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N-(2-hydroxypropyl)-3-trimethylammonium chitosan-poly(ε-caprolactone) copolymers and their antibacterial activity

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ABSTRACT

Chitosan-poly(ε-caprolactone) (CPC) copolymers were synthesized via an amino-group-protection method. Selected CPCs with poly(ε-caprolactone) content less than 50 wt.% were further modified by introducing quaternary ammonium groups. It was found that the maximum degree of quaternization for some quaternized CPCs (q-CPCs) could reach around 38% under present synthesis conditions. The optimized q-CPCs showed various antibacterial activities in vitro, and they were able to completely prevent growth of *Staphylococcus aureus* and *Escherichia coli* at different concentrations of about 0.2% and 0.25%, respectively. At lower concentrations, these optimal q-CPCs had higher antibacterial activities against both bacteria as compared to chitosan. The optimized q-CPCs were also processed into membranes for tensile mechanical investigations, and the resulting membranes exhibited notably higher strength and modulus in wet state but much lower strength and modulus in dry state when they were compared with chitosan membranes. Results suggested that the optimal q-CPCs with proper compositional proportions could have the potential for certain kinds of antibacterial applications where desirable tensile strength in wet state is required.

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1. Introduction

Chitosan has been widely used in different biomedical areas due to many of its advantageous properties (Lim & Hudson, 2003). Nevertheless, its mechanically weak features in wet state limit its applications (Madihally & Matthew, 1999). One of the main strategies for improving mechanical strength of chitosan-based materials is to blend it with other mechanically strong polymers such as polylactide (PLA) (Chen, Dong, & Cheung, 2005; Wan, Wu, Yu, & Wen, 2006) and poly(ε-caprolactone) (PCL) (Cruz, Ribelles, & Sanchez, 2008; Wan, Lu, Dalai, & Zhang, 2009). However, the reported results revealed that these blends showed inferior miscibility. Apart from directly blending chitosan with above mentioned polyesters, grafting PLA or PCL onto chitosan chains has also been tried (Liu, Chen, & Fang, 2006; Qu, Wirsen, & Albertsson, 1999). It has been found that chitosan-poly(ε-caprolactone) (CPC) copolymers could have tailorable properties with the merits of both chitosan and PCL components, depending on their composition.

One of the important characteristics of chitosan is its antimicrobial properties against a variety of bacteria and fungi because of polycationic features of chitosan (Muzzarelli, 2009). However, the antimicrobial activity of chitosan is limited to acidic conditions due to its poor solubility above pH ~ 6.5, where chitosan starts to lose its cationic nature. To improve solubility of chitosan in aqueous media, many studies have been focused on the preparation of soluble chitosan derivatives over a wide pH range (Lim & Hudson, 2004). Some reports have suggested that quaternary ammonium salts of chitosan with appropriate degrees of quaternization are soluble in neutral or even slightly alkaline aqueous solvents, and meanwhile, they also show markedly improved antimicrobial activities as compared to unmodified chitosan in pH-regulated environments (Jia, Shen, & Xu, 2001; Jintapattanakit, Mao, Kissel, & Junyaprasert, 2008; Sadeghi et al., 2008; Seong, Whang, & Ko, 2000). However, in spite of merits of the enhanced antibacterial activity, the increasing soluble or highly swelling properties of quaternized-chitosan derivatives in aqueous environments would in turn make themselves nearly inapplicable to some instances where the basic mechanical strength in the wet state is essential. Based on the adjustable properties of CPCs aforementioned, in the present study, selected CPCs were quaternized to aim at achieving desirable antimicrobial materials and their antimicrobial activity and mechanical properties were mainly investigated.

2. Experimental

2.1. Materials

Chitosan was purchased from Fluka. To obtain highly deacetylated chitosan, the obtained chitosan powder was treated in a

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50 wt.% NaOH solution at 100 °C for 2 h, and the alkali treatment was repeated again using a method described elsewhere (Wan, Cao, Wu, Zhang, & Wang, 2008). The degree of deacetylation and viscosity average molecular weight of chitosan were measured as 93.8 (±1.9) % and 1.04 (± 0.15) × 10^5 according to our previous method (Wan et al., 2006). *Escherichia coli* (*E. coli*, ATCC 25922) and *Staphylococcus aureus* (*S. aureus*, ATCC 29213) bacteria were obtained from ATCC. Other chemicals were purchased from Sigma–Aldrich and Fisher, and used as received.

2.2. Synthesis of CPCs

CPCs were synthesized following a protection–grafting–deprotection route and using reported methods (Liu, Wang, Shen, & Fang, 2005; Liu et al., 2006). In brief, 5 g of chitosan and 13.8 g of phthalic anhydride were dispersed in 100 mL of anhydrous DMF and the mixture was stirred at 90 °C for 8 h to produce phthaloylchitosan (PHCS). The collected products were washed with water, ethanol and diethyl ether, consecutively, and dried in vacuum. Grafting caprolactone onto PHCS was carried out under N_2 in the medium of DMF with stirring at 100 °C for various periods of time. The obtained PHCS-poly(ε-caprolactone) was extracted with acetone in a Soxhlet apparatus for at least 24 h to remove the homopolymer. After that, PHCS-poly(ε-caprolactone) was deprotected by eliminating phthaloyl groups using hydrazine monohydrate under nitrogen and the resulting CPCs were repeatedly washed with water and ethanol, lyophilized at −75 °C and dried again in vacuum.

2.3. Synthesis of quaternized CPCs (q-CPCs)

CPCs were cryogenically milled into very fine powder in a mill cooled with liquid nitrogen and vacuum-dried prior to grafting synthesis. In a typical experiment, 5 g of selected CPC powder was dispersed in 60 mL of distilled water and various amounts of glycidyl trimethyl-ammonium chloride (GTMAC) were introduced with stirring under N_2 atmosphere at 80 °C for at least 12 h. The resulting products were cooled down to 0 °C in an ice-water bath, and a given amount of precooling acetone was added with additional stirring. The collected precipitates were washed twice with cooled acetone and ethanol, and filtered. The unreacted GTMAC and oligomer were removed by extracting the products with ethanol using a Soxhlet apparatus for 24 h. The final products of q-CPCs (named as q-CPC-I(*i*), *i* = a, b, c, d; and q-CPC-II(*j*), *j* = a, b, c, d) were dried until to the constant weight. Some quaternized-chitosan samples (referred as q-CH-(*k*), *k* = a, b, c, d) were also synthesized using the same method and used as controls.

2.4. Preparation of membrane samples

Chitosan, CPCs and selected quaternized samples were processed into membranes for the measurements of X-ray diffractograms and tensile test. They were dissolved in 0.15% acetic acid (HAc) to prepare 2.0 wt.% solutions, respectively. Complete dissolution of samples was ensured by using a Tissuemiser Homogenizer (Fisher Scientific). The resulting solutions were cast onto Teflon dishes and dried in air. The resulting membranes were immersed in a 1.0% NaOH aqueous solution for their neutralization. These membranes were then washed thoroughly with deionized water until a neutral pH was reached, dried in air and again in vacuum. All dry membranes had a mean thickness of about 220 μm.

2.5. Characterization

IR spectra of q-CPCs were recorded on a spectrometer (IRPrestige-21, Shimadzu) in transmission mode. All samples were prepared as KBr pellets and scanned against a blank KBr pellet background.

Chitosan, CPC or q-CPC samples were dissolved in D_2O/CF_3COOD (98:2, v/v), respectively, and introduced into a 5-mm NMR tube. ^1H NMR measurements were performed on a Bruker Advance 600 spectrometer 60 °C.

The weight percent of PCL in CPCs was measured using a Perkin Elmer PE 2400 II elemental analyzer. PCL content in CPCs was also measured via a gravimetric method described elsewhere (Feng & Dong, 2006) for comparison.

Degree of quaternization (DQ) was determined via a titration method. q-CPC was dissolved in 0.1 M HAc solution and DQ was determined by titrating the amount of Cl^- ions on the q-CPC with an aqueous $AgNO_3$ solution according to a reported method (Wu, Su, & Ma, 2006).

An X-ray diffractometer (SCINTAG X1) was used to inspect diffractograms of the membranes. The X-ray source was Ni-filtered Cu-Kα radiation. Dry membranes were mounted on aluminum frames and scanned at a speed of 2°/min from 5° to 40° (2θ). To measure the relative crystallinity percentage (X_c) of the membranes, the amorphous areas and the areas of the crystalline peaks were measured, and X_c was calculated from the diffractograms of the membranes based on the following relationship (Wan et al., 2006):

$$X_c = \left[\frac{A_c}{A_c + A_a}\right] \times 100\% \quad (1)$$

where A_c and A_a are the areas of the crystalline and amorphous regions, respectively.

2.6. Evaluation of antibacterial properties

A loopful of *E. coli* or *S. aureus* was spread on nutrient agar and incubated at 37 °C for 24 h to give the single colonies. A representative bacteria colony was picked off with a wire loop, placed in pre-sterilized Difco™ nutrient broth and then incubated overnight at 37 °C for 12 h. Cells were harvested by centrifugation. By appropriately diluting with sterile distilled water, the cultures of *E. coli* and *S. aureus* containing ~10^8 CFU/mL were prepared according to McFarland standards. Each bacterium suspension was diluted with autoclaved nutrient broth and sterile distilled water, and the suspension was adjusted to the matched optical absorbance of 0.2 prior to the antibacterial test (Liu et al., 2006).

Selected antibacterial agents (chitosan, CPC-2, CPC-3, q-CH-(a), q-CPC-I(c) and q-CPC-II(d)) were respectively dissolved in sterile nutrient broth containing pre-sterilized 0.15% acetic acid to prepare different solutions with varied concentrations. Complete dissolution of the antibacterial agents was ensured by using a Tekmar Tissuemiser that has a sonicating function and is able to grind up those gelled particles. After that, these solutions were inoculated with *E. coli* or *S. aureus* and incubated with shaking at 37 °C for 48 h. The optical density (OD) of samples was measured spectrophotometrically on a Cary 5000 UV-vis-NIR spectrometer at 610 nm. All experiments were performed in triplicates against each tested bacterium. The agent-control groups contained antibacterial agent and nutrient broth, and the bacterium-control groups consisted of bacterium suspension and nutrient broth without any antibacterial agents (Sajomsang, Tantayanon, Tangpasuthadol, & Daly, 2009).

2.7. Tensile properties of membranes

Tensile mechanical parameters of the membranes were measured using an INSTRON universal testing machine (model 5865) at ambient temperature. Dry specimens were cut into strips with 50 mm length and 20 mm width. The strips were strained to failure on employing a crosshead speed of 2 mm/min. In the cases of hydrated samples, after being immersed in water for 2 h, the

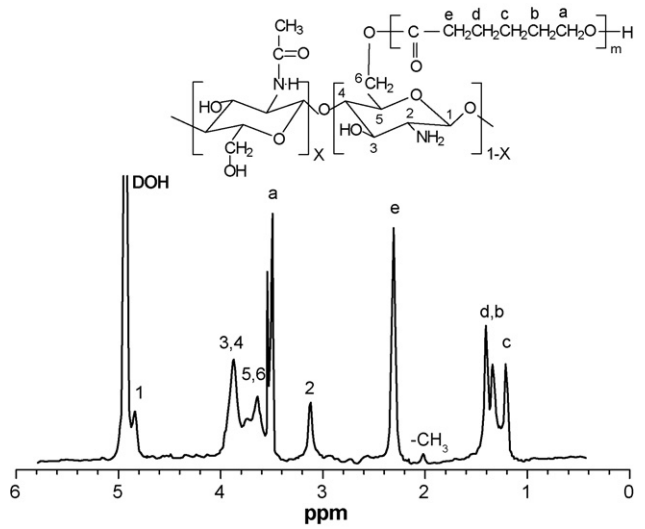

Fig. 1. ^{1}H NMR spectrum of chitosan–PCL (PCL content: 44.2 wt.%).

samples were taken out and the excess of water on the surface of membranes was removed using blotting paper. The hydrated samples were also cut into strips with the same dimensions described above and tested under the same conditions.

2.8. Statistical analysis

Analysis of variance (ANOVA), using commercially available statistical software (SPSS 15.0 for Windows), was performed to determine whether significant differences existed among the measured data. The differences in the data were considered as statistical significance at $p < 0.05$.

3. Results and discussion

3.1. CPCs and relevant parameters

To leave amino groups at the C-2 sites free for further quaternization, in the present study, PCL side chains were selectively grafted onto the C-6 sites using a group-protection method in which the amino groups at C-2 sites of chitosan were protected beforehand by phthaloyl groups.

Fig. 1 shows a representative ^{1}H NMR spectrum of CPC samples. The chemical shifts at δ: 3.1 (H-2), 3.6–3.9 (H-3, -4, -5, -6), and 4.8 (H-1) can be attributed to the chitosan, and the others at δ: 1.2 (c-CH2), 1.35–1.42 (b, d-CH2), 2.3 (e-CH2) and 3.5 (a-CH2) should be ascribed be to the PCL component. These data are well in agreement with the reported results (Liu et al., 2005b, 2006), suggesting that PCL side chains have been successfully grafted on the chitosan main chains.

It was found that PCL content in CPCs was notably dependent on both the feed ratios of caprolactone to chitosan and the synthesis conditions. The maximum weight percent of PLC in CPCs could reach around 74 wt.% by using present synthesis technique. Different CPCs with various PCL weight percentages were synthesized and relevant parameters are summarized in Table 1. Since PCL is a hydrophobic component, as expected, CPCs showed quite various soluble properties in different solvents, depending on PCL content. Although the weight percentage of PCL in CPCs could reach higher than 70 wt.%, the PCL content has to be well controlled because the resultant CPCs would show the characteristics more similar to pure PCL if PCL content is too high. To take full advantages of both chitosan and PCL components, and consider the feasibility for quaternization by using aqueous solvent, some CPCs containing PCL less than 50 wt.%, namely, CPC-2 and CPC-3, were hence selected for the followed quaternization. In addition, as mentioned in the experimental section, PCL content was measured using either a gravimetric method or an elemental analysis technique. Results in Table 1 indicate that data obtained by two different methods for PCL content exhibited reasonable consistency, confirming that the present synthesis technique was stable and well controllable.

Table 1
Parameters of CPC copolymers.

Samples	PCL content in CPC (wt.%)[a]		Soluble feature[b]		
	Gravimetric method	Elemental analysis	DMSO	0.5% HAc	Water
CPC-1	25.4 (±1.37)	26.3 (±1.41)	–	+	±
CPC-2	36.6 (±1.48)	35.5 (±1.39)	±	+	–
CPC-3	45.3 (±1.53)	46.1 (±1.45)	±±	±±	–
CPC-4	54.7 (±1.61)	53.8 (±1.27)	±±	±±	–
CPC-5	63.2 (±1.46)	62.3 (±1.42)	+	±	–
CPC-6	72.8 (±1.59)	73.5 (±1.53)	+	–	–

[a] PCL content was controlled by mainly changing the ratios of caprolactone to phthaloylchitosan, reaction time and the volume of media;
[b] DMSO (dimethyl sulfoxide), "–", "±", "±±", and "+" indicate that CPCs are insoluble, partially swelled, partially soluble or highly swelled, and soluble, respectively.

3.2. IR and NMR analysis of q-CPCs

Fig. 2 shows IR spectra of chitosan, CPC-2 and q-CPC. There was a shoulder-like absorption peak at around 1656 cm^{-1} for chitosan, which corresponds to the C=O stretch of the secondary amide and is usually shown for chitosan with a high DDA (Wan et al., 2006); another clear absorption peak at 1592 cm^{-1} is belonged the N–H bending of the primary amine of chitosan (Wan et al., 2006). Several new absorption bands at 2928, 1726, 1240 and 1178 cm^{-1} appeared in the spectrum of CPC-2 as compared to that of chitosan and they could be assigned for the characteristic absorption of ester corresponding to PCL side chains (Liu, Li, Fang, & Chen, 2005). It should be also noted from Fig. 2 that the peak situated at about 1588 cm^{-1} and originally corresponding to the amino groups in chitosan was still well remained, suggesting that PCL chains were grafted onto the hydroxyl groups at C-6 positions of chitosan backbone, while the amino groups of chitosan were reserved.

The IR spectrum of q-CPC in Fig. 2 shows evidence of the introduction of quaternary ammonium groups on chitosan backbone, which appeared at 1478 cm^{-1}, corresponding to the C–H bend-

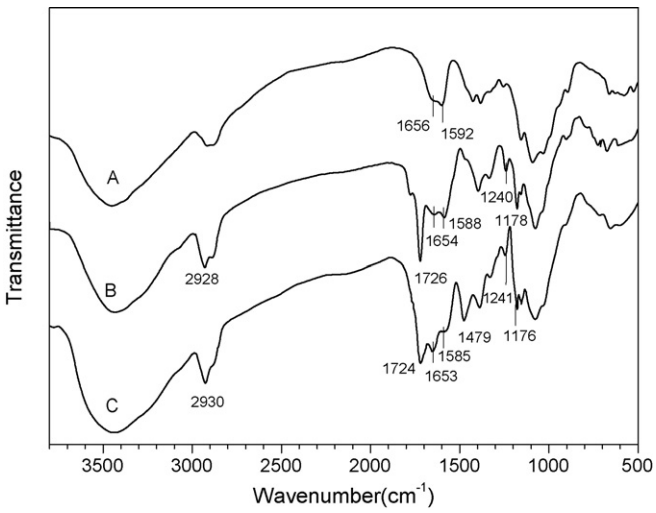

Fig. 2. IR spectra of chitosan (A), CPC-2 (B, PLC content in CPC: 36.7 wt.%) and q-CPC (C, PCL content in CPC: 35.9 wt.%, DQ: 35.1%).

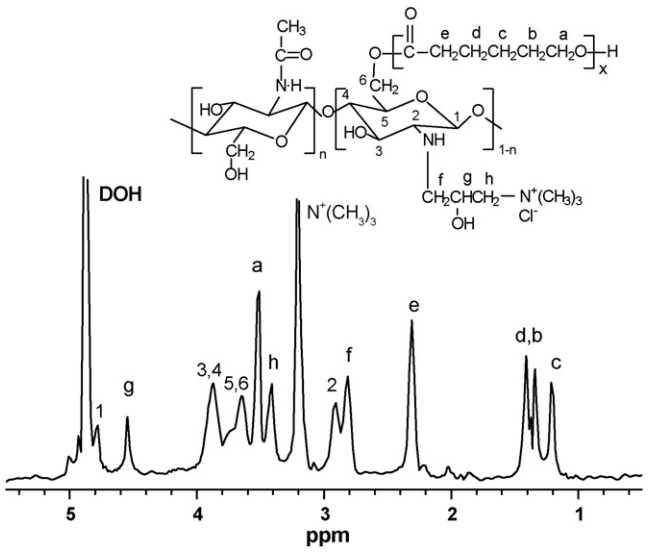

Fig. 3. ^{1}H NMR spectrum of q-CPC (PCL content in CPC: 35.2 wt.%, DQ: 34.3%).

ing of trimethylammonium group (Lim & Hudson, 2004; Wu et al., 2006). A shoulder originally matched to the secondary amide of chitosan component (see Fig. 2(A)) turned to a clear absorption band at 1653 cm^{-1}, and the peak initially corresponding to the primary amine of chitosan became a shoulder, implying that GTMAC already connected with amino groups on chitosan backbone so that some primary amine was changed into secondary amine (Pavia, Lampman, & Kriz, 1996).

Fig. 3 presents ^{1}H NMR spectrum of q-CPC. A very strong peak at around 3.2 ppm was observed, which confirms the presence of methyl groups in the quaternary ammonium side chains (Lim & Hudson, 2004). Several other peaks at δ: 2.8, 3.21 and 4.53 could be ascribed to protons in quaternized side chains, respectively (Kim, Choi, & Yoon, 1998; Seong et al., 2000). The signals in the spectrum for chitosan backbone and PCL side chains were maintained almost the same with exception of a small shift for H-2 (δ: 2.93) which originally positioned at δ: 3.1 (see Fig. 1). On the basis of IR and ^{1}H NMR spectra of q-CPC, it can be reached that some amounts of amino groups on the C-2 sites of chitosan backbone have been quaternized.

3.3. Basic parameters of q-CPCs

Based on many trials, it was attained that by changing GTMAC/CPC feed ratio and selecting appropriate reaction temperature and time, DQ of q-CPCs could be effectively controlled. Two sets of q-CPCs were synthesized by using CPC-2 and CPC-3 and altering the GTMAC/CPC ratios, and relevant data are summarized in Table 2. Another set of quaternized-chitosan (q-CH) samples was also prepared with the same method and used as controls. Their parameters are concomitantly listed in Table 2. Data for the samples in the control set revealed that chitosan could be easily quaternized and DQ of q-CH increased with GTMAC/chitosan feed ratio until the ratio reached a value of around 4.5, and after that, it showed a slow-down change. In contrast to these observations, it is noted that DQ of q-CPCs in set-one or set-two was much lower than that of the matched one in the control set even the GTMAC/CPC ratio was arranged to increase in a same proportional manner. In addition, DQ of samples in the set-one was measurably greater than that of the corresponding one in the set-two.

As indicated in Table 1, CPC-2 or CPC-3 had its PCL content up to 35 wt.% or higher. The PLC side chains in CPC-2 would inevitably generate steric hindrance to hinder some GTMAC molecules from reacting with amino groups on chitosan main chains, leading to much less effective quaternization of q-CPC samples in set-one as compared to that of q-CH. As regards the relatively low DQ of q-CPC samples in set-two when comparing with that of the matched one in set-one, it should be reasonable because the PCL content in CPC-3 was higher than that of CPC-2, and the increased PCL content would contribute enhanced steric hindrance, resulting in lower DQs.

Table 2
Parameters of q-CPC.

Samples	Feed ratio of GTMAC to chitosan or CPC[a] (wt/wt)	DQ (%)	Soluble feature[b]	
			0.15% HAc	Water
q-CH-(a)[c]	2.5:1	34.3(±2.41)	+	±±
q-CH-(b)	3.5:1	59.7(±2.68)	+	±±
q-CH-(c)	4.5:1	81.4(±3.05)	+	+
q-CH-(d)	5.5:1	92.6(±3.12)	+	+
q-CPC-I(a)[d]	2.5:1	17.2(±1.59)	±±	±
q-CPC-I(b)	3.5:1	29.6(±2.07)	±±	±
q-CPC-I(c)	4.5:1	34.8(±2.36)	±±	±
q-CPC-I(d)	5.5:1	37.9(±2.13)	±±	±
q-CPC-II(a)[e]	2.5:1	13.5(±1.42)	±	±
q-CPC-II(b)	3.5:1	25.1(±2.16)	±	±
q-CPC-II(c)	4.5:1	31.3(±2.21)	±±	±
q-CPC-II(d)	5.5:1	35.2(±2.29)	±±	±

[a] CPC-2 (PCL content in CPC-2: 35.4 wt.%) and CPC-3 (PCL content in CPC-3: 45.1 wt.%) were selected for quaternization (see Table 1 for CPC-2 and CPC-3).
[b] "+", "±±" and "±": soluble; partially soluble or highly swelled; partially swelled, respectively.
[c] Control set.
[d] Set-one was denoted by letter "I"; samples in the set-one were synthesized using CPC-2.
[e] Set-two was denoted by letter "II"; samples in the set-two were synthesized using CPC-3.

Table 2 indicates that q-CPCs were only partially swollen in water, and on the other hand, q-CHs with different DQs could be soluble in water, partially soluble or highly swelled in water, implying that the presently obtained q-CPCs will have better mechanical strength in the wet state than q-CHs. It can be seen from Table 2 that under the present synthesis conditions, the maximum DQ for q-CPC samples in set-one or set-two could reach around 38%. To evaluate the antibacterial properties of these quaternized derivatives on the same baseline, q-CH-(a), q-CPC-I(c) and q-CPC-II(d) were thus selected for the following examinations because they had a similar DQ of around 35%.

3.4. X-ray diffraction analysis

X-ray diffractograms of chitosan, CPC and q-CPC membrane samples are illustrated in Fig. 4(a). Chitosan membrane showed two typical peaks at around 10° and 20.5° (Wan et al., 2006). CPC-2 and CPC-3 membranes exhibited similar diffraction patterns. Two narrow peaks at angle 2θ of around 21° and 23° in Fig. 4(a) (B and C) for CPC-2 and CPC-3 appeared to be superposed over the remnant wide peak of chitosan component, which can be attributed to the crystalline PCL component (Liu et al., 2005b). In the case of q-CH membrane, the original peak of chitosan at around 10° completely vanished, and the strength of another typical peak originally situated at about 20.5° was significantly decreased. The X-ray patterns of q-CPC-I(c) and q-CPC-II(d) membranes became quite flat and only displayed a trace of the peak of chitosan component. The crystallinity of different membrane samples was calculated and relevant data are presented in Fig. 4(b). These bar-graphs showed that crystallinity of CPC-2 or CPC-3 membranes was remarkably lower than that of pure chitosan membrane; after quaternization, crystallinity of quaternized membranes was further significantly reduced, and the very low crystallinity of q-CPC-I(c)

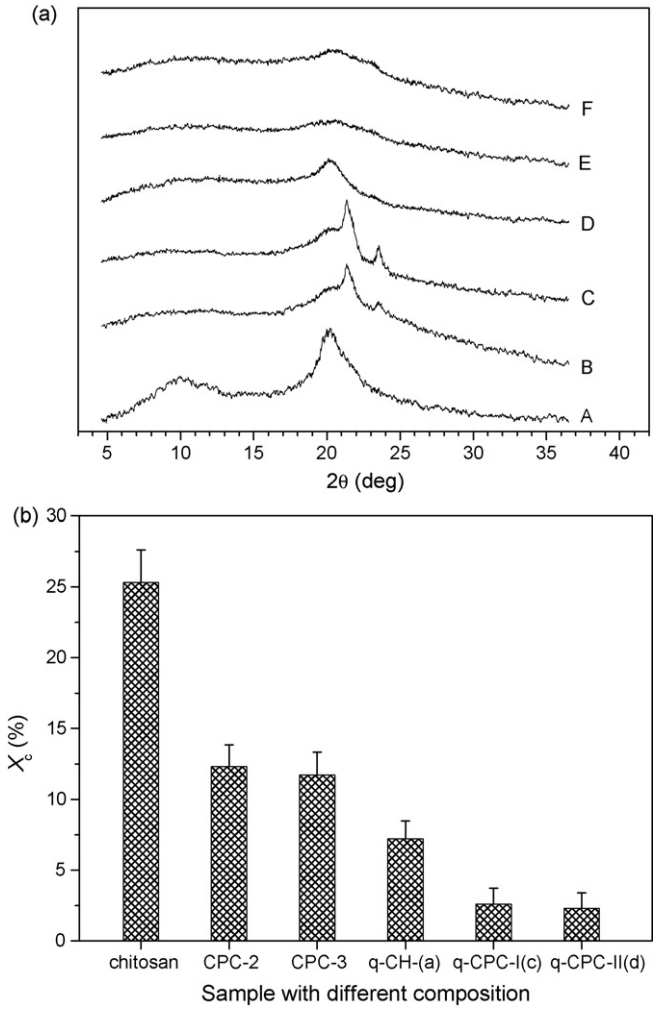

Fig. 4. X-ray diffractograms (a) and crystallinity (b) of different membranes ((a): (A) chitosan, (B) CPC-2, (C) CPC-3, (D) q-CH-(a), (E) q-CPC-I(c) and (F) q-CPC-II(d)).

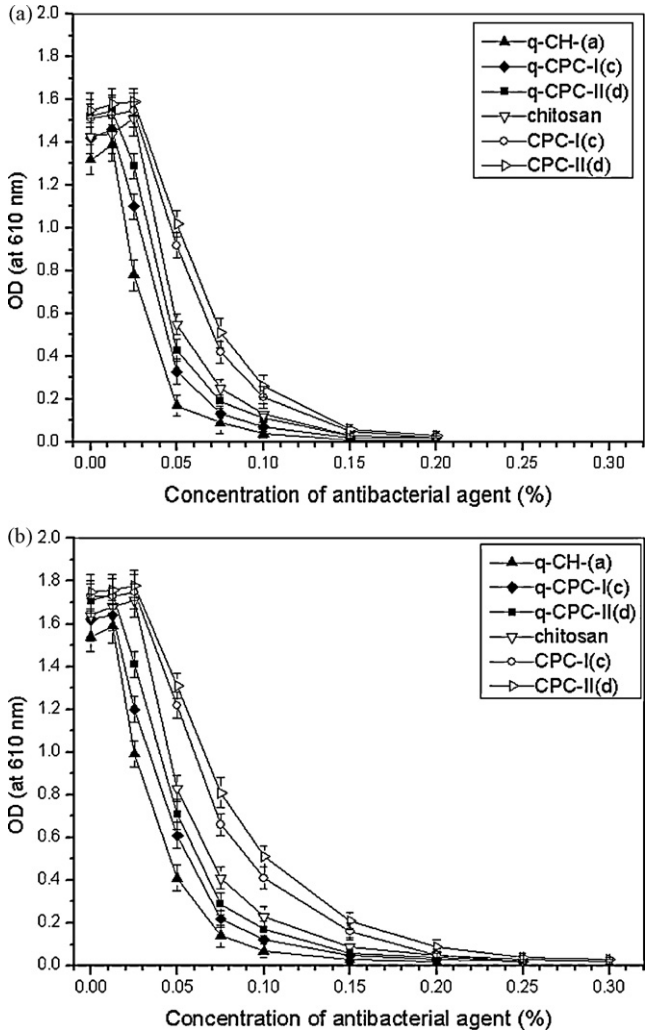

Fig. 5. Antibacterial activity of selected antibacterial agents against *S. aureus* (a) and *E. coli* (b).

and q-CPC-II(d) membranes indicated that they became almost amorphous. It is known that both chitosan and PCL are crystalline polymers. However, the interactions resulted from intermolecular entanglement between PCL side chains and chitosan chains in CPCs could obstruct crystallization of each component, leading to notably lower crystallinity in CPC-2 and CPC-3 membranes. The rigid structure of chitosan is known to be mainly stabilized by hydrogen bonds (Nishimura, Kohgo, Kurita, & Kuzuhara, 1991). In the case of unmodified chitosan, the intramolecular hydrogen bonds are usually formed by amino groups at C-2 and hydroxyl groups at C-3 positions, and the intermolecular hydrogen bonds can be created between hydroxyl groups at C-6 and C-3 positions through absorbed water molecules. However, after quaternization, these hydrogen bonds would be drastically destroyed, resulting in great decrease in crystallinity of q-CH-(a) membranes. With respect to q-CPC-I(c) and q-CPC-II(d) membranes, as compared to their precursors, namely, CPC-2 and CPC-3, the further reduction in their crystallinity is reasonable because introduction of quaternary ammonium side chains onto the C-2 positions of chitosan backbone would inevitably escalate the entanglement among chitosan chains, PCL side chains and quaternary ammonium side chains, which can become so tangly that q-CPC-I(c) and q-CPC-II(d) membranes are endowed with almost amorphous features.

3.5. Antibacterial properties

Fig. 5(a) shows concentration dependence of the antibacterial activities of selected agents against *S. aureus*. It can be observed from Fig. 5(a) that when concentrations were higher than 0.2%, all antibacterial agents were able to completely prevent growth of *S. aureus*. The inhibitory concentration of chitosan was about 0.1% and it is basically in agreement with reported results (Li et al., 2007). CPC-2 and CPC-3 had significantly lower ($p < 0.01$) antibacterial activities as compared to chitosan, which can be ascribed to their much lower content of amino groups due to the substitution of PCL side chains. q-CH-(a) exhibited remarkable enhancement on its antibacterial activities when comparing with chitosan, which can be attributed to the contribution of quaternary ammonium groups because amino groups on chitosan can only function as relatively weak positive charge centers whereas the grafted trimethylammonium groups on q-CH-(a) have much greater positively electronic strength than that of amino groups. It is specially worth noting that q-CPC-I(c) and q-CPC-II(d) acquired notably improved antibacterial activities in contrast to CPC-2 and CPC-3, respectively, and they even showed effectively inhibitory abilities against *S. aureus* at lower concentrations ($p < 0.05$) than that of chitosan.

The effect of concentrations on the antibacterial activity of selected agents against *E. coli* is illustrated in Fig. 5(b). OD values of these agents showed trends closely similar to that shown in Fig. 5(a)

Table 3
Tensile parameters of dry and hydrated membranes[a].

Sample	Dry state			Hydrated state		
	Tensile strength (MPa)	Breaking-elongation (%)	Young's modulus (MPa)	Tensile strength (MPa)	Breaking-elongation (%)	Young's modulus (MPa)
Chitosan	25.3 ± 2.4	14.2 ± 3.1	189.4 ± 19.3	0.31 ± 0.06	52.5 ± 4.9	3.9 ± 0.5
CPC-2	12.7 ± 1.4	27.6 ± 3.8	96.6 ± 8.7	6.2 ± 0.9	61.9 ± 6.3	53.4 ± 5.7
CPC-3	13.5 ± 1.2	29.9 ± 4.2	114.2 ± 10.8	8.3 ± 1.1	65.2 ± 6.7	87.1 ± 6.1
q-CH-(a)[b]	8.1 ± 1.1	24.7 ± 3.5	63.1 ± 8.4	–	–	–
q-CPC-I(c)	6.4 ± 0.9	32.5 ± 3.2	48.3 ± 5.1	1.09 ± 0.08	68.2 ± 7.4	8.4 ± 1.1
q-CPC-II(d)	72.3 ± 1.1	34.1 ± 3.6	56.7 ± 5.3	1.16 ± 0.09	72.2 ± 7.2	9.5 ± 1.2

[a] Dry membranes had a mean thickness of about 220 μm; the thickness of hydrated membranes was measured using a vernier caliper.
[b] Hydrated q-CH-(a) membranes were highly swelled so that the tensile measurements for them were not able to be conducted.

but it can be seen in Fig. 5(b) that the plots were integrally moved towards higher concentrations, and the concentration of agents for completely inhibitory growth of *E. coli* was about 0.25%, possibly revealing that these agents impose stronger antibacterial effects on *S. aureus* than on *E. coli*. These observations are rational and supported by some other reports in which chitosan was found to show higher antibacterial activity against *S. aureus* than against *E. coli* due to the main differences between the cell wall structures of *S. aureus* and *E. coli* (No, Park, Lee, & Meyers, 2002).

It is noted in Fig. 5(a) and (b) that OD values for q-CH-(a) and chitosan were slightly increased when their concentrations were lower than 0.025%. It has been reported that chitosan is quite susceptible to a wide range of enzymes originated from various bacteria and fungi (Hirano, Tsuchida, & Nagao, 1998). Therefore, chitosan and q-CH-(a) could be hydrolyzed by some enzymes synthesized by *S. aureus* and *E. coli* and used as a kind of energy when their concentrations are lower than a critical value, resulting in a slight increase in their OD values.

As shown in Table 2, q-CPC-I(c) and q-CPC-II(d) were partially swollen in water while q-CH-(a) was not fully soluble in water, a 0.15% HAc solution was thus employed for these agents during the antibacterial test in the present study. As a result, the plots in Fig. 5(a) and (b) should reflect the cooperative effect of antibacterial agents and 0.15% HAc since acetic acid also has antibacterial activity. Many other studies on the antibacterial activity of chitosan in HAc solutions with various concentrations ranging from 0.25 to 1.0% have been done and results indicate that the antibacterial effect mainly comes from chitosan (Avadi, Sadeghi, Tahzibi, Bayati, & Pouladzadeh, 2004; Liu et al., 2006; Qi, Xu, Jiang, Hu, & Zou, 2004). In the present instance, the concentration of HAc solution was very low, therefore, results presented in Figs. 5(a) and (b) should suggest that these tested agents mainly contribute their antibacterial activity against *S. aureus* and *E. coli* and q-CPC-I(c) and q-CPC-II(d) could be suitable for antibacterial applications due to their higher antibacterial activity as compared to chitosan.

3.6. Tensile properties

Since these antibacterial agents will possibly be used in a wet environment their tensile properties therefore, become an important issue. Chitosan, CPC-2, CPC-3, q-CH-(a), q-CPC-I(c) and q-CPC-II(d) were thus processed into membranes and tensile properties of these membranes were examined in both dry and wet states. Relevant parameters obtained from mechanical measurements are provided in Table 3.

The data in Table 3 reveal that in the case of dry state, chitosan membranes had much higher ($p < 0.01$) tensile strength and modulus but significantly lower ($p < 0.01$) breaking-elongation than other membranes; and after quaternization, tensile strength and modulus of q-CH-(a), q-CPC-I(c) and q-CPC-II(d) membranes further decreased and their breaking-elongation was lengthened when they were correspondingly compared with chitosan, CPC-2 and CPC-3 membranes, respectively. These differences could be ascribed to different structures and composition of membranes. Fig. 4(a) and (b) indicates that chitosan membranes had much higher crystallinity than others, and thus the mechanical strength of chitosan membranes should be significantly higher in contrast to other membranes because crystalline properties of polymer are highly linked to its strength, and usually, higher crystallinity will endow a polymer material with higher strength and modulus in comparison with those having lower crystallinity. On the other hand, the much lower crystallinity due to chain entanglement in CPC-2, CPC-3, q-CPC-I(c) and q-CPC-II(d) membranes could allow them to partially reorganize the molecule chains into an alignment arrangement during the tensile test, leading to significantly lengthened breaking-elongation. With respect to q-CH-(a) membranes, their lower strength and modulus as well as longer breaking-elongation in the day state when comparing with chitosan membranes could be assigned to the effect of N-[(2-hydroxy-3-trimethylammonium)propyl] side chains which certainly obstruct the formation of intermolecular and intramolecular hydrogen bonds inside the membrane.

In the case of hydrated state, q-CH-(a) membranes swelled so that they were not able to withstand any mechanical loadings. Data in Table 3 indicates that tensile strength and modulus of all other membranes sharply decreased and breaking-elongation of membranes was greatly increased. It is known that chitosan is hydrophilic because of its polar groups. In a wet state, some microcrystalline domains originally formed in the dry chitosan membrane through hydrogen bonds could be highly destroyed due to hydration and followed swelling (Kumar, 2000), and thus tensile strength and modulus of hydrated chitosan membranes would be remarkably reduced, accompanied by increased breaking-elongation. With respect to hydrated CPC-2 and CPC-3 membranes, their tensile properties should be closely associated with the properties of PCL component because hydrated chitosan was very mechanically weak, as indicated in Table 3. Since an acidic aqueous solution was employed as the solvent to prepare CPC-2 and CPC-3 membranes, PCL chains in CPC-2 or CPC-3 could turn inward and aggregate together while chitosan chains would stretch outward because PCL component is highly hydrophobic. As a result, many hydrophobic domains constructed by PCL chains would possibly act as physically crosslinked sites, as described in the case of chitosan-polylactide copolymers (Qu et al., 1999), resulting in enhanced tensile strength and modulus for hydrated CPC-2 and CPC-3 membranes. Their slightly lengthened breaking-elongation can be partially attributed the excellent ductility of PCL component (Wan et al., 2009). It is worthy of noting that the strength and modulus of hydrated q-CPC-I(c) and q-CPC-II(d) membranes were around three-fold higher ($p < 0.01$) than that of hydrated chitosan membranes, suggesting that q-CPC-I(c) and q-CPC-II(d) have

acceptable wet mechanical strength for biomedical applications in soft tissues repair or regeneration (Shalaby, 1994).

On the basis of above examinations and considering mechanical properties and antibacterial activity of quaternized CPC copolymers together, the obtained results may suggest that some of them, for example, q-CPC-I(c) and q-CPC-II(d), can serve as desirable candidates for certain kinds of antibacterial applications where the basic tensile strength in the wet state is required.

4. Conclusions

Under the optimized synthesis conditions, a degree of quaternization (DQ) of around 38% for selected q-CPCs with weight percentage of poly(ε-caprolactone) component less than 50 wt.% could be achieved. Some optimal q-CPCs with poly(ε-caprolactone) content ranging from about 35 to 45 wt.% and having a DQ of around 35% showed different in vitro antibacterial activities against *S. aureus* and *E. coli*, and they were able to completely prevent growth of the former at a concentration of about 0.2% and the latter at around 0.25%, respectively. At lower concentrations, these optimal q-CPCs had higher antibacterial activities against both bacteria as compared to chitosan. The result obtained from tensile evaluations revealed that in a dry state, the membranes made from the optimal q-CPCs exhibited notably lower strength and modulus in contrast to chitosan membranes but hydrated q-CPC membranes had significantly higher strength and modulus when comparing with hydrated chitosan membranes. These optimal q-CPCs had potential for antibacterial applications where mechanical strength in the wet state is essential.

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