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Alginate/quaternized carboxymethyl chitosan/clay nanocomposite microspheres: preparation and drug-controlled release behavior

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Drug-delivery systems, using natural drug carriers, have become increasingly important because of their nontoxicity and biodegradability. In this study, firstly, quaternized carboxymethyl chitosan (QCMC) was intercalated into the interlayer of organic montmorillonite (OMMT) to obtain the QCMC/OMMT nanocomposites, their structure, morphology, and thermal stability were investigated. Next, crosslinked alginate/QCMC/OMMT (AQCOM) microsphere was obtained by crosslinking with CaCl_2 , and the drug-controlled release behavior was evaluated with bovine serum albumin (BSA) as model drug. The results suggested that, carboxyl groups in alginate and QCMC crosslinked with Ca^{2+} , quaternary ammonium groups in QCMC or OMMT electrostatically interacted with carboxyl groups in alginate, and there was stable three-dimensional network in AQCOM microsphere. The swelling ratio of AQCOM microspheres decreased with the increase of OMMT content, the lowest one was only about 45% compared to the microsphere without OMMT of 197%. Besides, the *in vitro* release results for BSA indicated that the AQCOM microsphere displayed more excellent encapsulation and controlled release capacities than the microsphere without OMMT. The *in vitro* active cutaneous anaphylaxis test was carried out on Guinea pigs, which revealed that AQCOM microsphere did not cause anaphylaxis. Therefore, QCMC/OMMT nanocomposites from natural materials are considerably suitable to apply as drug-controlled release carriers.

Keywords: quaternized carboxymethyl chitosan; organic montmorillonite; nanocomposite; microsphere; drug-controlled release

Introduction

Currently, natural materials are important global research topic as there is a genuine interest both in academia and in industry. Montmorillonite (MMT) is one of the most abundant natural inorganic minerals and is proved to be safety as food additives in an evaluation report published by European Food Safety Authority [1], and considerable interest has been recently focused on finding new applications for biopolymer/MMT nanocomposites [2,3].

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Chitosan is a linear cationic amino polysaccharide and the second most abundant biopolymer in nature with unique structure and biodegradability [4]. Chitosan or its derivatives, such as quaternized chitosan and carboxymethyl chitosan, has been intercalated into MMT to obtain the nanocomposites, which showed great potential applications as the medical bionics material [5], antimicrobial agent [6,7], gene delivery carrier [8], flocculants [9], etc.

Quaternized carboxymethyl chitosan (QCMC) is an amphiprotic hydrosoluble derivative of chitosan, which has active hydroxyl, carboxymethyl, and quaternary ammonium groups on the molecular chain [6], thus it shows excellent drug encapsulation and controlled release ability [10]. Besides, due to high surface area and strong adsorption capacity, organo-MMT (OMMT, MMT modified by surfactant) also shows good drug encapsulation ability [11]. As the biopolymer/layered silicate nanocomposites are known to combine the physical and chemical properties of both inorganic and organic material [12], there is an interesting way to combine the drug-controlled release advantages of QCMC with OMMT. However, up to present, there is no report about QCMC/OMMT nanocomposite.

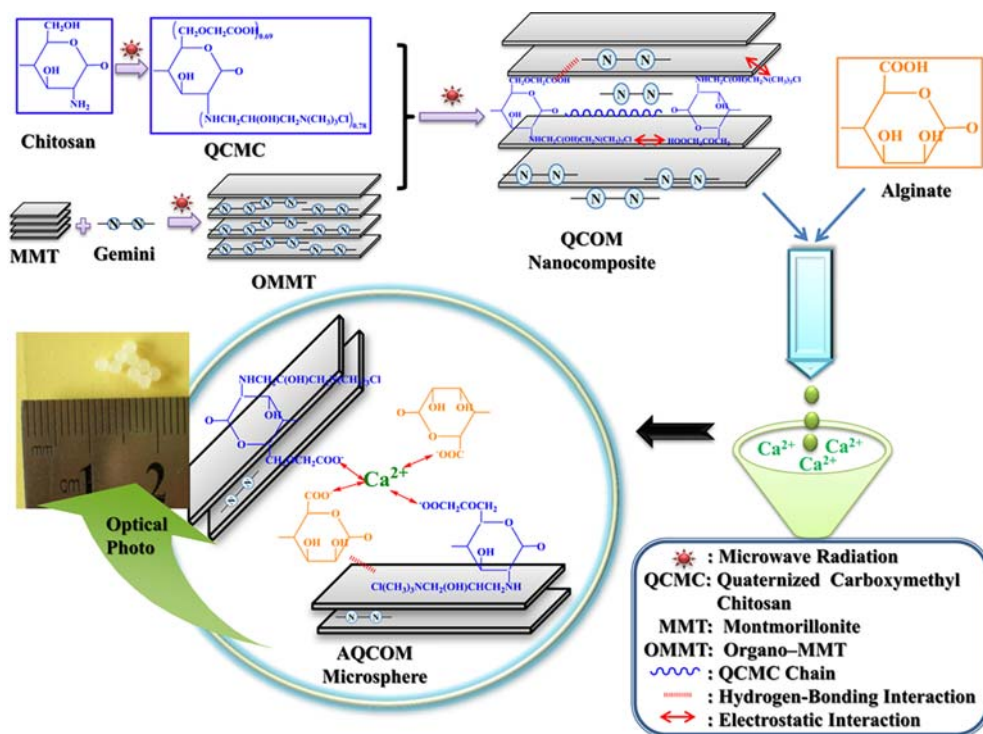
Sodium alginate as a naturally occurring nontoxic polysaccharide has been widely used in medicine and cosmetics field. The carboxylic groups of alginate extending through the backbone molecule have the capacity of crosslinking in the presence of divalent cations such as calcium (Ca^{2+}), barium (Ba^{2+}), or zinc (Zn^{2+}) thus resulting in gelation [7], which rather complex with G–G sequences in the polymer chain to form the ‘egg box junctions.’ So, alginate crosslinked microspheres are common drug carrier [13]. From mentioned point of view, a combination of chitosan-based layered silicate nanocomposite with alginate as an efficient drug-controlled release system is quite attractive, and there is still lack of related research.

In the present work, firstly QCMC/OMMT nanocomposites were prepared via the microwave radiation technique. The structure and morphology of QCMC/OMMT nanocomposites were evaluated by X-ray diffraction (XRD) and transmission electron microscopy (TEM), and the thermal behavior was analyzed by using thermogravimetric analysis method (TGA). Then, alginate/QCMC/OMMT (AQCOM) microspheres based on QCMC/OMMT nanocomposites were obtained through ion-crosslinking with alginate by Ca^{2+} ; their structural and morphology were observed by Fourier transform infrared (FT-IR) and scanning electron microscope (SEM), the swell ratio was evaluated in NaCl solution. Using bovine serum albumin (BSA) as a model protein drug, the encapsulation capacity and *in vitro* drug-controlled release properties of AQCOM microspheres with different amounts of OMMT were studied. The workflow is shown in Scheme 1.

Materials and methods

Materials and equipment

Chitosan was obtained from Jinan Haidebei Marine Bioengineering Co., Ltd. (Shandong, China), its degree of deacetylation was 85% and weight-average molecular weight was 2.0×10^5 . Sodium-MMT was supplied by Jianrong Mineral Refining Plant (Zhejiang, China) with cation exchange capacity (CEC) value of 87 mmol/100 g as reported by suppliers. Its 2,3-epoxypropyltrimethyl ammoniumchloride (ETA) was purchased from Dongying Guofeng Fine Chemical Co. Ltd (Shandong, China). Gemini surfactant (18–3–18, $\text{C}_{43}\text{H}_{92}\text{N}_2\text{Cl}_2$, FW: 707) was purchased from Henan Titaning Chemical Technology Co., Ltd (Henan, China). BSA with molecular weight of 68,000 was supplied by Shanghai Bio Science and Technology Co., Ltd. (Shanghai, China). Chitosan, MMT, and Gemini surfactant were used without any further purification, and all other chemicals were of analytical grade.



Scheme 1. The schematic representation of the preparation of QCMC, OMMT, QCMC/OMMT nanocomposites, and AQCOM microsphere.

A XH-100B microwave synthesis system was purchased from Beijing XiangHu Sci.–Tech. Dept. Co. Ltd (Beijing, China).

Synthesis of QCMC

QCMC was prepared by grafting carboxymethyl groups and quaternary ammonium groups on chitosan under microwave irradiation. Briefly, the carboxymethylation of chitosan was performed by using chloroacetic acid as modification agent at 800 W and 70 °C for 25 min, and then the carboxymethyl 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, QCMC was obtained by dialysis against distilled water and then lyophilization at -50°C . The weight-average molecular weight of the prepared QCMC was 9.85×10^4 which was determined by gel permeation chromatography method. The degree of substitution of carboxymethyl groups and quaternary ammonium groups were 0.69 and 0.78, respectively, which was determined by deposit-titration method.

Preparation of QCMC/OMMT nanocomposites

QCMC/OMMT nanocomposites were synthesized via the intercalation of obtained QCMC into organo-MMT (OMMT). Firstly, OMMT was prepared by the following procedure. MMT

(4.0 g) was dispersed in 250 mL distilled water to obtain clay suspension using a magnetic stirrer at 500 rpm for 24 h, and then put the clay suspension in the microwave synthesis system. Gemini surfactant (1.5 g, 0.5 CEC) was dissolved in 150 mL isopropanol aqueous solution (50%, v/v), and then dropped slowly into the clay suspension. The microwave synthesis system was operated at 800 W and 85 °C under stirring. After reacting for 1 h, the precipitate was collected by centrifugation and then washed with isopropanol aqueous solution (50%, v/v) until no chloride anion was tested by AgNO₃ solution (0.1 M). OMMT were obtained after lyophilization at −45 °C.

Subsequently, OMMT (0.2 g) was dispersed in 20 mL of distilled water by vigorous stirring for 30 min. An aliquot of obtained QCMC solution (5 mg/mL) were dropped slowly into OMMT suspension. QCMC/OMMT nanocomposites were obtained under microwave irradiation at 800 W and 80 °C for 70 min. The nanocomposites were freeze-dried at −45 °C and ground to powder. In order to control the QCMC loading in the silicate layers, the weight ratios of QCMC to OMMT were set at 0.5:1, 1:1, 2:1, 4:1, and 8:1, which were labeled as QCMC/OMMT-1, QCMC/OMMT-2, QCMC/OMMT-3, QCMC/OMMT-4, and QCMC/OMMT-5, respectively.

Preparation of AQCOM microspheres

AQCOM microspheres were prepared according to drop method with CaCl₂ as crosslinking agent. Alginate (20 mg/mL) was slowly added to QCMC/OMMT nanobiocomposite (10 mg/mL) under stirring with volume ratio of 2:1. The mixture was degassed for 1 h, and then extruded through an injector (0.45 mm diameter) into a coagulating bath containing CaCl₂ (10 mg/mL) under slightly stirring at room temperature. The as-spun microspheres were washed with distilled water, until no free chloride anion was tested by AgNO₃ solution (0.1 M). AQCOM microspheres were obtained after vacuum drying. The QCMC/OMMT-1, QCMC/OMMT-2, QCMC/OMMT-3, QCMC/OMMT-4, and QCMC/OMMT-5 nanobiocomposites were corresponded to AQCOM-1, AQCOM-2, AQCOM-3, AQCOM-4, and AQCOM-5 microspheres, respectively.

Swelling experiment

The swelling ratio (SR) of AQCOM microspheres was determined by immersing the dried microspheres in NaCl solution (0.1 M) at 5.7 pH value and 37 °C. After reaching the swelling equilibrium (24 h), AQCOM microspheres were taken out and weighed after carefully blotting with tissue paper to remove surface water. The SR (%) of AQCOM microspheres was calculated by Equation (1) [14]:

$$SR(\%) = \frac{W_t - W_d}{W_d} \times 100\% \quad (1)$$

where W_d (g) and W_t (g) represent the weight of microspheres before and after swelling experiment, respectively.

Drug loading analysis

Encapsulation efficiency (EE) of AQCOM microspheres was determined by amount of free BSA in the supernatant. Firstly, BSA-loaded microspheres were prepared via the same way to AQCOM microsphere preparation by adding 1.0 mg/mL BSA to the mixture of alginate with

QCMC/OMMT nanocomposite. After the above suspension was centrifuged for 20 min at 2000 rpm, the amount of free BSA in the CaCl_2 solution was determined by TU-1810 UV–Vis spectrophotometer (Beijing Puxi General Apparatus Ltd., Beijing, China) with the analytical wavelength of 278 nm at room temperature ($24 \pm 1^\circ\text{C}$). The EE (%) was calculated by using Equation (2) [15]:

$$EE(\%) = \frac{(D_t - D_f)}{D_t} \times 100\% \quad (2)$$

where D_t (g) represents the doses of total added BSA (g) and D_f (g) represents the doses of free BSA in the supernatant.

***In vitro* BSA release experiment**

In vitro release experiment was carried out in simulated physiological environment of gastrointestinal. Briefly, dried BSA-loaded AQCOM microspheres (0.2 g) were immersed in 100 mL HCl solution medium (0.1 M, pH=1.2) for 4 h at $37 \pm 1^\circ\text{C}$, and then transferred into phosphate buffer solutions (0.1 M, pH=7.4) for 8 h [16]. At proper time intervals, 5 mL of samples were withdrawn from the dissolution medium and immediately back after measuring the absorbency at 278 nm. The concentration of BSA was determined from different calibration curve at pH 1.2 and 7.4, respectively, and then the cumulative release percentage of BSA was calculated. Each experiment was repeated in three times and mean values were reported.

Active cutaneous anaphylaxis test

Guinea pig model was established for determination of the influence of AQCOM microsphere on active cutaneous anaphylaxis (ACA). Ten AQCOM-3 microspheres were applied to the skin of Guinea pigs (250–300 g, $n=6$), and the induce contact lasted for seven days. Then, two microspheres were used in a challenge exposure of this ACA at the seventh day. At last, the challenge exposure result was observed at the eighth day. Saline and 2,4-dinitrophenol solutions were applied as the negative control and positive control, respectively. The 0.1 and 0.01 mg/mL of 2,4-dinitrophenol solution were used in induce contact and challenge exposure, respectively. The test skin was taken photos.

Instrumental characterization

The d-spacing of the nanocomposites was investigated by using a D8 advance X-ray diffractometer (Bruker, Germany) with a Cu $K\alpha$ radiation ($\lambda=0.15418\text{ nm}$) at 40 kV and 50 mA. The relative intensity was recorded in the scattering range of 1° – 10° (2θ) at a scanning rate of $0.5^\circ/\text{min}$. The d-spacing was calculated by using Bragg's equation ($n\lambda = 2d \cdot \sin \theta$). A JEM-2010HR TEM (JEOL, Japan) was used to investigate the microstructural and surface morphology of OMMT and QCMC/OMMT-3 nanocomposite at an accelerating voltage of 200 kV. OMMT 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. QCMC/OMMT-3 nanocomposite for TEM studies was prepared by cutting from the epoxy block (UCL/FC6, LEICA, Austria) with the embedded nanocomposites sheet at room temperature.

The thermal analysis of QCMC/OMMT nanocomposites was investigated by using TGA. TGA was carried out on a TGA Q500 (TA, USA). Samples were heated from room temperature to 700°C at heating rate of $10^\circ\text{C}/\text{min}$ in high-purity flowing nitrogen atmosphere ($50\text{ mL}/\text{min}$).

FT-IR spectra were conducted on Vector 33 spectrophotometer (Bruker, Germany) under a dry air at room temperature by the KBr pellets method. The spectra were collected over the range from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} .

The surface morphology of microsphere was obtained by S-3700 N SEM (Hitachi, Japan). Samples were cryo-fractured from liquid nitrogen, and for morphological studies the beads were mounted on metal grids, using double-sided adhesive tape, and coated with gold under vacuum.

Results and discussion

Structure and morphology characterization of QCMC/OMMT nanocomposites

The prepared QCMC/OMMT nanocomposites were characterized by both XRD and TEM, which are the most frequently used and approachable methods to study the structure of nanocomposites [17].

The intercalation of surfactant or polymer in the clay interlayer was firstly confirmed by the decrease of 2θ values from XRD results [3]. Figure 1 gives the XRD patterns of MMT, OMMT, and QCMC/OMMT nanocomposites. The d_{001} basal spacing of pristine MMT was calculated to be 1.51 nm from a diffraction peak at $2\theta = 5.85^\circ$ by using Bragg's equation while the interlayer distance of OMMT was calculated to be 2.24 nm (Fig. 1(b)), it indicates that Gemini 18-3-18 has been intercalated into the silicate layers of MMT [18,19]. The surface character of MMT was changed from hydrophilic to hydrophobic, which is beneficial to further polymer intercalation [7,8,15].

Correspondingly, the d_{001} peaks of five QCMC/OMMT nanocomposites shifted towards lower angle in comparison with OMMT (Fig. 1(c)–(g)), which was an indicative of significant intercalation of QCMC into OMMT [20]. Interestingly, the degree of shift was dependent on the QCMC loading. There were more shifts with the increase of the mass ratio of QCMC to OMMT in the QCMC/OMMT nanocomposites, that is to say, the interlayer spacing of QCMC/OMMT nanocomposites was enlarged gradually with the increasing content of QCMC. When the mass ratio of QCMC to OMMT was 8:1, the largest interlayer distance of 4.16 nm was obtained. The fact can be explained that the driving force for intercalation was stronger with the increase of QCMC content, so more QCMC molecules can enter into the interlayer of clay.

Noteworthy, XRD patterns of all five QCMC/OMMT nanocomposites had two peaks. The first peak was d_{001} peak corresponding to paraffin-bilayer arrangement with tilt angle of QCMC chains. The second peak ($2\theta = 4.56^\circ$) could be either the alkyl chains assumed as paraffin-monolayer arrangement in the (002) reflection of MMT or due to the collapse of the MMT particles [21–23].

The TEM images of OMMT and QCMC/OMMT-3 nanocomposites are shown in Fig. 2 (a)–(d) which can directly exhibit the dispersion of OMMT in QCMC/OMMT nanocomposite and the intercalation of QCMC into the interlayer of OMMT. Figure 2 showed that both OMMT and QCMC-3 nanocomposites comprised a typical intercalated structure. Dark section is the cross section of MMT layers and the gray area is the polymer matrix, respectively. From TEM images with either lower magnification of Fig. 2(a) and (c) or higher magnification of Fig. 2(b) and (d), the enlarged interlayer of QCMC/OMMT-3 nanocomposite can be seen clearly. Besides, the whole OMMT in the QCMC-3 nanocomposite was found to be changed into silicate rock fragments but not exfoliated thoroughly, which is well in agreement with the XRD results.

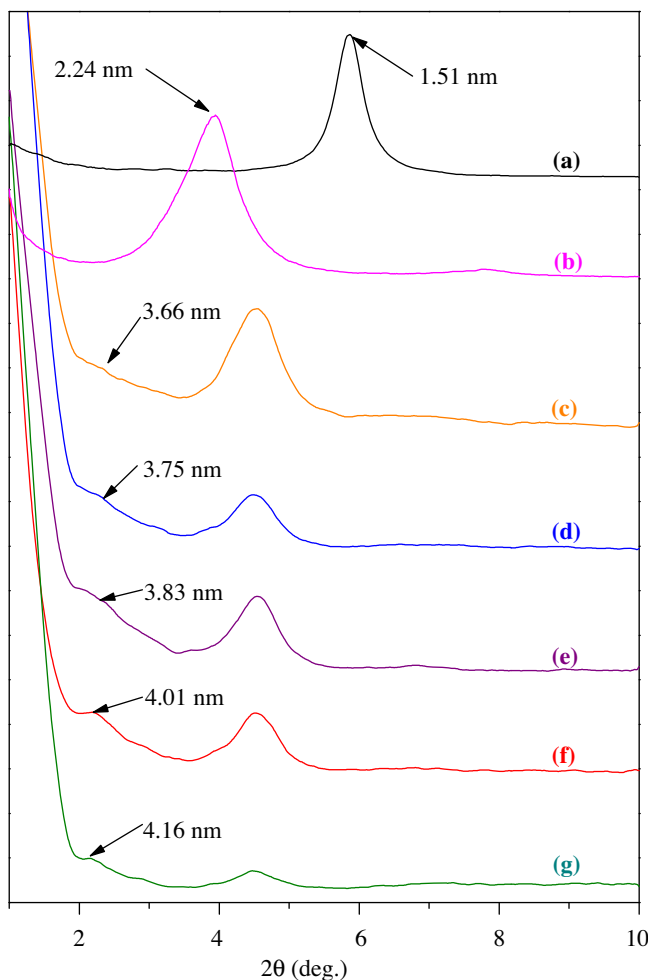


Figure 1. XRD patterns of MMT, OMMT, and QCMC/OMMT nanocomposites: (a) MMT, (b) OMMT, (c) QCMC/OMMT-1, (d) QCMC/OMMT-2, (e) QCMC/OMMT-3, (f) QCMC/OMMT-4, and (g) QCMC/OMMT-5.

On the basis of the XRD results and TEM images, the QCMC/OMMT nanocomposites have been successfully prepared through the simple solution-intercalation method under microwave irradiation. It can be explained that microwave irradiation may rapidly induce the thermal motion of QCMC molecules, the QCMC molecules were equally agitated and had the same energy to align themselves linearly under the influence of polar forces and embraced snugly to each other through hydrophobic interaction, in this case, this type of interaction made QCMC molecules enter into the layer space of OMMT [12,24].

Thermal analysis of QCMC/OMMT nanocomposites

Figure 3 shows the thermogravimetric (TG) curves of OMMT, QCMC, and QCMC/OMMT nanocomposites. Obviously, five curves of QCMC/OMMT nanocomposites are similar to that of QCMC, which exhibits two discrete weight losses corresponding to the loss of water and

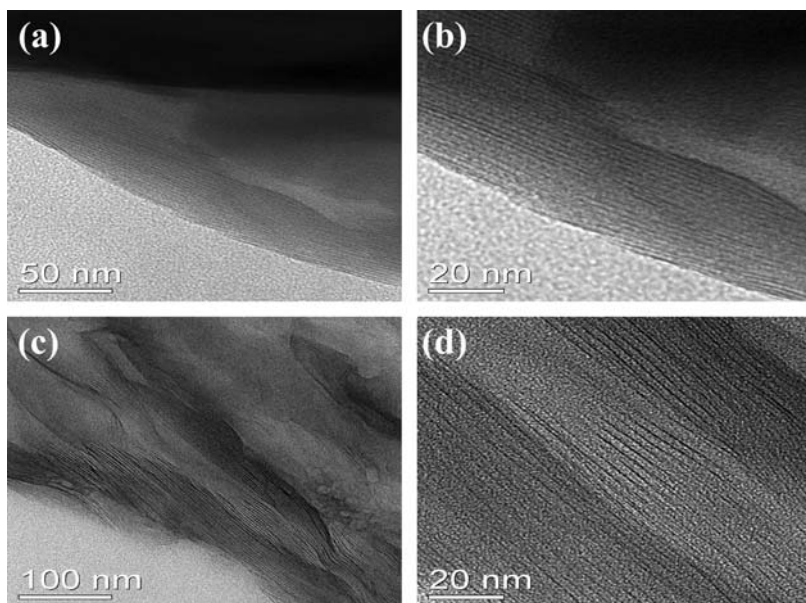


Figure 2. (a) and (b) TEM images of OMMT and (c) and (d) QCMC/OMMT-3 nanocomposite.

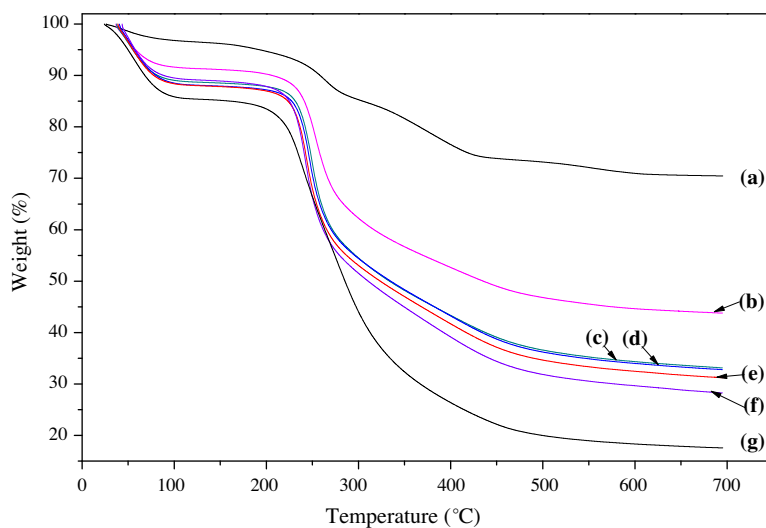


Figure 3. TG curves for OMMT, QCMC, and QCMC/OMMT nanocomposites: (a) OMMT, (b) QCMC/OMMT-1, (c) QCMC/OMMT-2, (d) QCMC/OMMT-3, (e) QCMC/OMMT-4, (f) QCMC/OMMT-5, and (g) QCMC.

the degradation of polymer chains [25]. All nanocomposites had lower mass loss than QCMC below 200 °C, which is an indicative of less free water in the nanocomposites. This fact can be explained that QCMC/OMMT nanocomposites contained less water than QCMC in the presence of clay, and their water content gradually reduced with the increase of the OMMT amount [26].

The curves of all five prepared QCMC/OMMT nanocomposites had a thermal degradation process among 200–600 °C, which were mainly due to the degradation of QCMC. The $T_{\max}$ (the temperature when the rate of weight loss reaches a maximum) of QCMC was observed to be at about 240 °C, in contrast, $T_{\max}$ of the QCMC/OMMT nanocomposites were higher, which were at 253, 250, 246, 243, and 242 °C for QCMC/OMMT-1 nanocomposite to QCMC/OMMT-5 nanocomposite, respectively. The enhancement of $T_{\max}$ may be ascribed to the QCMC lodged into the OMMT hollow, which is a good reservoir not only to protect the QCMC but also to encapsulate the degradation products, delaying the mass transport [26]. The fact primarily reveals that as the loading level of OMMT increased, the thermal stability of QCMC/OMMT nanocomposites was gradually improved.

Besides, it can be found from TG curves that the remaining weight of QCMC at 700 °C was only 18%, while those were 44, 34, 33, 31, and 28% for QCMC/OMMT-1 nanocomposite to QCMC/OMMT-5 nanocomposite, respectively. But the amount of OMMT in QCMC/OMMT nanocomposites was 68, 50, 33, 20, and 11%, respectively. So it can deduce that the remaining weight at 700 °C was not only related to the amount of OMMT in the composite, but QCMC chains in the interlayer of OMMT may retain at 700 °C, especially for QCMC/OMMT-3, QCMC/OMMT-4, and QCMC/OMMT-5. It further confirms that the thermal stability of the nanocomposites was higher than pristine QCMC [27].

Structural and morphology characterization of the AQCOM microsphere

FT-IR spectra of QCMC, OMMT, QCMC/OMMT-3 nanocomposites, alginate, and AQCOM-3 microsphere are shown in Fig. 4. Compared to OMMT and QCMC spectra, the spectrum of the QCMC/OMMT-3 nanocomposite (Fig. 4(b)) not only shows the characteristic peaks of MMT (Si–O–Si and Al–O–Si bending vibration, 470 and 524 cm^{-1}) but also exhibits the characteristic peaks of QCMC which are the asymmetrical stretching band of carboxyl groups and the vibration band of the methyl in quaternary ammonium groups [28]. The results further indicate that the QCMC was successfully inserted into OMMT.

The spectrum of AQCOM-3 microsphere included all the characteristic adsorption peaks of QCMC, OMMT, and alginate. Differently, in contrast with the FT-IR spectra of QCMC and alginate, the peak near 3400 cm^{-1} which was attached to N–H bonded and O–H of AQCOM-3 microsphere (Fig. 4(e)) became stronger and sharper and moved between that of QCMC (Fig. 4(a), 3371 cm^{-1}) and alginate (Fig. 4(d), 3423 cm^{-1}). This was a strong proof of hydrogen bonding between –OH group in alginate and –NH– and –OH groups in QCMC [15].

In the spectrum of alginate (Fig. 4(d)), the characteristic peaks were observed at 1615 and 1419 cm^{-1} , which were ascribed to the asymmetric and symmetric –COO[–] stretching vibration of the salified carboxyl group, respectively [15]. However, the two –COO[–] peaks weakened obviously after crosslinking by Ca²⁺ (Fig. 4(e)), which can be explained that the contact between alginate and Ca²⁺ in solution immediately induced ionic crosslinking with the –COONa of alginate, and then formed hydrogel beads [29]. Moreover, –COO[–] groups may electrostatically interact with –N⁺(CH₃) group in QCMC or OMMT.

In the spectra of OMMT (Fig. 4(b)) and QCMC (Fig. 4(a)), a characteristic peak at 1480 cm^{-1} was assigned to the vibration band of the methyl in quaternary ammonium groups [6], but the absorption band disappeared in spectrum of AQCOM-3 microsphere as shown in Fig. 4(e), which further suggests that the –N⁺(CH₃) groups in QCMC or OMMT electrostatically interacted with –COO[–] groups in alginate.

As a result, the conclusion can be drawn that dropwise addition of QCMC/OMMT nanocomposite and alginate intermixture into CaCl₂ solution immediately induced ionic crosslink-

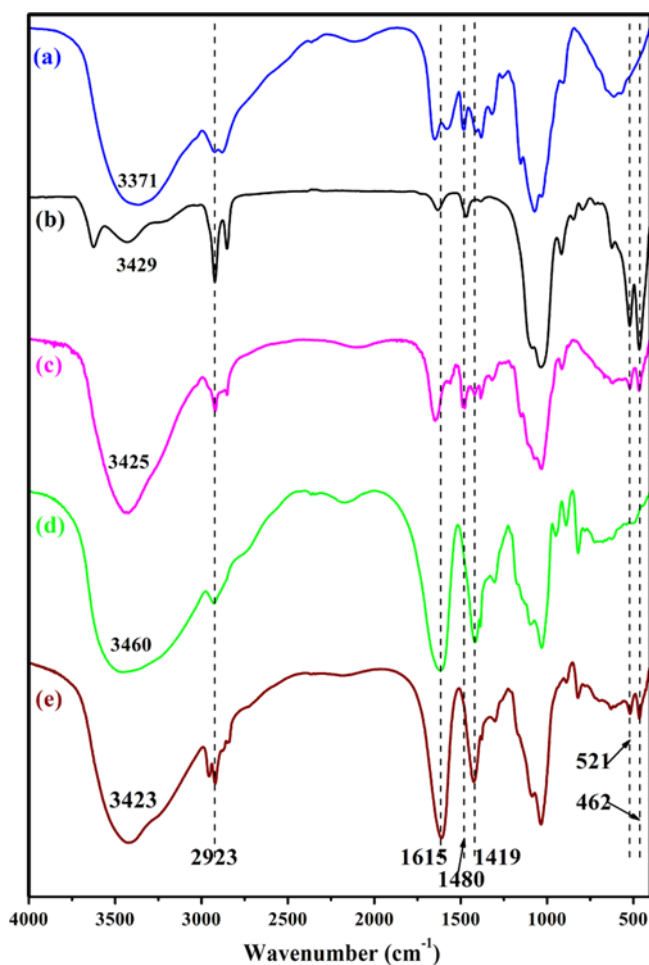


Figure 4. FT-IR spectra of (a) QCMC, (b) OMMT, (c) QCMC/OMMT-3 nanocomposite, (d) alginate, and (e) AQCOM-3 microsphere.

ing of carboxylate ions ($-\text{COO}^+$) on alginate and QCMC chains [30], thus forming cross-linked microsphere with interpenetrating three-dimensional network [16].

SEM micrographs of the dry crosslinked microsphere are shown in Fig. 5, which depicts the particle size, surface (Fig. 5(a) and (b)) and internal (Fig. 5(c) and (d)) morphology of the AQCOM-3 microsphere. Obviously, the dry AQCOM-3 microsphere was spherical in shape with a rather compact surface, and the average size was about $700\text{ }\mu\text{m}$ (Fig. 5(a)). The internal of microsphere was a loose three-dimensional network structure (Fig. 5(c)), which is in agreement with the FT-IR results. The highly microporous surface (Fig. 5(b)) may provide a high ability for the BSA release, while the poriferous structure (Fig. 5(d)) is advantage for swelling [16]. Additionally, the observation of freshly prepared AQCOM-3 microsphere (as shown in Scheme 1) indicates that wet crosslinked microsphere had good sphericity and uniform size.

Swelling behavior of AQCOM microsphere

Figure 6 shows variation of the swelling behavior (SR) for different AQCOM microspheres in 0.1 M NaCl buffer solution ($\text{pH} = 5.7$). It was found that QCMC–alginate microsphere had

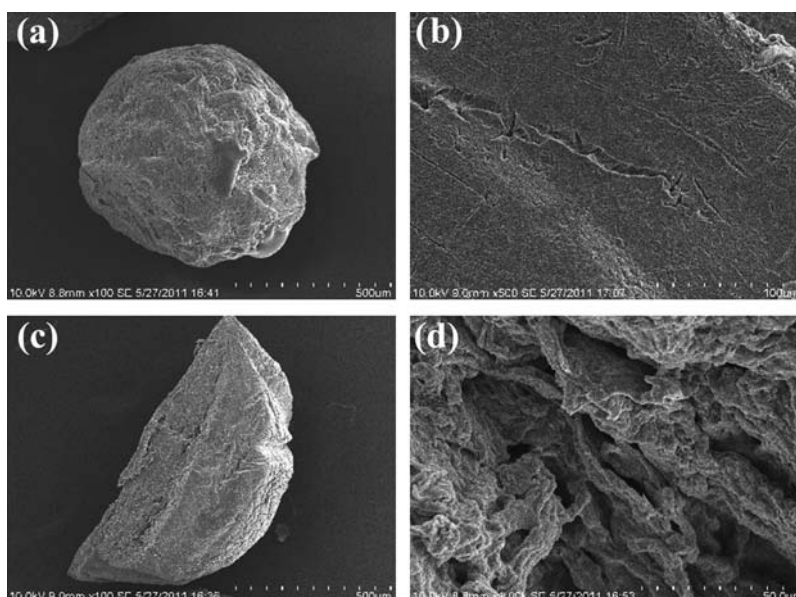


Figure 5. Surface (a) and (b) and internal (c) and (d) SEM images of AQCOM-3 microsphere.

the highest SR, and with the increase of the mass ratio of QCMC to OMMT, the SR of AQCOM microspheres was lower. This suggests that OMMT played an important effect on the swelling behavior of AQCOM microsphere. The higher content of OMMT weakened the water absorption of crosslinked AQCOM microsphere, which coincides with the TG result. These behaviors are interpreted as follows: QCMC/OMMT nanocomposite contained some hydrophilic groups in QCMC, such as carboxymethyl groups and hydroxyl groups; the hydrophilic group in the polymer structure formed an intermolecular stronger H-bond with water

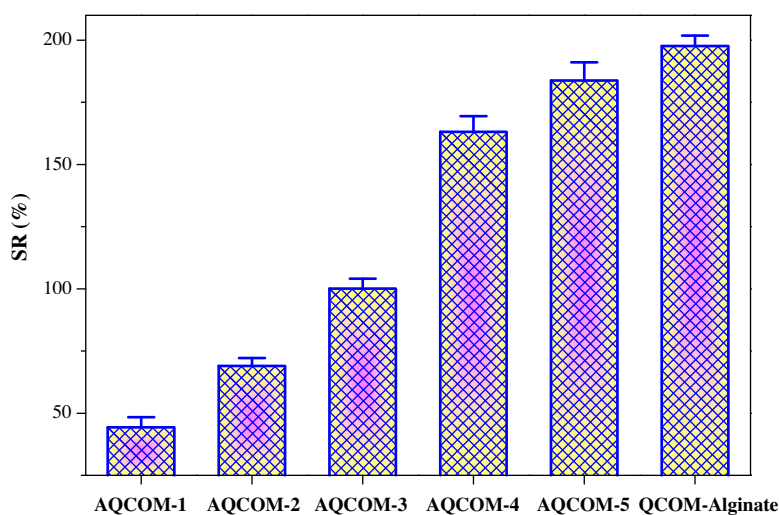


Figure 6. SR (%) of AQCOM microspheres, data shown are the mean $\pm$ standard deviation ($n = 3$).

molecules [14]. Besides, $-\text{COOH}$ groups of QCMC can dissociate to $-\text{COO}^-$ groups in 0.1 M NaCl solution, and formed the electrostatic repulsion with the $-\text{COO}^-$ groups of alginate, which increased the surface area of AQCOM microsphere and made the microsphere dramatically swell [15]. However, with the addition of OMMT consisting of some hydrophobic groups such as $-\text{CH}_2-$ and $-\text{CH}_3$, the above interaction was gradually decreased. Moreover, the decreasing SR was presumed due to interpenetrating three-dimensional network confirmed by FT-IR and SEM results.

Drug EE of AQCOM microsphere

Figure 7 shows the BSA encapsulation efficiencies of different AQCOM microspheres. The EE of QCMC–alginate microsphere (only QCMC without OMMT in the nanocomposite) was $65 \pm 6\%$, which demonstrates that QCMC and alginate played a major role in encapsulation of BSA. For AQCOM microspheres, except QCMC/OMMT-1 nanocomposite with high proportion of OMMT (67%), other QCMC/OMMT nanocomposites resulted in the increasing load capacity for BSA, and the EE improved with the increase of the interlayer distance of OMMT. AQCOM-4 and AQCOM-5, which interlayer distance was more than 4 nm, displayed more excellent encapsulation capacities. The fact reveals that both the interlayer distance and the content of OMMT had an important effect on the EE of the microspheres. The certain content of OMMT on the microspheres was helpful to load the BSA, in this case, the interaction between QCMC, OMMT, and alginate may have taken place, which formed more networks to encourage BSA loading in microspheres [8], but the large amount of OMMT resulted in the collapse of the structure [8], so that AQCOM-1 microsphere cannot load enough BSA. Because OMMT with large specific area had good adsorption capacity, and this capacity was stronger as the interlayer distance increased, so AQCOM-5 with the largest d_{001} spacing exhibited the highest EE [31,32].

Controlled release behavior of AQCOM microspheres

The *in vitro* cumulative release profiles of BSA from the loaded microspheres with different QCMC/OMMT nanocomposites are shown in Fig. 8. The release profiles appeared to have

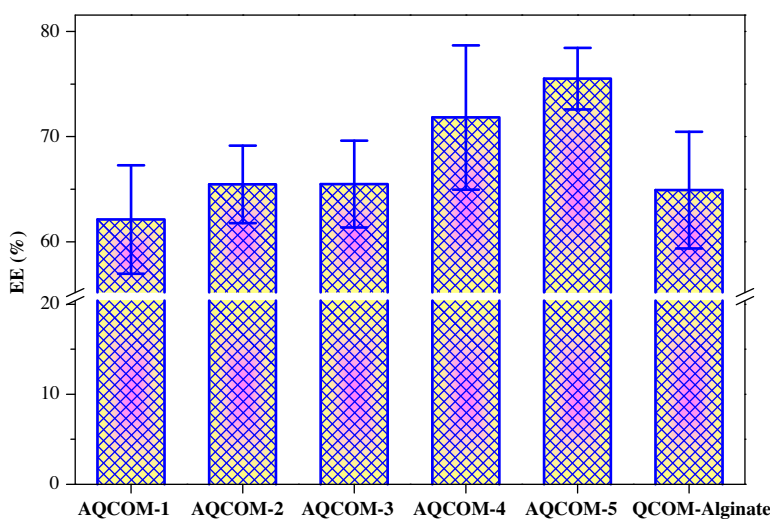


Figure 7. EE (%) of AQCOM microspheres, data shown are the mean $\pm$ standard deviation ($n = 3$).

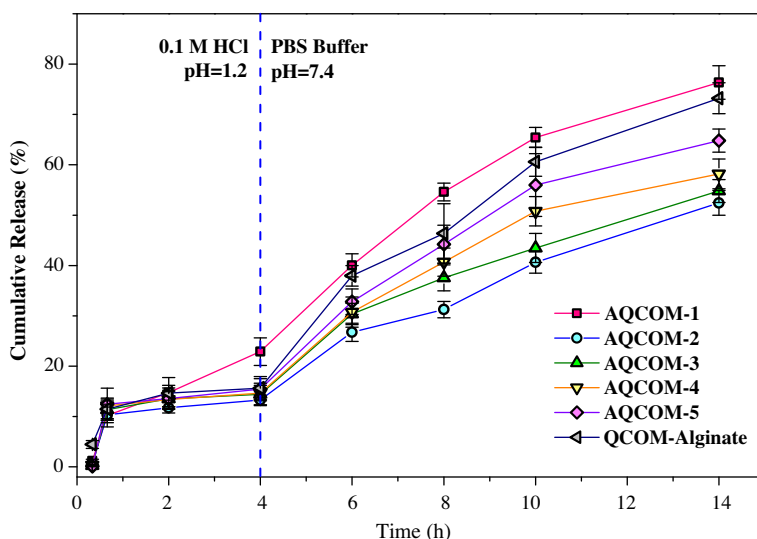


Figure 8. Cumulative release (%) of BSA from AQCOM microspheres in 0.1 M HCl (pH=1.2) and 0.1 M PBS (pH=7.4), data shown are the mean $\pm$ standard deviation ($n=3$).

two phases in acidic condition within the first 4 h. The first phase was a rapid burst release and all samples released about 12% BSA in the prior period. This rapid releasing process was mainly due to the adsorbed drugs on microspheres surface which can diffuse easily in the initial 40 min [33]. The second phase was that the loaded microspheres kept a steady state until 4 h, which implied that the obtained microsphere had a good ability to load BSA under acidic conditions, such as in the stomach. Furthermore, the release profiles also had two phases in pH 7.4 PBS buffer condition within the following 10 h. As shown in Fig. 8, another burst release was observed after the loaded microspheres were immersed into the PBS buffer at the beginning of 6 h. In the following 4 h, the crosslinked AQCOM microspheres formed a swelling-controlled and sustained release system [8]. The total BSA cumulative release QCMC–alginate microsphere and AQCOM-1 microsphere to AQCOM-5 microsphere was 73 ± 3 , 76 ± 3 , 52 ± 2 , 55 ± 2 , 58 ± 3 , and $65 \pm 2\%$, respectively. Obviously, the AQCOM microspheres, except the AQCOM-1, had better controlled release behavior than QCMC–alginate microsphere. The cumulative releases from AQCOM-5 to AQCOM-2 microspheres decreased gradually, so the conclusion can be drawn that the presence of OMMT had the positive effect on the drug-controlled release property of the microsphere, but too high content of OMMT in AQCOM-1 microsphere induced the reduction of this property.

Result from FT-IR confirmed that there was a strong network structure between the QCMC and alginate which can create tortuous paths. Therefore, it is suggesting that due to concentration gradient, free volume spaces of tortuous paths within microsphere assisted the transportation of BSA molecules to the surface of microsphere, thereby releasing into the dissolution medium. With the suitable increase of OMMT, the stronger network was formed, free volume spaces of tortuous paths were smaller, which prevented the BSA release gradually. But the large amount of OMMT resulted in the collapse of the structure [31].

Furthermore, it is clear that the cumulative release of AQCOM microspheres except AQCOM-1 was accordance with their swelling behavior, which decreased with the increase of OMMT content. The low SR can impulse a great number of BSA molecules to diffuse out from the crosslinked microsphere and pass into the release medium through numerous pores

and channels in the microspheres [34], so the lower SR induced more BSA cumulative release, this can further explain why AQCOM microspheres displayed the increased drug-controlled release ability according to their swelling behavior.

In addition, as mentioned above, MMT with large specific area possessed good adsorption capacity; accordingly it can absorb BSA well, and with the appropriate increase of the clay content, magnified specific area was obtained and this capacity became stronger [32], thus AQCOM-2 microsphere (a half of OMMT in QCMC/OMMT nanocomposite) had the lowest cumulative release.

Therefore, the order of BSA cumulative release from AQCOM microspheres was AQCOM-2 < AQCOM-3 < AQCOM-4 < AQCOM-5 < AQCOM-1.

In addition, in our experiment, after release of BSA, the AQCOM microsphere remained intact. As mentioned in FT-IR and SEM results, AQCOM microspheres were crosslinked microsphere with interpenetrating three-dimensional network, there were hydrogen bonding between $-OH$ group in alginate and $-NH-$ and $-OH$ groups in QCMC, and the $-N^+(CH_3)$ groups in QCMC or OMMT electrostatically interacted with $-COO^-$ groups in alginate. Therefore, the nanocomposite has not decomposed when BSA was released, and it is promising for the application of AQCOM microspheres.

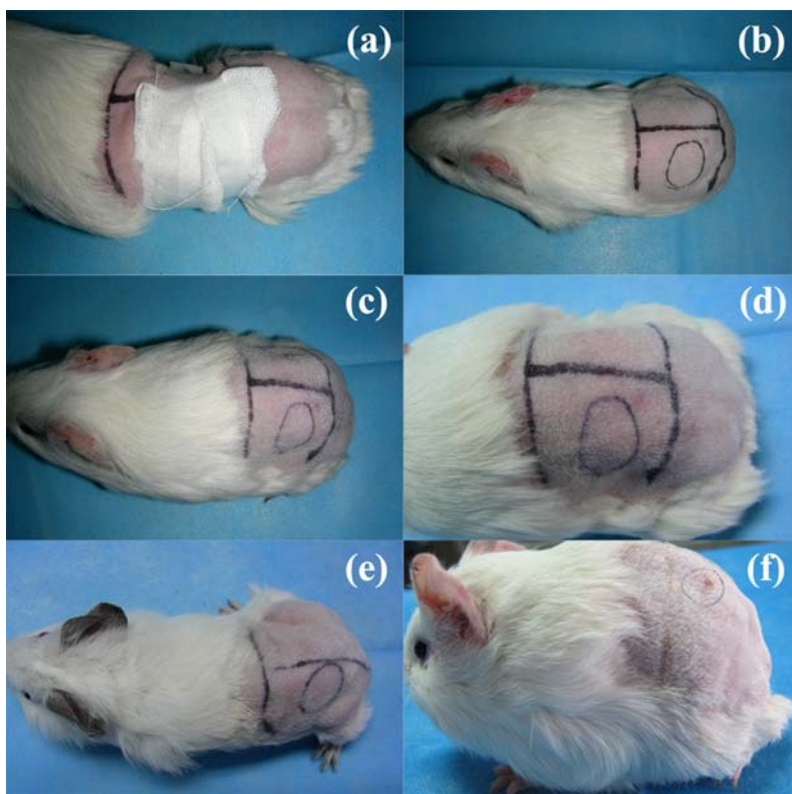


Figure 9. Photos of Guinea pigs by ACA test: (a) experimental procedure, (b) the first day after treating by AQCOM-3 microsphere, (c) the seventh day after treating by AQCOM-3 microsphere, (d) the eighth day after treating by AQCOM-3 microsphere, (e) the eighth day after negative control, and (f) the eighth day after positive control.

ACA test

The ACA of AQCOM-3 microsphere was tested by using Guinea pig model. Figure 9 gives the photos of Guinea pigs after ACA test. Significant anaphylaxis induced by 2,4-dinitrophenol was observed, such as swelling and even ulcerated, as shown in Figure 9(f). Interestingly, the induce contact and even challenge exposure on Guinea pig treated by AQCOM-3 microsphere did not induce the anaphylaxis (Fig. 9(b)–(d)), which demonstrates that the AQCOM microsphere did not cause anaphylaxis.

Conclusions

Natural drug delivery system of AQCOM microspheres based on QCMC/OMMT nanocomposite and alginate was produced by Ca^{2+} crosslinking method. Firstly, QCMC molecules entered into the layer space of OMMT under microwave radiation and QCMC/OMMT nanocomposites with higher thermal stability were obtained. Then AQCOM microspheres with three-dimensional network were prepared because carboxyl groups on alginate and QCMC chains crosslinked with Ca^{2+} , and quaternary ammonium groups in QCMC or OMMT electrostatically interacted with carboxylate groups in alginate, the SRs of AQCOM microspheres were lower than that of QCMC–alginate microsphere because of the addition of OMMT, which is in agreement with TG results. Except QCMC/OMMT-1 nanocomposite with high proportion of OMMT, other QCMC/OMMT nanocomposites resulted in the increasing load capacity for BSA, because the larger specific area of OMMT was obtained with the increase of the interlayer distance. Furthermore, *in vitro* release results showed that with the increase of OMMT content in AQCOM microspheres, the stronger network structure between QCMC and alginate and the lower SR were obtained, which induced better controlled release behavior. Besides, *in vitro* ACA test results showed that AQCOM microsphere did not cause anaphylaxis. Therefore, QCMC based clay nanocomposite may be used as potential natural carrier for drug delivery.

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References

- [1] European Food Safety Authority (EFSA). Scientific opinion on the safety and efficacy of bentonite (dioctahedral montmorillonite) as feed additive for all species. EFSA Journal. 2011;9:2007–30.
- [2] Pavlidou S, Papaspyrides CD. A review on polymer-layered silicate nanocomposites. Progress in Polymer Science. 2008;33(12):1119–98.
- [3] Darder M, Colilla M, Ruiz-Hitzky E. Biopolymer-clay nanocomposites based on chitosan intercalated in montmorillonite. Chemistry of Materials. 2003;15(20):3774–80.
- [4] Kean T, Thanou M. Biodegradation, biodistribution and toxicity of chitosan. Advanced Drug Delivery Reviews. 2010;62(1):3–11.
- [5] Katti KS, Katti DR, Dash R. Synthesis and characterization of a novel chitosan/montmorillonite/hydroxyapatite nanocomposite for bone tissue engineering. Biomedical Materials. 2008;3(3).

- [6] Sun LP, Du YM, Fan LH, Chen X, Yang JH. Preparation, characterization and antimicrobial activity of quaternized carboxymethyl chitosan and application as pulp-cap. *Polymer*. 2006;47(6):1796–804.
- [7] Dong Z, Wang Q, Du Y. Alginate/gelatin blend films and their properties for drug controlled release. *Journal of Membrane Science*. 2006;280(1–2):37–44.
- [8] Fang YY, Wang LJ, Li D, Li BZ, Bhandari B, Chen XD, Mao ZH. Preparation of crosslinked starch microspheres and their drug loading and releasing properties. *Carbohydrate Polymers*. 2008;74(3):379–84.
- [9] Yang ZH, Huang J, Zeng GM, Ruan M, Zhou CS, Li L, Rong ZG. Optimization of flocculation conditions for kaolin suspension using the composite flocculant of MBFGA1 and PAC by response surface methodology. *Bioresource Technology*. 2009;100(18):4233–9.
- [10] Liang XF, Wang HJ, Luo H, Tian H, Zhang BB, Hao LJ, Teng JI, Chang J. Characterization of novel multifunctional cationic polymeric liposomes formed from octadecyl quaternized carboxymethyl chitosan/cholesterol and drug encapsulation. *Langmuir*. 2008;24(14):7147–53.
- [11] Sirousazar M, Kokabi M, Hassan ZM. In vivo and cytotoxic assays of a poly(vinyl alcohol)/clay nanocomposite hydrogel wound dressing. *Journal of Biomaterials Science, Polymer Edition*. 2011;22(8):1023–33.
- [12] Park JH, Jana SC. Mechanism of exfoliation of nanoclay particles in epoxy-clay nanocomposites. *Macromolecules*. 2003;36(8):2758–68.
- [13] Watthanaphanit A, Supaphol P, Tamura H, Tokura S, Rujiravanit R. Wet-spun alginate/chitosan whiskers nanocomposite fibers: preparation, characterization and release characteristic of the whiskers. *Carbohydrate Polymers*. 2010;79(3):738–46.
- [14] Mi FL, Tan YC, Liang HC, Huang RN, Sung HW. In vitro evaluation of a chitosan membrane cross-linked with genipin. *Journal of Biomaterials Science, Polymer Edition*. 2001;12(8):835–50.
- [15] Wang Q, Zhang J, Wang A. Preparation and characterization of a novel pH-sensitive chitosan-g-poly (acrylic acid)/attapulgit/sodium alginate composite hydrogel bead for controlled release of diclofenac sodium. *Carbohydrate Polymers*. 2009;78(4):731–7.
- [16] Tavakol M, Vasheghani-Farahani E, Dolatabadi-Farahani T, Hashemi-Najafabadi S. Sulfasalazine release from alginate-N,O-carboxymethyl chitosan gel beads coated by chitosan. *Carbohydrate Polymers*. 2009;77(2):326–30.
- [17] Zia KM, Zuber M, Barikani M, Jabbar A, Khosa MK. XRD pattern of chitin based polyurethane bio-nanocomposites. *Carbohydrate Polymers*. 2010;80(2):539–43.
- [18] Xi YF, Frost RL, He HP. Modification of the surfaces of Wyoming montmorillonite by the cationic surfactants alkyl trimethyl, dialkyl dimethyl, and trialkyl methyl ammonium bromides. *Journal of Colloid and Interface Science*. 2007;305(1):150–8.
- [19] Zheng Q, Xu B, Song YH, Yang HM, Pan Y. Interlayer structure of organically modified montmorillonites: effect of surfactant loading. *Journal of Materials Research*. 2005;20(2):357–63.
- [20] Rhim JW, Hong SI, Park HM, Ng PKW. Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *Journal of Agriculture and Food Chemistry*. 2006;54(16):5814–22.
- [21] Gianni AD, Amerio E, Monticelli O, Bongiovanni R. Preparation of polymer/clay mineral nanocomposites via dispersion of silylated montmorillonite in a UV curable epoxy matrix. *Applied Clay Science*. 2008;42(1–2):116–24.
- [22] Zhao F, Bao XJ, McLauchlin AR, Gu JJ, Wan CY, Kandasubramanian B. Effect of POSS on morphology and mechanical properties of polyamide 12/montmorillonite nanocomposites. *Applied Clay Science*. 2010;47(3–4):249–56.
- [23] Hashemifard SA, Ismail AF, Matsuura T. Effects of montmorillonite nano-clay fillers on PEI mixed matrix membrane for CO₂ removal. *Chemical Engineering Journal*. 2011;170(1):316–25.

- [24] Kabiri K, Mirzadeh H, Zohuriaan-Mehr MJ. Chitosan modified MMT-poly(AMPS) nanocomposite hydrogel: heating effect on swelling and rheological behavior. *Journal of Applied Polymer Science*. 2010;116(5):2548–56.
- [25] Luo JW, Wang XY, Xia B, Wu J. Preparation and characterization of quaternized chitosan under microwave irradiation. *Journal of Macromolecular Science, Pure*. 2010;47(9):952–6.
- [26] Cavallaro G, Lazzara G, Milioto S. Dispersions of nanoclays of different shapes into aqueous and solid biopolymeric matrices. Extended physicochemical study. *Langmuir*. 2011;27(3):1158–67.
- [27] Wang ZF, Wang B, Qi N, Zhang HF, Zhang LQ. Influence of fillers on free volume and gas barrier properties in styrene-butadiene rubber studied by positrons. *Polymer*. 2005;46(3):719–24.
- [28] Wang XY, Liu B, Ren JL, Liu CF, Wang XH, Wu J, Sun RC. Preparation and characterization of new quaternized carboxymethyl chitosan/rectorite nanocomposite. *Composites Science and Technology*. 2010;70(7):1161–7.
- [29] Gonzalez-Rodriguez ML, Holgado MA, Sanchez-Lafuente C, Rabasco AM, Fini A. Alginate/chitosan particulate systems for sodium diclofenac release. *International Journal of Pharmaceutics*. 2002;232(1–2):225–34.
- [30] Wang J, Yang F, Tan JJ, Liu GP, Xu J, Sun DJ. Pickering emulsions stabilized by a lipophilic surfactant and hydrophilic platelike particles. *Langmuir*. 2010;26(8):5397–404.
- [31] Wang XY, Liu B, Wang XH, Sun RC. Amphoteric polymer-clay nanocomposites with drug-controlled release property. *Current Nanoscience*. 2011;7(2):183–90.
- [32] Wang XY, Tang YF, Li Y, Zhu Z, Du YM. The rheological behaviour and drug-delivery property of chitosan/rectorite nanocomposites. *Journal of Biomaterials Science, Polymer Edition*. 2010;21(2):171–84.
- [33] Ji J, Hao S, Wu D, Huang R, Xu Y. Preparation, characterization and in vitro release of chitosan nanoparticles loaded with gentamicin and salicylic acid. *Carbohydrate Polymers*. 2011;85(4):803–8.
- [34] Liu KH, Liu TY, Chen SY, Liu DM. Drug release behavior of chitosan-montmorillonite nanocomposite hydrogels following electrostimulation. *Acta Biomaterialia*. 2008;4(4):1038–45.