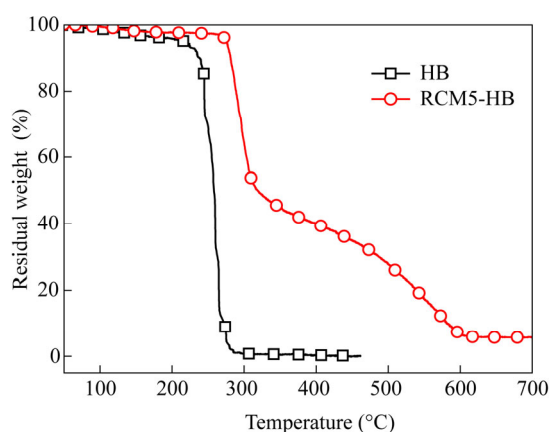


Fig. 8 TGA curves of the HB and RCM5-HB composite film under air atmosphere

Evaluation for Antibacterial Activity of the Composite Films

The antibacterial activity of these materials is very important for their practical applications. It has been reported that cellulose/Ag nanocomposites, quaternary ammonium polyethylenimine nanoparticles and chitosan-based silver nanoparticles^[28–30] have been used as antibacterial materials. To evaluate the prospects for the application of the present composite films as antibacterial materials, an antimicrobial test was carried out according to the international standard method. Figure 9 shows the inhibitory zone of RC, RCM5, RCM-HB and RCM5-HB films after 24 h contact with microbial culture. The bacteria concentration was 500 mg/L, and the visible bacteria colonies were approximately 10^5 – 10^6 cfu/mL. It was noted that the RC, RCM5 and RC-HB films exhibited no antibacterial activities against the *E. coli* and *S. aureus* microorganisms. This could be explained that RC and RCM5 did not contain antimicrobial agent HB, whereas HB in RC-HB was lost, as a result of absence of MMT to fix it in the cellulose/MMT. Interestingly, the RCM5-HB exhibited very clear inhibitory zones around the specimen (Fig. 9d), and with the inhibition zones of the RCM5-HB for *E. coli* and *S. aureus* of 12.6 and 18.2 mm, respectively. This result indicated their good antibacterial activities against *E. coli* and *S. aureus*. This phenomenon was caused by the effect of the release of HB from the RCM-HB film. These results indicated that the RCM5-HB composite film had strong antibacterial activities against Gram-positive and Gram-negative

bacteria. The zone of inhibition showed the diffusion of the HB from treated MMT into the agar. Thus, the possible account involved the release of the antibacterial agents from the MMT and the application of the antibacterial activity from hexadecyl pyridine bromide.

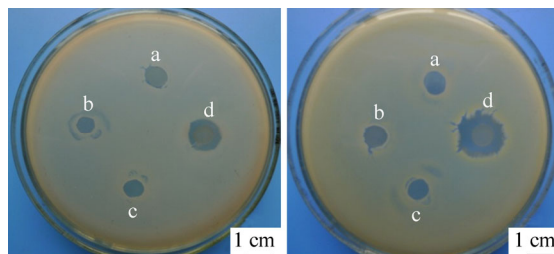


Fig. 9 Antibacterial activities of RC (a), RCM5 (b), RC-HB (c), and RCM5-HB (d) films against *E. coli* (left) and *S. aureus* (right)

CONCLUSIONS

Cellulose/MMT/HB composite films were prepared successfully by blending MMT, hexadecylpyridine bromide and cellulose solution in precooled alkaline/urea aqueous solvent. The MMT could be dispersed evenly in the cellulose matrix, forming organic/inorganic hybrid hydrogels and films through strong inter-facial bonding between MMT and the cellulose. Hexadecylpyridine bromide could be fixed tightly in the cellulose/MMT matrix through cation exchange. The cellulose/MMT/HB composite films exhibited excellent mechanical properties and high antibacterial activities against *E. coli* and *S. aureus*. This material has potential applications in the biomedical field and antibacterial packaging fields.

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