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Preparation, characterization and properties of aminoethyl chitin hydrogels

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ABSTRACT

Aminoethyl chitins (AEC) with different amino contents were synthesized from chitin and 2-chlorethylamine hydrochloride, and the AEC hydrogels were prepared by crosslinking with glutaraldehyde. The microstructures, swelling behaviors and antibacterial activities of the hydrogels were investigated. The results of Fourier transform infrared spectroscopy (FTIR), ^{1}H nuclear magnetic resonance (^{1}H NMR) spectrum and scanning electron microscopy (SEM) showed that the hydrogels were prepared by forming the Schiff base from AEC and glutaraldehyde. The aminoethyl chitin hydrogels were sensitive to acidic environment. The swelling ratio changed with the amino content of AEC, declined with the increase of the crosslinking agent concentration and increased with the increase of the AEC concentration. In addition, the antibacterial results of the hydrogels against *Staphylococcus aureus* (*S. aureus*) indicated that the hydrogels had good antibacterial activities, and the antibacterial properties were affected by the amino content of AEC and the crosslinking agent concentration.

1. Introduction

Environment sensitive hydrogels are smart or intelligent hydrogels that can alter their volumes and properties in response to external stimulations, and they have attracted extensive research attentions in recent years (Lee et al., 2008; Qiu & Park, 2001; Wu et al., 2008). They have hydrophilic polymer network structures that may uptake water from 10% to 20% (an approximately lower limit) up to thousands of times their dry weight in water (Arndt, Knörgen, Richter, & Schmidt, 2008; Hoffman, 2002). The network structures of polymer hydrogels can also be classified as chemical and physical ones by covalent bonds and inter-molecular forces that have formed in the preparation of the hydrogels (Bhattarai, Gunn, & Zhang, 2010; Hoare & Kohane, 2008). Some hydrogels have good biocompatibilities and excellent physicochemical properties (Lee & Mooney, 2001). Among these hydrogels, pH sensitive hydrogels are frequently used to develop the drug controlled-release systems for oral administration (Velasco, Elvira, & Román, 2008).

Chitin, poly(β-(1–4)-*N*-acetyl-D-glucosamine), is the second most important natural polymer in the world, next to cellulose. As a result of intra- and inter-molecular hydrogen bonds, as well as the regularity and rigidity of the crystal structure, the non-solubility of chitin in almost all common solvents has been a stumbling block in its appropriate utilization (Prashanth & Tharanathan, 2007). Therefore, the modifications of chitin derivatives have been used to enlarge their applications. Due to their biocompatibility, biodegradability, and non-toxicity, together with their antimicrobial activity and low immunogenicity, chitin and its derivatives are known as immense potential materials widely applied in biomedical and other industrial areas (Crini & Badot, 2008; Je & Kim, 2006; Pillai, Paul, & Sharma, 2009; Yang, Cai, Hu, Li, & Du, 2012). Especially the researches on the applications of their antibacterial activities for the agriculture, food, medicine, cosmetics, tissue engineering, biomaterials and drug controlled release systems have been broadly reported in the past decade (Costa-Júnior, Barbosa-Stancioli, Mansur, Vasconcelos, & Mansur, 2009; Kim et al., 2007; Liang, Liu, Huang, & Yam, 2009; Yang & Su, 2011). Although the exact antibacterial mechanisms of the chitin derivatives are still debated, the disruption of the cell membrane appears to be an acceptable mode of action. The interactions between positively charged polysaccharide molecules and negatively charged bacterial cell membranes lead to the leakage of the proteinaceous and other intracellular constituents, which cause the deformations of the cell structures (Jung, Kim, Choi, Lee, & Kim, 1999; Lou et al., 2011; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). However, the probable antibacterial mechanism of the hydrogel is different from that of the antibacterial agents. The surface roughness of the hydrogel can lead to the physical effect of bacterial adhesion (Tsao et al., 2010).

In this study, the aminoethyl chitins (AEC) with different amino contents were synthesized by grafting 2-chlorethylamine hydrochloride to the chitin molecule, improving the solubility of the chitin. In addition, the aminoethyl chitin pH-sensitive hydrogels were prepared by crosslinking with glutaraldehyde, and the

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swelling properties and antibacterial activities of the hydrogels against *Staphylococcus aureus* (*S. aureus*) were also investigated.

2. Experimental

2.1. Materials

Chitin was purchased from Golden-shell Biochemical Corp. Ltd. (Zhejiang, China). 2-Chlorethylamine hydrochloride was purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Peptone, beef extracts and agar were biological reagents. And all other solvents and reagents were of analytical grade, obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), and were used without further purification. *S. aureus* was provided by State Key Laboratory of Agriculture Microbiology, Huazhong Agricultural University, China.

2.2. Synthesis of aminoethyl chitin

Chitin (2 g) suspended in NaOH solution was kept at −20 °C for 12 h. Then the alkalized chitin was dispersed in isopropanol, and 4.5 g of 2-chlorethylamine hydrochloride was added into the reaction system in portions in 1 h. After that, the mixture was stirred at room temperature overnight (Je & Kim, 2006; Ngo, Qian, Je, Kim, & Kim, 2008). After dialysizing against deionized water, the product was vacuum dried at room temperature. The aminoethyl chitins with different amino contents were obtained by altering the starting amount of the 2-chlorethylamine hydrochloride.

The potentiometric titration method was used to determine the amino content (AC) of the aminoethyl chitin. It was dissolved in 40 mL 0.1 mol/L HCl solution. Then 0.1 mol/L NaOH solution was dropping into it for titration. Then the amino content (AC) was calculated by using the formula below:

$$\mathrm{AC}(\%) = \frac{V \times C/1000 \times M}{W} \times 100\% \quad (1)$$

where V(mL) is the volume of NaOH solution, C(mol/L) is the concentration of NaOH solution, M is the molar mass of the amino group, and W(g) is the weight of the sample used in the potentiometric titration.

The results indicated that the amino content of aminoethyl chitin ranged from 1.5% to 7.4%.

2.3. Preparation of aminoethyl chitin hydrogels

The aminoethyl chitin was dissolved in 5% (W/V) acetic acid to obtain 4% (W/V) solution. Then the glutaraldehyde (2%, W/V) was added into the AEC solution and stirred rapidly. The mixture was placed in a beaker at 25 °C for 24 h. Until the hydrogel was formed, the hydrogel was cut into the cylinders by using a punch, with a diameter of about 5 mm and a height of about 5 mm. Then the hydrogels were washed with deionized water for several times, and dried in a vacuum desiccator to constant weights at room temperature. And by changing the aminoethyl chitins with different amino contents, glutaraldehyde concentrations and aminoethyl chitin concentrations, a series of AEC hydrogels with different microstructures were prepared.

2.4. Characterization of aminoethyl chitin and hydrogels

Fourier transform infrared (FTIR) spectra were used to characterize the AEC and the hydrogels by using an instrument (Avator 360, Nicolet, MA, USA). Aminoethyl chitin and the hydrogels were dried under vacuum freeze drying condition and prepared as KBr pellet and scanned against a blank KBr pellet background at range 500–4000 cm^{-1}.

The ^{1}H nuclear magnetic resonance (^{1}H NMR) spectrum was recorded with a NMR spectrometer (Varian, USA) at 400 MHz. Deuterated hydrochloride was used as the solvent for dissolved AEC sample. Chemical shifts were given in ppm using tetramethylsilane (TMS) as an internal reference.

The interior morphology of the hydrogel was observed by scanning electron microscopy (SEM). The hydrogel sample used for SEM characterization was dried under vacuum freeze drying condition. For magnification, a JSM-5610LV (JEOL, Japan) scanning electron microscope was operated at 20 kV acceleration voltages. Prior to imaging, the dried AEC hydrogel was coated with gold–palladium in a JFC-1600 sputter coater (JEOL, Japan).

2.5. Estimation of swelling properties

The swelling ratio (SR) of the hydrogel was measured by weighing method. The hydrogel was immersed completely in pH 1.0 and 7.4 buffer solutions, and weighed at intervals after absorbing the surface moisture with a filter paper. Then the SR was calculated by using the equation below:

$$\mathrm{SR}(\%) = \frac{W_s - W_d}{W_d} \times 100\% \quad (2)$$

where W_d is the initial weight of dry gel and W_s is the weight of the gel swollen in the buffer solution.

2.6. Determination of antibacterial activity

S. aureus, a Gram-positive bacterium commonly found on the human body, was chosen as the tested bacterium (Ye et al., 2005). The bacteria were grown in nutrient agar. Single representative microbe colony was picked off with a wire loop, placed in sterile nutrient broth (peptone 10 g, beef extracts 5 g, NaCl 10 g in deionized water 1000 mL, pH 7.0–7.2) and incubated at 37 °C overnight. The culture was diluted to a suitable concentration before using for the antimicrobial tests (Liu, Guan, Yang, Li, & Yao, 2001).

The optical density method was used for determining the antibacterial effect of AEC against *S. aureus*. 1 mL of the culture was inoculated to the medium (24 mL) containing the AEC hydrogels, the bacteria concentration of which was about 10^6–10^7 CFU/mL, and then incubated in a shaking bed (150 rpm) at 37 °C for 24 h (Li, Zhuang, Liu, Guan, & Yao, 2002; Zhao et al., 2003). During the incubation, the turbidity of the medium was measured at 600 nm with an UV spectrophotometer (SPSIC 722N, Shanghai, China) at 8 h, 16 h and 24 h respectively, and the formula for calculating the inhibitory ratio (IR) was the following:

$$IR(\%) = 1 - \frac{A_{AEC} - A_{AEC0}}{A_{medium} - A_{medium0}} \times 100\% \quad (3)$$

where A_{AEC} is the absorbance of the bacteria medium with the AEC hydrogel after incubated, A_{AEC0} is the absorbance of the bacteria medium with the AEC hydrogel before incubated, A_{medium} is the absorbance of the bacteria medium after incubated, and $A_{medium0}$ is the absorbance of the bacteria medium before incubated (Liu, Bao, Du, Zhou, & Kennedy, 2006; Rurián-Henares & Morales, 2008).

3. Results and discussion

3.1. FTIR of aminoethyl chitin and hydrogels

FTIR spectrum of the aminoethyl chitin is presented in Fig. 1a. The spectrum showed intense bands around 3440 cm^{-1} corresponding to —OH and —NH_2. The absorptions at 2962 cm^{-1} and 2900 cm^{-1} were for stretching vibration of —CH_3 and —CH_2. The characteristic absorptions for stretching vibration of C=O (amide I band) appeared at 1656 cm^{-1}, and 1560 cm^{-1} and 1317 cm^{-1} were

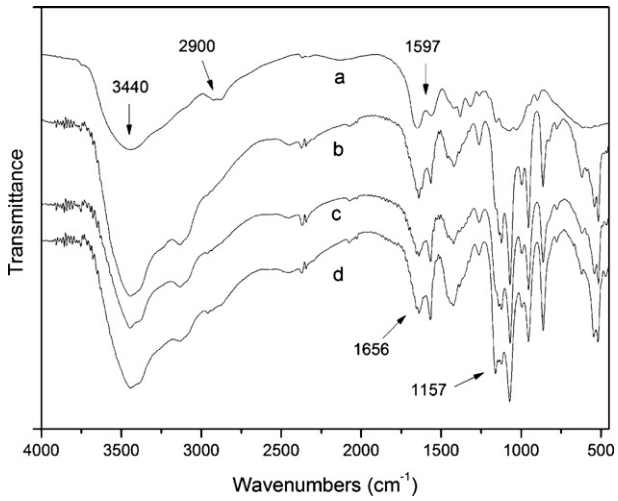

Fig. 1. FTIR spectra of (a) aminoethyl chitin, (b) the dried gel, (c) the dried gel after swelling in pH 1.0 buffer solution for 12 h and (d) the dried gel after swelling in pH 7.4 buffer solution for 12 h.

assigned to bending vibration of N—H (amide II band) and stretching absorption of C—N (amide III band) respectively (Brunner et al., 2009; Tian, Liu, Hu, & Zhao, 2004). The peak appeared at 1597 cm^{-1} attributed to the characteristic absorption of amino group, which indicated that amino group was introduced into the molecule of chitin successfully. And the peaks at 1157 cm^{-1} and 894 cm^{-1} for the characteristic peaks of β-(1–4)-glucoside bond in AEC were both shown in Fig. 1a, which demonstrated that the synthesis of AEC did not destroy the structure of glucoside molecule.

FTIR spectra of the dried gels after swelling in the buffer solutions of different pH are presented in Fig. 1b–d respectively. The chemical crosslinking of AEC and glutaraldehyde was expected to occur at the amino site and form the structure of Schiff base (C=N). The characteristic peaks for amide bonds in chitin (amide I) and the characteristic band for the Schiff base were both located at about 1655 cm^{-1} (Dragan & Perju, 2010). After gelation by using the glutaraldehyde, the peak at 1656 cm^{-1} increased, which could indicate that the aminoethyl chitin had reacted with the glutaraldehyde successfully to form the hydrogel. When the hydrogel swelled at pH 1.0, the characteristic peak of dried gel appeared at 1548 cm^{-1}, which illustrated the presence of $-NH_3^+$ groups. While in the spectrum of the dried gel after swelled at pH 7.4, the characteristic peak of $-NH_3^+$ at 1548 cm^{-1} diminished, indicating the transition of $-NH_3^+$ to $-NH_2$ (Wang, Turhan, & Gunasekaran, 2004).

3.2. ^1H NMR of aminoethyl chitin

Fig. 2 shows the ^1H NMR spectrum (400 MHz) for AEC in deuterated hydrochloride solvent. It was known that the signals at δ 3.00–4.00 ppm were assigned to the H2–H6 protons of chitin. As shown from the figure, the typical signals of chitin saccharide units at 3.00–4.00 ppm (H2, H3, H4, H5 and H6) did not change significantly after the reaction, and the new peak at 2.85 ppm represented the protons of the methylene, which confirmed the substitution of the aminoethyl group (Je & Kim, 2006).

3.3. Morphology study of aminoethyl chitin hydrogel

In order to investigate the interior microstructure of the AEC hydrogel, scanning electron microscopy (SEM) measurement was performed. As shown in Fig. 3, the hydrogel sample exhibited obvious porous structure. The interior was filled with interconnected circular of elliptical macropores, which illustrated that high water content of the hydrogel probably resulted in highly three-dimensional macroporous network frame after freeze drying (Zhao et al., 2009; Zhou, Ma, Shi, Yang, & Nie, 2011).

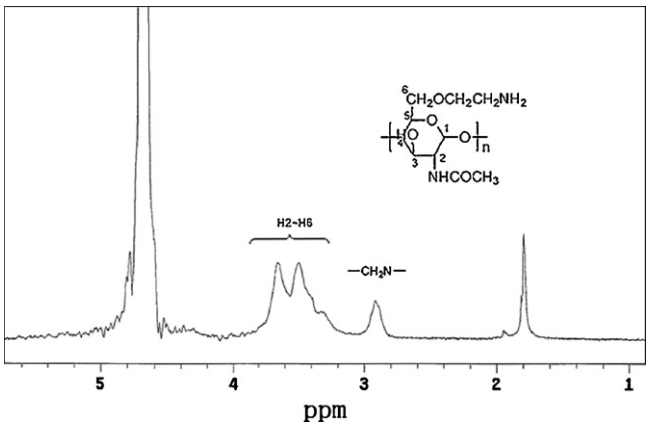

Fig. 2. ^1H NMR spectrum of aminoethyl chitin.

3.4. Swelling properties of aminoethyl chitin hydrogels

The swelling behavior is a very important property that describes the volume transition of the environment sensitive hydrogel network. The swelling behaviors of ionic pH sensitive hydrogels are mainly decided by the degree of ionization, crosslinking density and amount of the sensitive group. Therefore, the swelling tests of the aminoethyl chitin hydrogels were conducted with different amino contents of the AEC, crosslinking agent concentrations and AEC concentrations.

3.4.1. Effect of amino content of aminoethyl chitin

Effect of amino content of AEC on swelling kinetics of the hydrogels in pH 1.0 and pH 7.4 buffer solutions at 37 °C are shown in Fig. 4. We could see that the swelling properties of the hydrogels were better at pH 1.0 compared with that at pH 7.4. In the acidic environment, there was more H^+ to make the formation of ammonium, which led to the osmotic pressure and charge repulsive force in the crosslinked network of the hydrogels to make them swell, as well as made the polymer chain in networks stretch leading to contact with solvent molecule more sufficiently. Therefore, the swelling ratio was significantly higher in acidic buffer solution than in alkaline one, which could illustrate that the aminoethyl chitin hydrogels had good pH-sensitive properties.

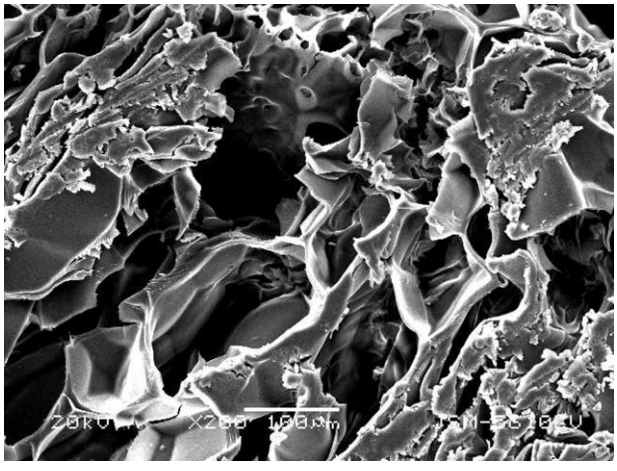

Fig. 3. The interior SEM photograph of the AEC hydrogel.

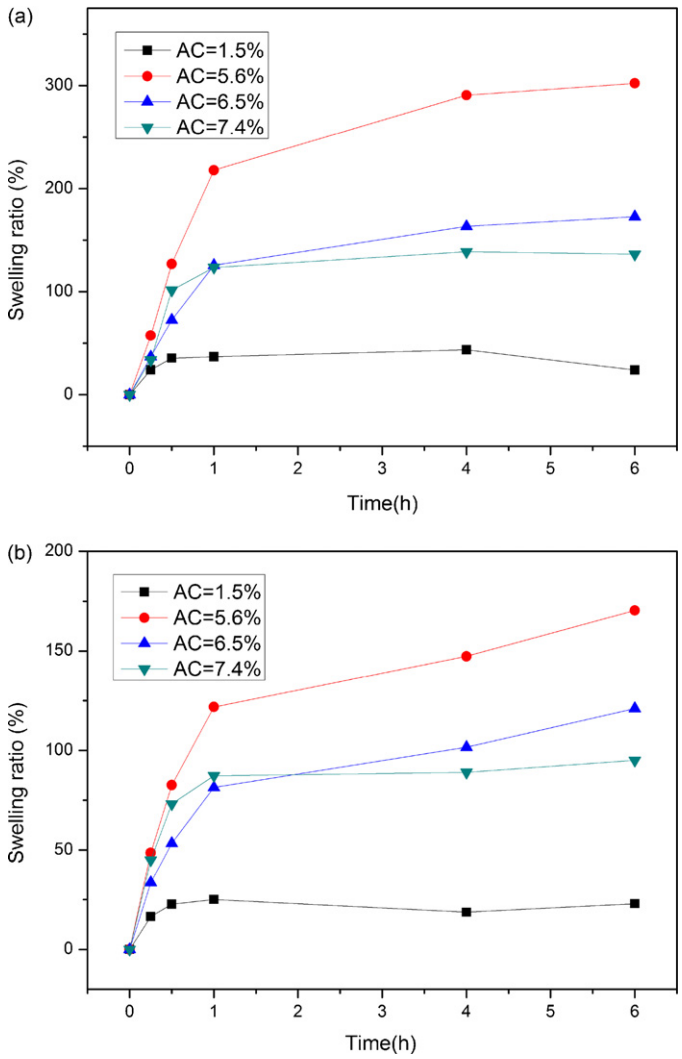

Fig. 4. The effect of the amino content of AEC on the swelling kinetics of the hydrogels (AEC concentration = 4%, crosslinking agent concentration = 2%) in (a) pH 1.0 and (b) pH 7.4 buffer solutions at 37 °C.

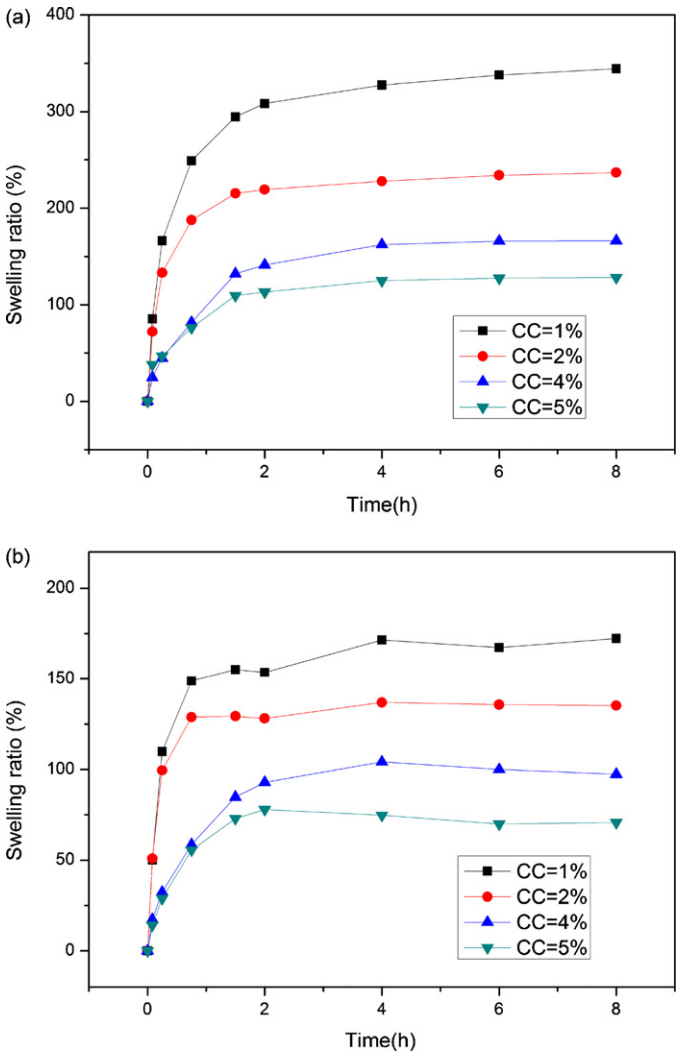

Fig. 5. The effect of the crosslinking agent concentration (CC) on the swelling kinetics of the hydrogels (AEC concentration = 4%, amino content of AEC = 5.6%) in (a) pH 1.0 and (b) pH 7.4 buffer solutions at 37 °C.

The AEC hydrogels reached swelling equilibrium after about 4 h, and with the increase of the aminoethyl chitin amino content from 1.5% to 7.4%, the equilibrium swelling ratio increased first, and then began to decrease both at pH 1.0 and pH 7.4 respectively. When the amino content of AEC was too low, the hydrogels showed poor swelling performances. Because there was few or almost no hydrophilic free amino groups to swell by contacting with H_2O molecules, and less effect on the swell ratio by forming ammonium ions leading repulsion among polymer molecule chains (Gong et al., 2011; Rao, Naidu, Subha, Sairam, & Aminabhavi, 2006; Tanuma et al., 2010). The increase of the amino content could increase the hydrophility of the AEC, and the osmotic pressure and charge repulsive force both improved the swelling ratio. When the amino content of AEC was too high, although there were more amino groups left after crosslinking, the hydrogel had more crosslinking points, and it competed with the repulsive force among ammonium ions, therefore, the swelling ratio of the hydrogels decreased.

3.4.2. *Effect of crosslinking agent concentration*

The swelling kinetics of the aminoethyl chitin hydrogels with different crosslinking agent concentration in pH 1.0 and pH 7.4 buffer solutions at 37 °C are shown in Fig. 5. As seen from the figures, the hydrogels reached swelling equilibrium after about 2 h, and the swelling rates decreased both at pH 1.0 and pH 7.4 respectively when changing the crosslinking agent concentration from 1% to 5%. This was attributed to that the hydrogels had the same original composition, so the crosslinking density should be the predominant factor to affect the swelling behaviors. When the crosslinking agent concentration increased, a more rigid network was formed by the reactions that had occurred among the polymer chains and the glutaraldehyde, reducing the flexibility and the amount of amino groups of the hydrogels (Costa-Júnior et al., 2009; Wang, Xie, Zhang, Zhang, & Wang, 2010). These made the polymer chains stretch not enough, reducing the osmotic pressure and the electrostatic repulsion between $-NH_3^+$. Moreover, the distance between crosslinking points became shorter, which made the space in polymeric networks smaller. All these resulted in the decrease of the swelling properties of the AEC hydrogels.

3.4.3. *Effect of aminoethyl chitin concentration*

The swelling kinetics of the hydrogels with different aminoethyl chitin concentrations in pH 1.0 and pH 7.4 buffer solutions at 37 °C are shown in Fig. 6. The solutions with a concentration of 1%, 2%, 3%, 4% and 5% were used in the experiment of hydrogel preparation, but the hydrogel could not be formed by using the 1% and 2% AEC solutions, or the strengths of the hydrogels were very low.

Table 1
The inhibitory ratio of the AEC hydrogels against *S. aureus*.

Sample	AEC concentration (%)	Amino content of AEC (%)	Crosslinking agent concentration (%)	Inhibitory ratio (%)		
				8 h	16 h	24 h
1	4	5.6	2	74.2	73.9	59.0
2	4	6.5	2	75.6	79.4	72.0
3	4	7.4	2	77.5	85.4	80.0
4	4	5.6	3	59.2	57.1	47.3
5	4	5.6	4	58.2	55.5	45.5
6	4	5.6	5	55.9	51.1	38.5

While the AEC concentrations were beyond 5%, the solution viscosities were too high and it was difficult to obtain the homogenous AEC solutions. Therefore, the final swelling tests were performed with a concentration of 3%, 4% and 5%. Seen from the figures, with the increase of the aminoethyl chitin concentration, the swelling rate and maximum swelling ratio both increased at pH 1.0 and pH 7.4, and the hydrogels reached swelling equilibrium after about 3 h. The hydrogels of high aminoethyl chitin concentrations had more hydrophilic amino groups, which had a rapid absorption of water to make the gels swell. Moreover, when adding the same amount of glutaraldehyde in forming the hydrogels, the hydrogel prepared from higher aminoethyl chitin concentration reserved more $-NH_3^+$ at pH 1.0, which caused higher osmotic pressure and more charge repulsion leading to the gradual increase of the swelling properties (Teng et al., 2010).

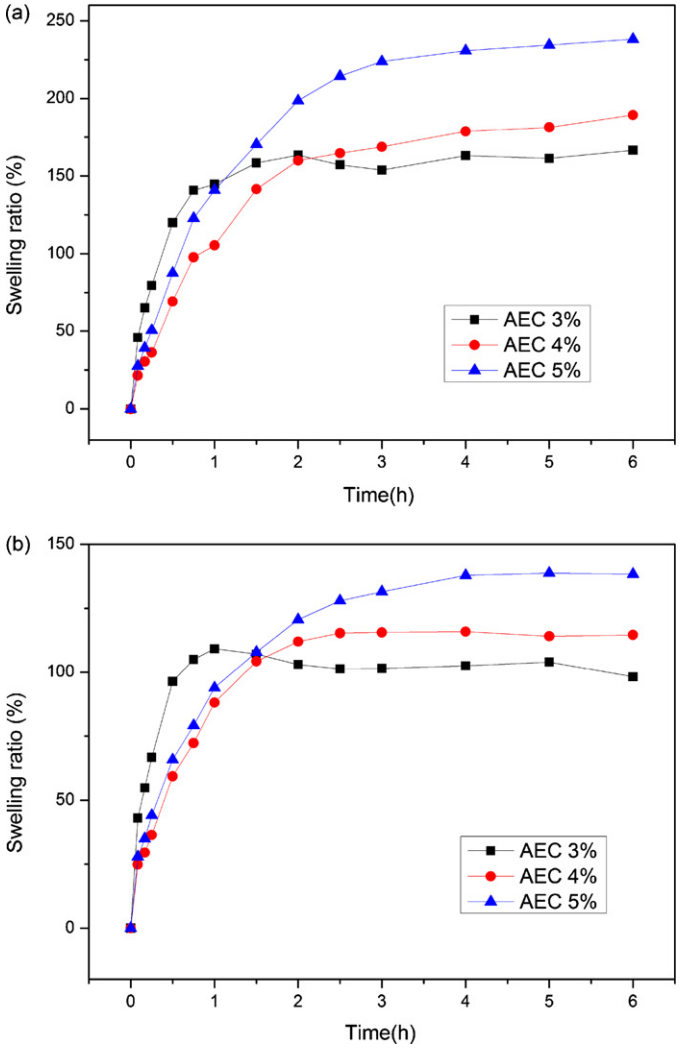

Fig. 6. The effect of the AEC concentration on the swelling kinetics of the hydrogels (amino content of AEC = 5.6%, crosslinking agent concentration = 2%) in (a) pH 1.0 and (b) pH 7.4 buffer solutions at 37 °C.

3.5. Antibacterial activities of aminoethyl chitin hydrogels

The inhibitory ratio results are presented in Table 1, which demonstrate the inhibitory ratio versus corresponding culture time for the aminoethyl chitin hydrogels against *S. aureus*. The results showed that the hydrogels had the good antibacterial activities. The inhibitory ratio increased with the amino content of AEC increasing from 5.6% to 7.4%, but decreased as the crosslinking agent concentration increasing from 3% to 5%. As the most important chitin derivative, the antibacterial activity of chitosan was generated from the amino groups, so the antibacterial effects of the AEC hydrogels were perhaps due to the remained amino groups after forming the hydrogels, and the more of the amino group amount, the higher was the antibacterial activity of the AEC.

4. Conclusions

In the present study, aminoethyl chitins (AEC) with different amino contents were synthesized by etherification reaction from chitin and 2-chlorethylamine hydrochloride. The results characterized by FTIR, ^{1}H NMR and SEM confirmed that the amino groups of aminoethyl chitin had reacted with the glutaraldehyde to form the hydrogels. The hydrogels were sensitive to acidic environment. The swelling ratio changed as the AEC amino content increased, decreased as the crosslinking agent concentration increased, and increased as the concentration of aminoethyl chitin increased. The antibacterial activities of the hydrogels against *S. aureus* were strengthened with the increase of the amino content or the decrease of the crosslinking agent concentration. Therefore, the aminoethyl chitin hydrogels have potential use as the materials for biomedical applications, etc.

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