Journal of Innovative Technology, Vol. 1, No. 2, pp. 63-68 September 2019
http://doi.org/10.29424/JIT.201909_1(2).0010

PREPARATION AND ANTIMICROBIAL ACTIVITY OF QUATERNARY AMMONIUM CHITOSAN/MONTMORILLONITE COMPOSITES

Kuo-Yu Chen [1*], You-Cheng Jhang [2], and Guan-Ming Chen [3]

ABSTRACT

Montmorillonite (MMT) is a mineral that is capable of adsorbing bacteria due to its large specific surface area. Chitosan (CS), a biodegradable and biocompatible natural polysaccharide, has good antibacterial properties but only in an acidic medium. In this study, quaternary ammonium CS (QAC) was prepared by graft polymerization of 2-[(Acryloyloxy)ethyl]trimethylammonium chloride (AETMAC) monomers onto CS to increase the antimicrobial activity of CS in a neutral environment. Different amounts of CS and QAC were then mixed with MMT to form CS/MMT and QAC/MMT composites, respectively. The introduction of AETMAC into CS chains was confirmed by Fourier transform infrared spectroscopy. The X-ray diffraction analysis showed that QAC was better able to intercalate into the interlayer of MMT than CS due to the stronger electrostatic interaction between QAC and MMT. The antimicrobial test results demonstrated that the antimicrobial activity of CS was remarkably improved after graft polymerization of AETMAC. Moreover, the QAC exhibited a broad spectrum of antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Additionally, QAC/MMT had higher antimicrobial ratio than CS/MMT. The antimicrobial activity increased in proportion to the amount of QAC in QAC/MMT. Of particular note was the superior enhancement of antibacterial activity against *Staphylococcus aureus* that was observed when the QAC/MMT composite weight ratio was 1:10, suggesting its potential for antimicrobial applications.

Keywords: Montmorillonite; Chitosan; Quaternary ammonium; Graft polymerization; Antimicrobial activity; Composites.

1. INTRODUCTION

Bacterial and fungal infections pose an ongoing threat to human and animal health (Naimi *et al*. 2003). The overuse and misuse of antibiotics has led to the appearance of drug-resistant bacteria (Normanno *et al*. 2007). Montmorillonite (MMT), a naturally occurring clay, consists of silicate layers with a high aspect ratio. MMT is widely used in various fields, such as adsorption, catalysis, and biomedicine due to its high cation exchange capacity, large specific surface area, good absorbability, low toxicity, high availability, and ease of surface modification (Ghadiri *et al*. 2015; Srinivasan 2011; Varma 2002). A number of studies have shown that MMT is capable of adsorbing bacteria (Girardeau 1987; Hu and Xia 2006; Lemke *et al*. 1998). Recently, MMT-based materials have been actively exploited for their antimicrobial properties (Chen *et al*. 2016; Kleyi *et al*. 2016; Zhu *et al*. 2018). MMT can be modified by ion-exchange reactions with cationic modifiers, physical adsorption, and chemical grafting (Liu 2007; Martynková and Valášková 2014). Several investigators have attempted to modify MMT with metals, inorganic cations, and small molecules organic matter to enhance its antimicrobial activity (Bujdáková *et al*. 2018; Malachová *et al*. 2011; Yapar *et al*. 2017; Zhang *et al*. 2018). However, most of these modifying agents are toxic in varying degrees and have high consumption levels. Thus, it would be attractive to modify MMT with polymer having high antimicrobial activity and good biocompatibility to produce an environmentally friendly antimicrobial agent.

Chitosan (CS) is a natural biopolymer derived by partial deacetylation of chitin. It has been widely used in biomedical applications owing to its biodegradability, biocompatibility, nontoxicity, antimicrobial activity, hemostatic properties, and susceptibility to chemical modifications (Cheung *et al*. 2015). CS contains cationic charges in acidic media (pH < 6.5) because of protonation of its amino groups (Thandapani *et al*. 2017). However, it only dissolves in an acid aqueous solution, which limits its application.

The solubility of CS can be significantly improved by modification of its hydroxyl and amine groups (Jin *et al*. 2017; Liu *et al*. 2012; Upadhyaya *et al*. 2013). The introduction of quaternary ammonium groups into CS chains not only enhanced the water solubility of CS, but also increased its positive charge density and antimicrobial activity (Peng *et al*. 2010). The hydroxyl and amine groups of CS can be grafted with vinyl monomers carrying quaternary ammonium groups through redox reaction (Hassan 2015). The graft modification of CS with long pendant

Manuscript received January 31, 2019; revised July 8, 2019; accepted July 31, 2019.
[1*] Associate Professor (corresponding author), Department of Chemical and Materials Engineering, Nation Yunlin University of Science and Technology, Douliou, Yunlin, Taiwan, 64002, R.O.C. (e-mail: chenkuo@yuntech.edu.tw).
[2] Master student, Department of Chemical and Materials Engineering, Nation Yunlin University of Science and Technology, Douliou, Yunlin, Taiwan, 64002, R.O.C. (e-mail: M10515037 @yuntech.edu.tw).
[3] Master student, Department of Chemical and Materials Engineering, Nation Yunlin University of Science and Technology, Douliou, Yunlin, Taiwan, 64002, R.O.C. (e-mail: M10615049 @yuntech.edu.tw).

side-chains improved the water solubility of CS (Chen and Zeng 2018).

MMT has been used to improve the thermal stability, mechanical properties, and barrier properties against water and gases of CS (Giannakas *et al*. 2014, Wang *et al*. 2005). Some studies have used CS to enhance the antimicrobial property of MMT (Han *et al*. 2010; Lertsutthiwong *et al*. 2012). Moreover, few reports demonstrated that quaternary ammonium chitosan (QAC) could increase the antimicrobial activity of MMT in a weak acidic or weak basic medium (Wang *et al*. 2008). However, the content of CS or QAC in the composites was usually greater than that of MMT. Moreover, little research has been conducted on MMT modification using CS-grafted vinyl monomers with quaternary ammonium groups. In this study, QAC/MMT composites with weight ratios of 1:10, 1:50, and 1:100 were developed as antimicrobial materials. Firstly, quaternary ammonium-containing monomers were graft polymerized onto CS by redox polymerization with ammonium persulfate. QAC/MMT composites were then prepared by simple solution-mixing. The materials were characterized using Fourier transform infrared spectroscopy (FTIR) and powder X-ray diffraction (XRD). Finally, the antimicrobial activity of the composites against Gram-negative bacteria (*Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus*), and fungi (*Candida albicans*) was examined by colony counting.

2. MATERIALS AND METHODS

2.1 Materials

MMT was kindly supplied by Houchi Chemical Co. (Taipei, Taiwan). CS, with a molecular weight of 50 to 190 kDa and a deacetylation degree of 75% to 85%, was purchased from Sigma-Aldrich (St. Louis, MO, USA), and 2-[(Acryloyloxy)ethyl] trimethylammonium chloride (AETMAC) was also purchased from Sigma-Aldrich. Ammonium persulfate, which was used as the redox initiator, was obtained from Showa Chemicals (Tokyo, Japan). Acetic acid and sodium hydroxide were purchased from Katayama Chemical (Osaka, Japan). Acetone and methanol were supplied by Echo Chemical (Miaoli, Taiwan). All chemicals were used as received with no further purification.

2.1 Synthesis of QAC

QAC was synthesized by graft polymerization of AETMAC onto CS according to our previous work (Chen and Zeng 2017). Briefly, a 1 wt% CS solution was prepared by dissolving CS powder in 2 vol% aqueous acetic acid at 80 °C using a mechanical stirrer. After purging with nitrogen for 30 min, 0.012 M of AETMAC and 0.03 M of ammonium persulfate were added dropwise successively to carry out graft polymerization at 80 °C under nitrogen purging. The reaction was continued for 3 h. The reaction solution was then cooled down to room temperature and precipitated in an excess of acetone. The precipitate was flushed thoroughly with methanol to remove unreacted AETMAC monomer and poly([2-(acryloyloxy)ethyl]-trimethylammonium chloride (PAETMAC) homopolymer. Finally, the purified product (QAC) was dried at 60 °C under vacuum. The grafting ratio (GR) of QAC was calculated by gravimetric calculation using the equation (Li *et al*. 2013):

$$GR(\%) = \left(\frac{W_g - W_c}{W_c} \right) \times 100\% \tag{1}$$

where W_g and W_c represent the weights of QAC and CS, respectively.

2.2 Preparation of CS/MMT and QAC/MMT composites

A 2 wt% MMT colloid suspension was prepared by vigorously dispersing MMT in deionized water. CS and QAC were dissolved in 0.2 vol% aqueous acetic acid to prepare 0.2 wt% CS and QAC solutions, respectively. The pH of CS and QAC solutions were adjusted to 6.0 with 5N sodium hydroxide solution. Various volumes of MMT suspension were then added to CS and QAC solutions under vigorous stirring to prepare the CS/MMT and QAC/MMT dispersions, respectively. The weight ratios of CS/MMT or QAC/MMT were 1:10, 1:50, and 1:100. The concentration of CS/MMT and QAC/MMT was 1 wt%. The dispersions were then homogenized (IKA T18 basic, Ultra-Turrax, Germany) at 7,200 rpm for 10 min to disperse the CS/MMT and QAC/MMT particles and reduce aggregation. Subsequently, the dispersions were centrifuged, washed with deionized water, freeze-dried, and ground to obtain CS/MMT and QAC/MMT composite powders.

2.3 Characterization of materials

FTIR spectra of dry samples over the range of 4000–500 cm^{-1} were collected using KBr discs with a Bio-Rad FTS-40 spectrophotometer (Hercules, CA, USA) at a resolution of 2 cm^{-1} and by averaging 32 scans.

The powder XRD analysis was performed on a Rigaku Miniflex II X-ray diffractometer (Tokyo, Japan) with Cu-Kα (λ = 0.154 nm) radiation. The XRD patterns were recorded for 2θ from 2° to 40° at a scan rate of 2° min^{-1}.

2.4 Evaluation of the antimicrobial properties

The antimicrobial activity of the samples against Gram-negative *Escherichia coli* (ATCC 8739), Gram-positive *Staphylococcus aureus* (ATCC 6538), and *Candida albicans* (ATCC 24433) was determined according to the ASTM E2149-01 standard test method. The microbial strains were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). Luria-Bertani broth (LB broth, Miller; Difco, Detroit, MI, USA), Sabouraud dextrose broth (SDB broth, HiMedia, Mumbai, India), and nutrient agar (High Standard Enterprise Co., Ltd., Taichung, Taiwan) were dissolved in deionized water and sterilized in an autoclave at 121 °C for 20 min. The sterilized agar medium was cast on sterilized Petri dishes and allowed to solidify in a laminar flow hood. A loop of microbial cells was plated on the agar surface to give single colonies. Single colonies of bacterial and fungal cells were then suspended in 25 mL of LB broth and 50 mL of SDB broth, respectively, and cultured in a shaking incubator (120 rpm) at 37 °C. After incubating for 24 h, the optical density of the microbial suspension was determined at 600 nm in a Metertech SP-830+ spectrophotometer (Metertech Inc., Taipei, Taiwan). The microbial concentration of the suspension was adjusted by PBS to 1.1×10^5 colony-forming units (CFU) mL^{-1}.

The MMT, CS, QAS, CS/MMT, and QAC/MMT solutions were prepared in deionized water at a concentration of 1 wt%. The prepared solution was stirred for 10 min and sonicated for another 10 min. One hundred μL of sample solution and 900 μL of microbial suspension were added to 24-well tissue culture plates to achieve a final sample concentration of 0.1 % (w v^{-1}). In the control group, 100 μL of deionized water was mixed with 900 μL of microbial suspension. The mixtures were cultured at 37 °C with shaking at 120 rpm. After 0.5 h and 6 h of incubation, 100 μL of microbial culture was withdrawn, and serial dilutions with PBS were performed to determine the numbers of colonies. The agar plates were kept in an incubator at 37 °C for 24 h, and the CFUs were counted visually. Each experiment was done in triplicate. The antimicrobial ratio (AR, %) of the test sample was calculated according to the equation:

$$AR(\%) = \left(\frac{N_c - N_s}{N_c}\right) \times 100\% \quad (2)$$

where N_c and N_s are the CFU of control and test samples, respectively.

2.5 Statistical Analysis

All quantitative data are expressed as mean ± standard deviation. Statistical differences among samples were assessed by the least significant difference (LSD) test with the level of significance set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Characterizations of CS/MMT and QAC/MMT composites

The FTIR transmittance spectra of different samples are given in Fig 1. CS and QAC had a broad and intense band at 3100 – 3600 cm^{-1}, which can be attributed to the stretching vibrations of the O – H and N – H groups. The band at around 1645 cm^{-1} was ascribed to the C = O stretching vibration of amide I groups. The band for the N – H bending and C – N stretching vibration of amide II was located at around 1595 cm^{-1}. Compared with CS, QAC exhibited two additional characteristic absorption bands that appeared at 1734 cm^{-1} and 953 cm^{-1}, corresponding to the C = O stretching of ester groups and the C – N stretching of quaternary ammonium groups, respectively, in AETMAC, indicating the formation of graft copolymer onto the CS backbone (Hassan, 2015). Moreover, the decrease in the intensity of the band at 1595 cm^{-1} (amide II) suggests that the amine groups of CS were involved in the graft polymerization reaction (Abdel *et al*. 2015). The grafting ratio of AETMAC on CS was determined to be 22.1 wt% by gravimetric analysis. In the spectra of MMT, the bands near 3630 cm^{-1} and 1640 cm^{-1} corresponded to O – H stretching and O – H bending, respectively. MMT also exhibited broad and strong absorption at around 1045 cm^{-1} due to stretching vibration of Si – O (Özdemir *et al*. 2013). CS/MMT and QAC/MMT composites exhibited the same characteristic bands as MMT and no obvious difference in shifting or intensity variation was observed due to these composites having relatively high MMT content.

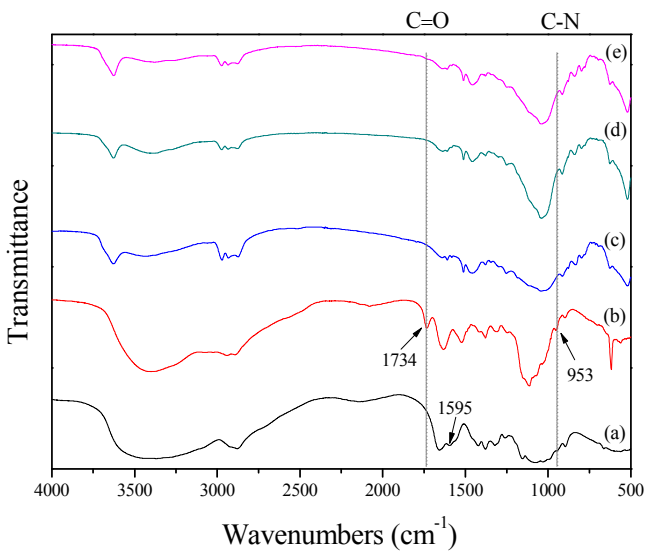

Fig. 1 FTIR spectra of (a) CS; (b) QAC; (c) MMT; (d) CS/MMT (1/10); and (e) QAC/MMT (1/10).

The XRD patterns of CS, QAC, MMT, and all of the composites are presented in Fig. 2. CS exhibited two major diffraction peaks at 2θ = 10.32° and 20.08°, which can be attributed to the (020) and (110) crystal planes, respectively (Salama *et al*., 2015). In the diffraction spectrum of QAC, the peak at 10.32° disappeared, while the intensity of the peak at 20.08° decreased and broadened, suggesting that the crystallinity of CS decreased with grafting of AETMAC monomers. The diffraction peak of MMT was observed at 2θ = 5.24°, which can be attributed to the (001) crystal plane. The d-spacing, interlayer distance, was 16.8 Å, which was calculated by the Bragg equation. As the results of the FTIR spectra, XRD patterns of all composites were similar to that of MMT since they had low CS and QAC content. However, a slight shift in the d001 diffraction peak of composites towards a

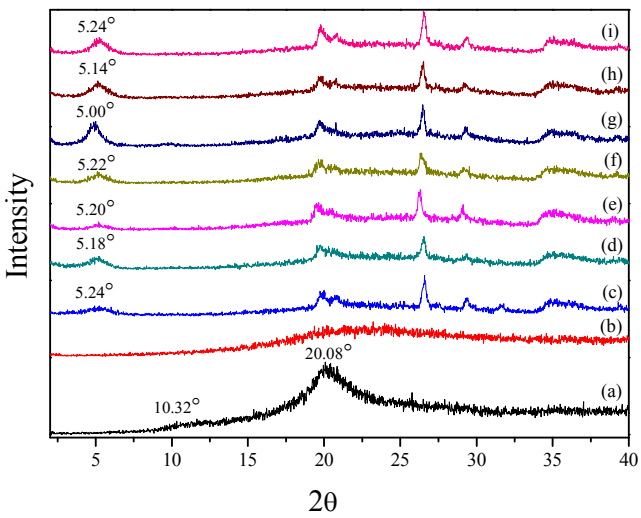

Fig. 2 XRD spectra of (a) CS; (b) QAC; (c) MMT; (d) CS/MMT (1/10); (e) CS/MMT (1/50); (f) CS/MMT (1/100); (g) QAC/MMT (1/10); (h) QAC/MMT (1/50); and (i) QAC/MMT (1/100).

lower angle in comparison with that of MMT was observed, revealing that a small amount of QAC and CS had intercalated into the MMT structure. The interlayer distance of MMT grew slightly as the amount of CS and QAC increased. The d001 peak of QAC/MMT slightly shifted towards lower angles as compared to the CS/MMT when the weight ratios of polymer/MMT were 10:1 and 50:1, indicating that the QAC chains could be more readily inserted into the MMT layer through a cationic exchange reaction. This phenomenon could be explained by the greater amount of cationic groups in QAC, which likely facilitated better interaction of QAC molecules with MMT. The largest interlayer distance of 17.7 Å was obtained when the weight ratio of QAC/MMT was 10:1.

3.2 Antimicrobial properties of CS/MMT and QAC/MMT composites

The antimicrobial properties of the CS, QAC, MMT, CS/MMT, and QAC/MMT composites in microbial suspension were evaluated comparatively against Gram-negative bacteria (*E. coli*), Gram-positive bacteria (*S. aureus*), and fungi (*C. albicans*). These three species were chosen because they are common nosocomial pathogens.

Table 1 presents the antimicrobial ratios of the test samples against *E. coli*, *S. aureus*, and *C. albicans* after 0.5 h and 6 h of incubation at 37 °C. All of the materials inhibited bacterial and fungal growth to a certain degree after 0.5 h of contact. MMT and CS/MMT composites exhibited good antibacterial activity against *E. coli*, whereas they showed slightly weaker antibacterial activity against *S. aureus* and low antifungal activity against *C. albicans*. CS also had a higher antibacterial activity against *E. coli* than against *S. aureus* and *C. albicans*. Interestingly, MMT showed a higher antibacterial activity than CS ($P < 0.05$). The positively charged amino group in CS was reported to be one of the main factors contributing to its antibacterial activity (Papineau *et al*. 1991). However, CS has a relatively low positive charge at a neutral pH, suggesting that it ought to exhibit low antimicrobial activity. This notion is consistent with a previous study that found that CS only manifested its antibacterial activity in an acidic environment because of its poor solubility above pH 6.5 (Ferfera-Harrar *et al*. 2014). The grafting of AETMAC with quaternary ammonium groups onto CS significantly improved the antimicrobial activity against bacteria and fungi ($P < 0.05$). The antimicrobial effects of quaternary ammonium compounds have been recognized for a long time. In the present study, QAC exhibited good antimicrobial activity and a broad spectrum of activity. Of particular note, the antimicrobial ratio of QAC against *C. albicans* reached 96.6% after 6 h of contact, even at a concentration of only 0.1 wt%. Additionally, QAC/MMT showed a stronger antimicrobial activity than CS/MMT composites at the same polymer/MMT weight ratios, owing to the higher antimicrobial activity of QAC relative to CS. The extent of microbial growth inhibition was dependent on the weight ratio of QAC to MMT, *i.e*., the larger the amount of QAC in the composites, the greater the antimicrobial ratio. The QAC/MMT composite with a weight ratio of 1:10 demonstrated the best antimicrobial activity among all of the composites. Of note, it showed a better inhibitory activity against *S. aureus* as compared to pure QAC or MMT ($P < 0.05$), indicating a synergistic effect between QAC and MMT. The large specific surface area of MMT might enhance the adsorption of microbial cells onto composites from the solution, and QAC then kills these cells (Wang *et al*. 2006). Accordingly, as a result of the dual actions of MMT and QAC, the composites were able to strongly inhibit the growth of microbes.

As shown in Table 1, the antimicrobial ratios of all materials against *E. coli* increased with contact time, whereas the antimicrobial activity of MMT and CS/MMT against *S. aureus* and *C. albicans* decreased with contact time. After contact for 6 h, CS, MMT, and CS/MMT did not exert any antifungal effect on *C. albicans* (Table 1 and Fig. 3). In contrast, the antimicrobial activity of QAC and QAC/MMT with a weight ratio of 1:10 increased with increasing contact time. No bacterial was found after 6 h of contact with QAC/MMT that had a weight ratio of 1:10. Moreover, the antimicrobial ratio of *C. albicans* reached 85.7%.

Compared with *E. coli* and *S. aureus*, *C. albicans* had poorer antimicrobial sensitivity to the test materials because fungal cells are protected by a rigid cell wall (Ling *et al*. 2013). These results are consistent with the findings of previous reports (Chen *et al*. 2016; Liu *et al*. 2013). However, the QAC/MMT composite with a weight ratio of 1/10 also exhibited a strong inhibitory effect against *C. albicans* growth (Table 1 and Fig. 3), suggesting that it has great potential in antimicrobial applications.

Table 1 Antimicrobial activity of materials

Materials	Antimicrobial ratio (%)					
	Escherichia coli		*Staphylococcus aureus*		*Candida albicans*	
	0.5 h	6 h	0.5 h	6 h	0.5 h	6 h
CS	52.9 ± 1.2	75.7 ± 4.0	22.1 ± 5.0	26.7 ± 2.2	15.0 ± 4.2	0.0 ± 0.0
QAC	99.5 ± 0.2	100.0 ± 0.0	80.9 ± 1.7	93.1 ± 0.5	43.8 ± 1.7	96.6 ± 0.5
MMT	83.3 ± 8.0	91.9 ± 2.6	58.7 ± 4.6	40.3 ± 1.9	16.3 ± 4.7	0.0 ± 0.0
CS/MMT (1/10)	88.9 ± 0.0	95.2 ± 1.9	39.8 ± 7.2	34.9 ± 1.4	13.4 ± 4.5	0.0 ± 0.0
CS/MMT (1/50)	88.9 ± 0.2	97.7 ± 0.9	49.7 ± 1.7	24.7 ± 5.5	18.5 ± 1.6	0.0 ± 0.0
CS/MMT (1/100)	78.5 ± 2.7	93.2 ± 1.8	54.7 ± 4.4	14.3 ± 4.1	16.3 ± 6.9	0.0 ± 0.0
QAC/MMT (1/10)	95.3 ± 1.2	100.0 ± 0.0	89.9 ± 0.8	100.0 ± 0.0	35.7 ± 6.0	85.7 ± 2.8
QAC/MMT (1/50)	97.0 ± 0.8	99.2 ± 0.2	61.2 ± 8.1	56.3 ± 5.3	27.1 ± 4.8	0.0 ± 0.0
QAC/MMT (1/100)	96.2 ± 2.0	93.6 ± 0.9	47.5 ± 7.7	55.8 ± 3.5	8.6 ± 4.6	0.0 ± 0.0

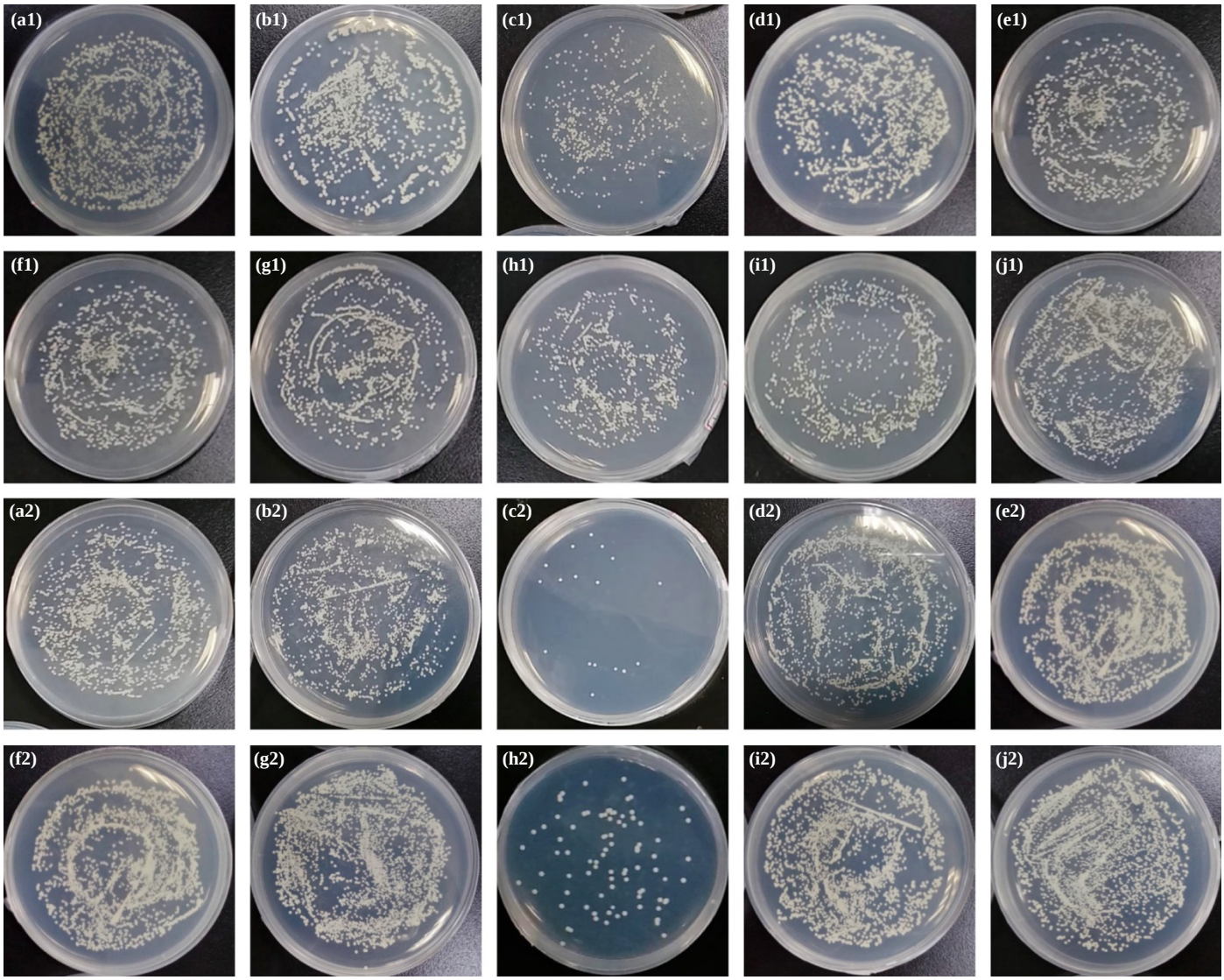

Fig. 3 Images of colonies of *C. albicans* after (1) 0.5 and (2) 6 h of treatment with various samples: (a) control; (b) CS; (c) QAC; (d) MMT; (e) CS/MMT (1/10); (f) CS/MMT (1/50); (g) CS/MMT (1/100); (h) QAC/MMT (1/10); (i) QAC/MMT (1/50); and (j) QAC/MMT (1/100).

4. CONCLUSION

In this investigation, quaternary ammonium monomers were successfully grafted onto CS. A series of CS/MMT and QAC/MMT composites were prepared by simple mixing and homogenization. XRD results showed the QAC had a stronger interaction with MMT than with CS. The antimicrobial tests revealed that the antimicrobial ratio of CS was significantly increased after grafting of AETMAC onto CS. Moreover, the QAC/MMT composites with small amounts of QAC had a higher antimicrobial activity than that of MMT alone. These results suggest that QAC could be used as an enhancer to improve the antimicrobial properties of MMT.

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

Abdel Aziz, M.S., Naguib, H.F., and Saad, G.R. (2015). "Nanocomposites based on chitosan-graft-poly(N-vinyl-2-pyrrolidone): Synthesis, characterization, and biological activity." *International Journal of Polymeric Materials and Polymeric Biomaterials*, **64**, 578-586.

Bujdáková, H., Bujdáková, V., Májeková-Koščová, H., Gaálová, B., Bizovská, V., Boháč, P., and Bujdák, J. (2018). "Antimicrobial activity of organoclays based on quaternary alkylammonium and alkylphosphonium surfactants and montmorillonite." *Applied Clay Science*, **158**, 21-28.

Chen, K., Ye, W., Cai, S., Huang, L., Zhong, T., Chen, L., and Wang, X. (2016). "Green antimicrobial coating based on quaternised chitosan/organic montmorillonite/Ag NPs nanocomposites." *Journal of Experimental Nanoscience*, **11**, 1360-1371.

Chen, K.Y. and Zeng, S.Y. (2017). "Preparation and characterization of quaternized chitosan coated alginate microspheres for blue dextran delivery." *Polymers*, **9**, 210.

Chen, K.Y. and Zeng, S.Y. (2018). "Fabrication of quaternized chitosan nanoparticles using tripolyphosphate/genipin dual cross-linkers as a protein delivery system." *Polymers*, **10**, 1226.

Cheung, R.C.F., Ng, T.B., Wong, J.H., and Chan, W.Y. (2015). Chitosan: An update on potential biomedical and pharmaceutical applications." *Marine Drugs*, **13**, 5156-5186.

Ferfera-Harrar, H., Aiouaz, N., Dairi, N., and Hadj-Hamou, A.S. (2014). "Preparation of chitosan-g-poly (acrylamide)/montmorillonite superabsorbent polymer composites: Studies on swelling, thermal, and antibacterial properties." *Journal of Applied Polymer Science*, **131**, 39747-39760.

Ghadiri, M., Chrzanowski, W., and Rohanizadeh, R. (2015). "Biomedical applications of cationic clay minerals." *RSC Advances*, **5**, 29467-29481.

Giannakas, A., Grigoriadi, K., Leontiou, A., Barkoula, N. M., and Ladavos, A. (2014). "Preparation, characterization, mechanical and barrier properties investigation of chitosan–clay nanocomposites." *Carbohydrate polymers*, **108**, 103-111.

Girardeau, J.P. (1987). "Smectite aggregation by Escherichia coli. Acta Gastro-Enterologica Belgica, **50**, 181-192.

Han, Y.S., Lee, S.H., Choi, K.H., and Park, I. (2010). "Preparation and characterization of chitosan–clay nanocomposites with antimicrobial activity." *Journal of Physics and Chemistry of Solids*, **71**, 464-467.

Hassan, M.M. (2015). "Binding of a quaternary ammonium polymer-grafted-chitosan onto a chemically modified wool fabric surface: Assessment of mechanical, antibacterial and antifungal properties." *RSC Advances*, **5**, 35497-35505.

Hu, C.H. and Xia, M.S. (2006). "Adsorption and antibacterial effect of copper-exchanged montmorillonite on Escherichia coli K88." *Applied Clay Science*, **31**, 180-184.

Jin, Z., Li, D., Dai, C., Cheng, G., Wang, X., and Zhao, K. (2017). "Response of live Newcastle disease virus encapsulated in N-2-hydroxypropyl dimethylethyl ammonium chloride chitosan nanoparticles." *Carbohydrate Polymer*, **171**, 267-280.

Kleyi, P.E., Ray, S.S., Abia, A.L.K., Ubomba-Jaswa, E., Wesley-Smith, J., and Maity, A. (2016). "Preparation and evaluation of quaternary imidazolium-modified montmorillonite for disinfection of drinking water." *Applied Clay Science*, **127**, 95-104.

Lemke, S.L., Grant, P.G., Phillips, T.D. (1998). "Adsorption of zearalenone by organophilic montmorillonite clay. *Journal of Agricultural and Food Chemistry*, **46**, 3789-3796.

Lertsutthiwong, P., Noomun, K., Khunthon, S., and Limpanart, S. (2012). "Influence of chitosan characteristics on the properties of biopolymeric chitosan-montmorillonite." *Progress in Natural Science: Materials International*, **22**, 502-508.

Li, G., Guo, L., Wen, Q., and Zhang, T. (2013). "Thermo-and pH-sensitive ionic-cross-linked hollow spheres from chitosan-based graft copolymer for 5-fluorouracil release." *International Journal of Biological Macromolecules*, **55**, 69-74.

Ling, Y., Luo, Y., Luo, J., Wang, X., and Sun, R. (2013). "Novel antibacterial paper based on quaternized carboxymethyl chitosan/organic montmorillonite/Ag NP nanocomposites." *Industrial crops and products*, **51**, 470-479.

Liu, P., 2007. "Polymer modified clay minerals: A review." *Applied Clay Science*, **38**, 64-76.

Liu, B., Shen, S., Luo, J., Wang, X., and Sun, R. (2013). "One-pot green synthesis and antimicrobial activity of exfoliated Ag NP-loaded quaternized chitosan/clay nanocomposites." *RSC Advances*, **3**, 9714-9722.

Malachová, K., Praus, P., Rybková, Z., and Kozák, O. (2011). "Antibacterial and antifungal activities of silver, copper and zinc montmorillonites." *Applied Clay Science*, **53**, 642-645.

Martynková, G.S. and Valášková, M. (2014). "Antimicrobial nanocomposites based on natural modified materials: A review of carbons and clays." *Journal of Nanoscience and Nanotechnology*, **14**, 673-693.

Naimi, T.S., LeDell, K.H., Como-Sabetti, K., Borchardt, S.M., Boxrud, D.J., Etienne, J., Johnson, S.K., Vandenesch, F., Fridkin, S., O'Boyle C. Danila, R.N., and Lynfield R. (2003). "Comparison of community and health care-associated methicillin-resistant Staphylococcus aureus infection." *Journal of the American Medical Association*, **290**, 2976-2984.

Normanno, G., Corrente, M., La Salandra, G., Dambrosio, A., Quaglia, N. C., Parisi, A., Greco, G., Bellacicco, A.L., Virgilio, S., and Celano, G.V. (2007). "Methicillin-resistant Staphylococcus aureus (MRSA) in foods of animal origin product in Italy." *International Journal of Food Microbiology*, **117**, 219-222.

Özdemir, G., Yapar, S., and Limoncu, M.H. (2013). "Preparation of cetylpyridinium montmorillonite for antibacterial applications." *Applied Clay Science*, **72**, 201-205.

Papineau, A.M., Hoover, D.G., Knorr, D., and Farkas, D.F. (1991). "Antimicrobial effect of water-soluble chitosans with high hydrostatic pressure." *Food Biotechnology*, **5**, 45-57.

Peng, Z.X., Wang, L., Du, L., Guo, S.R., Wang, X.Q., and Tang, T.T. (2010). "Adjustment of the antibacterial activity and biocompatibility of hydroxypropyltrimethyl ammonium chloride chitosan by varying the degree of substitution of quaternary ammonium." *Carbohydrate Polymer*, **81**, 275-283.

Salama, H.E., Saad, G.R., and Sabaa, M.W. (2015). "Synthesis, characterization and biological activity of Schiff bases based on chitosan and arylpyrazole moiety." *International Journal of Biological Macromolecules*, **79**, 996-1003.

Srinivasan, R. (2011). Advances in application of natural clay and its composites in removal of biological, organic, and inorganic contaminants from drinking water." *Advances in Materials Science and Engineering*, 2011, 872531.

Thandapani, G., Prasad, S., Sudha, P.N., and Sukumaran, A. (2017). "Size optimization and in vitro biocompatibility studies of chitosan nanoparticles." *International Journal of Biological Macromolecules*, **104**, 1794-1806.

Upadhyaya, L., Singh, J., Agarwal, V., and Tewari, R.P. (2013). "Biomedical applications of carboxymethyl chitosans." *Carbohydrate Polymer*, **91**, 452-466.

Varma, R.S. (2002). "Clay and clay-supported reagents in organic synthesis." *Tetrahedron*, **58**, 1235-1255.

Wang, S.F., Shen, L., Tong, Y.J., Chen, L., Phang, I.Y., Lim, P.Q., and Liu, T.X. (2005). "Biopolymer chitosan/montmorillonite nanocomposites: Preparation and characterization." *Polymer Degradation and Stability*, **90**, 123-131.

Wang, X., Du, Y., Yang, J., Wang, X., Shi, X., and Hu, Y. (2006). "Preparation, characterization and antimicrobial activity of chitosan/layered silicate nanocomposites." *Polymer*, **47**, 6738-6744.

Wang, X., Du, Y., Yang, J., Tang, Y., and Luo, J. (2008). "Preparation, characterization, and antimicrobial activity of quaternized chitosan/organic montmorillonite nanocomposites." *Journal of Biomedical Materials Research Part A*, **84**, 384-390.

Yapar, S., Ateş, M., and Özdemir, G. (2017). "Preparation and characterization of sodium lauroyl sarcosinate adsorbed on cetylpyridinium-montmorillonite as a possible antibacterial agent." *Applied Clay Science*, **150**, 16-22.

Zhang, L., Chen, J., Yu, W., Zhao, Q., and Liu, J. (2018). "Antimicrobial nanocomposites prepared from montmorillonite/Ag." *Journal of Nanomaterials*, 2018, 6190251.

Zhu, W., Li, J., Lei, J., Li, Y., Chen, T., Duan, T., Yao, W., Zhou, J., Yu, Y., and Liu, Y. (2018). "Silver nanoparticles incorporated konjac glucomannan-montmorillonite nacre-like composite films for antibacterial applications." *Carbohydrate Polymers*, **197**, 253-259.