Synthesis of Chitosan-Poly(acrylic acid) Complex Particles by Dispersion Polymerization and Their Applications in ph Buffering and Drug Release

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ABSTRACT: This research prepared polyelectrolyte complex particles of chitosan-poly(acrylic acid) (CS-PAA) through polymerization of acrylic acid (AA) in the presence of chitosan (CS). The prepared CS-PAA complex particles had positive zeta potential and dispersed very well in the aqueous solution. Structure and morphology of complex particles were investigated with the changes in the feeding molar ratio of glucosamine unit in CS to AA monomer. It was found at a molar ratio of 1.0/1.1, a hollow structure in complex particles was observed after polymerization. From solid state ^{13}C-NMR and FTIR analyses, the results confirmed that CS-*co*-PAA copolymer was also produced during polymerization in addition to the PAA homopolymer, and both constituted in the structure of complex particles. The synthesized complex particles were environmentally sensitive in which their mean diameter could change with the pH value of medium. Moreover, the complex particles showed a continuous release of the encapsulated doxycycline hyclate up to 8 days. These complex particles with environmentally sensitive properties are expected to be utilized in the hydrophilic drug delivery system. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 120: 1659–1670, 2011

Key words: chitosan; poly(acrylic acid); complex particles; hollow structure; drug delivery

INTRODUCTION

Recently, polymeric particles with a hollow structure have been widely investigated as carriers for drug delivery, cosmetics encapsulation and diagnostics.[1–4] Because of their hollow structure, they can encapsulate a large amount of guest molecules and release them at a later stage in a well-controlled manner.[5,6] Several different methods have been used to prepare polymeric hollow particles, such as the layer-by-layer deposition of polyelectrolyte on a template core,[7–10] polymerizing monomers in lipid vesicles,[11,12] self-assembly of block copolymers in an appropriate solvent,[13,14] and emulsion (or miniemulsion) polymerization.[15,16] For the biomedical applications, it would be better if polymeric hollow particles also have properties of biocompatibility and biodegradability. Consequently, polymeric hollow particles made of synthetic biodegradable polymers such as poly(lactic acid) (PLA), poly-ε-caprolactone (PCL), and their copolymers have attracted a lot of interest.[14] However, because of their hydrophobic properties, these polymeric hollow particles are not suitable for the delivery of hydrophilic drugs such as protein, antibiotic, and some anticancer drugs. Hence, hydrophilic polymeric hollow particles have been developed as hydrophilic drug carriers. One of these developed hydrophilic systems is made of poly(acrylic acid) (PAA) shell and PCL core.[17] The PCL core template is then removed by the use of an organic solvent to create a hollow structure, leaving the hydrophilic PAA shell. This system has a disadvantage that an organic solvent has to be used.

Chitosan (CS) is a high-molecular-weight polysaccharide composed mainly of β-(1,4) linked 2-deoxy-2-amino-D-glucopyranose units and partially of β-(1,4) linked 2-deoxy-2-acetamido-D-glucopyranose. CS can be dissolved in an acid solution and then becomes a cationic polymer due to the protonation of amino groups on the C-2 position of pyranose ring. Because of its advantageous properties including biodegradability, biocompatibility, antibacteria and nontoxicity, CS can be used in the fields of wastewater treatment, food processing, cosmetics, pharmaceuticals, biomaterials, and agriculture.[18–20] In the field of drug delivery, CS and its derivatives have been designed as carriers in various formulations, such as film, gel, and

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microsphere for delivery of vaccines, DNA, and insulin.[21–24] Many methods have been developed to prepare CS particles including emulsion, spray-drying, and emulsion-droplet coalescence technique. Recently, Cheng et al.[25] prepared CS-polypyrrole hollow spheres by using AgBr crystal as the core template. Using different shapes of AgBr crystal, the size and morphology of hollow spheres could be controlled. Hu et al.[4] reported the preparation of CS-PAA hollow spheres by an emulsion polymerization method without using a core template material. It is therefore possible to synthesize CS-based hollow spheres for biomedical applications.

In this study, CS was first dissolved in the acrylic acid (AA) solution. During dissolution, proton transfer from AA to CS occurred, and subsequently electrostatic interactions between protonated amino groups of CS and carboxylate groups of AA developed, leading to the formation of polymer-monomer complex micelles.[4,26] By the addition of potassium persulfate (KPS) initiator, polyelectrolyte complex particles were produced by the polymerization of AA monomer in the micelles. After polymerization, characterizations of CS-PAA complex particles were carried out including structure, morphology, particle size, and surface charge. In addition, the applications of CS-PAA complex particles in pH buffering and drug release were also studied and reported in this article.

EXPERIMENTAL

Materials

Chitosan (CS) with a degree of deacetylation of 95% and an average molecular weight of 200,000 g/mol was obtained from Kio-Tek (Taipei, Taiwan). Acrylic acid (AA) monomer from Acros (Geel, Belgium) was distilled under reduced pressure before use. Potassium persulfate (KPS, $K_2S_2O_8$), was a reagent-grade from Acros (Geel, Belgium). All the other chemicals were analytical-grade or above and used as received without further purification.

Synthesis of CS-PAA complex particles

A desired amount of CS was first dissolved in 20 mL aqueous solution containing 0.11 g (1.528 mmol) AA monomer. The solution was purged with nitrogen and heated to 80°C in an isothermal water bath. KPS (0.041g) was dissolved in 5 mL of water and preheated to 80°C, before it was poured into the solution. After 2 h of reaction, a dispersion aqueous-solution containing CS-PAA complex particles was obtained. The reaction conditions and the sample code of complex particles are listed in Table I. The molar ratio of CS to AA is based on the moles of glucosamine unit in CS to the moles of carboxylic acid group in AA.

TABLE I
Reaction Conditions and Sample Code of Final Products from Different Reaction Systems[a]

Sample	CS/AA (molar ratio)[b]	AA (g)	KPS (g)	H_2O (mL)
CS/AA = 0.2/1.1	0.2/1.1	0.11	0.041	25
CS/AA = 1.0/1.1	1.0/1.1	0.11	0.041	25
CS/AA = 2.0/1.1	2.0/1.1	0.11	0.041	25

[a] All reactions proceeded at 80°C for 2 h.
[b] The feeding molar ratio of CS/AA was based on the moles of glucosamine unit in CS to the moles of carboxylic acid group in AA.

Structure and morphology analysis

Structure analysis was carried out with a Fourier transform infrared (FTIR) spectrophotometer (FTS3000, Bio-Rad, Cambridge, MA) and solid state NMR spectrometer (DSX-400WB, Bruker, Billerica, MA). The dispersion solution containing CS-PAA complex particles was taken out for dialysis (in DI water, 72 h, 50 kDa cut-off) to remove the residual KPS and unreacted monomer. The dialyzed dispersion solution was then frozen at −20°C for 48 h, and lyophilized by a freeze-dryer to obtain dried CS-PAA complex particles. CS-PAA complex particles were mixed with potassium bromide and pressed to a transparent disk for obtaining FTIR spectra. The disk was scanned from 4000 to 400 cm^{-1} for 16 times to average the signal. Samples for obtaining solid-state NMR spectra were ground to fine powder and analyzed by a solid-state NMR instrument (100 MHz for ^{13}C nucleus).

The morphology of CS-PAA complex particles was observed with TEM (JEOL JSM-1230 EX II, Tokyo, Japan). One drop of CS-PAA dispersion solution, being diluted with deionized water, was dropped on a Formvar carbon-coated Cu grid. The residual water on the grid was removed by blotting with a filter paper from the bottom of the grid. The grid was then air-dried at room temperature. To observe the interior structure of CS-PAA complex particles, the dispersion solution was frozen at −20°C for 48 h, and lyophilized by a freeze-dryer to obtain dried CS-PAA complex particles. A few granules of dried CS-PAA were embedded in an acrylic resin and subjected to microtome to obtain an ultrathin section about 90 nm thick. The interior structure and composition of CS-PAA complex particles were analyzed by TEM-EDS (Tecnai F20 G2, FEI, Hillsboro, OR).

Particle size and ζ-potential of CS-PAA complex particles

The hydrodynamic diameter and size distribution of the synthesized complex particles were measured by a dynamic light scattering (DLS) method

(Zetasizer 3000HS, Malvern, UK). Before measurement, samples were all adjusted to a proper concentration. The results obtained were the mean value (±SD) (base on intensity) of 10 determinations and repeated three times. ζ-potential of complex particles was obtained with a Zetasizer 3000HS. Samples were diluted to a concentration of 1wt% in 0.01*M* NaCl solution. The results were the average of three runs.

pH-buffering ability

pH-buffering ability of CS-PAA complex particles was evaluated by the following method. Aqueous solutions with different pH values at 3, 5, 7, 9, and 11 were prepared first by using sodium hydroxide and hydrogen chloride. Then, 0.01 g of the dried CS-PAA complex particles powder was added and dispersed in the 10 mL aqueous solution. The variation of pH value of the solution with time was then recorded.

Preparation of drug-loaded CS-PAA complex particles

A specific amount of doxycycline hyclate was dissolved in 25 mL CS-PAA dispersion solution at 25°C. After incubation for 96 h, the drug-loaded complex particles were separated from the solution by centrifugation at 40,000 rpm (19,000*g*) and 4°C for 40 min (L8-70M, Beckmann, Fullerton, CA). The resulting drug-loaded CS-PAA complex particles were maintained in the wet state. To calculate the encapsulation efficiency and drug loading content of complex particles, the drug-loaded CS-PAA complex particles were dried in a vacuum oven at 60°C for 48 h and then weighed. Doxycycline hyclate concentration in the supernatant was also analyzed by the UV absorbance at a specific wavelength of 346 nm, using a UV–Visible spectrophotometer (Heλios-α, Thermo Spectronic, Waltham, MA). The measurement was performed in triplicate. The amount of the drug in the complex particles thus could be calculated by subtracting the amount of drug from the supernatant. The encapsulation efficiency and drug-loading content were calculated by the following equations.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Weight of the drug in complex particles}}{\text{Weight of the feeding drug}} \times 100 \quad (1)$$

$$\text{Drug-loading content (\%)} = \frac{\text{Weight of the drug in complex particles}}{\text{Weight of the complex particles}} \times 100 \quad (2)$$

Drug release experiment

Doxycycline hyclate-loaded CS-PAA complex particles (0.15 g) (wet state) was resuspended in a 5-mL normal-saline solution, which was then placed in a dialysis bag (3500 Da cut-off). The dialysis bag was then immersed in a 195-mL normal saline solution at 37°C. After a predetermined period, 3 mL of the normal saline solution was drawn out from the system for analysis and 3 mL fresh medium was added into the release system. The release amount of doxycycline hyclate was determined by a UV analysis at 346 nm with a calibration curve as described previously.

RESULTS AND DISCUSSION

Synthesis and structure of CS-PAA complex particles

CS-PAA polyelectrolyte complex was prepared through polymerization of AA monomer in the presence of CS. Before polymerization, CS was added into the AA aqueous solution first, and proton transfer from the carboxylic acid of AA to the amino group of CS occurred during dissolution due to the acid–base neutralization, thus producing anionic AA and cationic CS. Gradually, positive charges along CS chain exerted repulsive force which extended CS chain and rendered it to be dissolved in the aqueous solution. Gradually, micelles of CS-AA complex were formed due to the electrostatic interactions between protonated amino groups in CS with carboxylate groups of anionic AA, which will be discussed in detail in TEM section. As the KPS initiator was added into the CS/AA solution at the reaction temperature, it dissociated into two anionic sulfate radicals ($^{\bullet}SO_4^-$) which could initiate free radical polymerization of AA monomer to form PAA homopolymer. However, in the previous studies, it was found that the anionic sulfate radical ($^{\bullet}SO_4^-$) can degrade one CS chain into two shorter chains. One has a terminal carbonyl group and the other has a free radical at the scission end.[27,28] It is thus expected that if KPS was used to initiate the polymerization in the present system, it could also degrade CS and thus produce CS chain radical. Subsequently, AA was able to grow on the CS chain radical to produce CS-*co*-PAA copolymer, in addition to the PAA homopolymer.[28] Furthermore, when the extent of polymerization reached a certain level, polyelectrolyte complex would be formed between positively charged CS and negatively charged PAA chains. It was observed that at the early stage of polymerization, the reaction solution was still clear. Yet, as the polymerization continued, the solution became translucent, revealing the formation of CS-PAA complex particles.

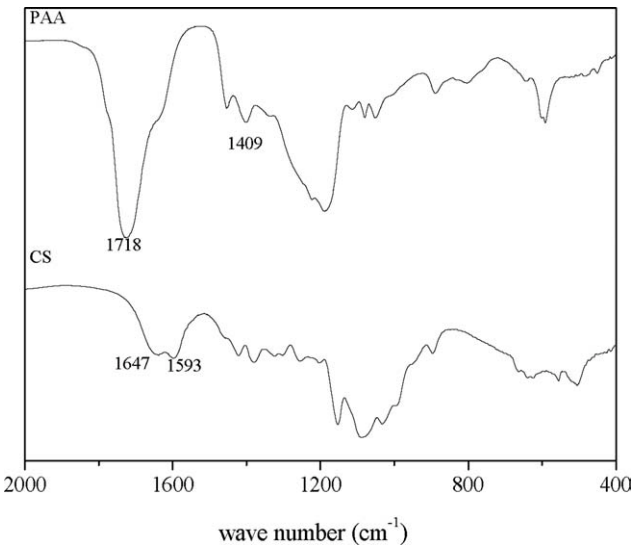

Figure 1 FTIR spectra of pure PAA and CS.

FTIR spectra of pure CS, PAA, and CS-PAA complex particles were analyzed to investigate their chemical structures. Figure 1 shows that the most important absorption peak in PAA is at 1718 cm^{-1}, caused by the C = O stretching vibration in carboxylic acid group. The characteristic peaks of CS are NH_2 bending vibration at 1593 cm^{-1} and C = O stretching vibration (amide I) at 1647 cm^{-1}.[29,30] Figure 2(a) shows the FTIR spectrum of a CS-PAA sample, CS/AA = 1.0/1.1. This sample was produced by feeding CS and AA at a molar ratio of 1.0/1.1 based on glucosamine unit in CS and carboxylic acid group in AA. In the spectrum, the characteristic peaks of the NH_3^+ absorption at 1628 cm^{-1} and COO^- symmetric stretching absorption at 1409 cm^{-1}

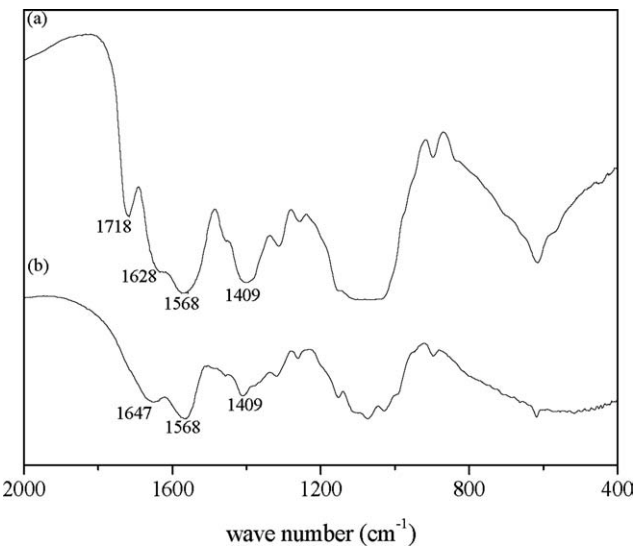

Figure 2 FTIR spectra of CS-PAA complex particles, CS/AA = 1.0/1.1 (a) before and (b) after 1*M* $NaOH_{(aq)}$ washing for 72 h.

are found.[30,31] This evidence confirms the proton transfer from the carboxylic acid of AA to the amino group of CS, resulting in the COO^- and NH_3^+ groups. Subsequently, anionic PAA and cationic CS form polyelectrolyte complex through ionic interaction.[30,32,33] On the other hand, CS-*co*-PAA copolymer was also produced during polymerization using the KPS initiator as explained previously. To prove the existence of copolymer, 1*M* $NaOH_{(aq)}$ solution was used to extract PAA homopolymer exhaustively from the complex particles. FTIR spectrum, Figure 2(b), shows that the COO^- symmetric stretching absorption peak (1409 cm^{-1}) still existed after 72 h of extraction. This result implies that the CS-*co*-PAA was produced during the polymerization.

A solid state ^{13}C-NMR spectrometer was also used to investigate the structure of CS-PAA complex particles. Figure 3 shows the absorption spectra of pure

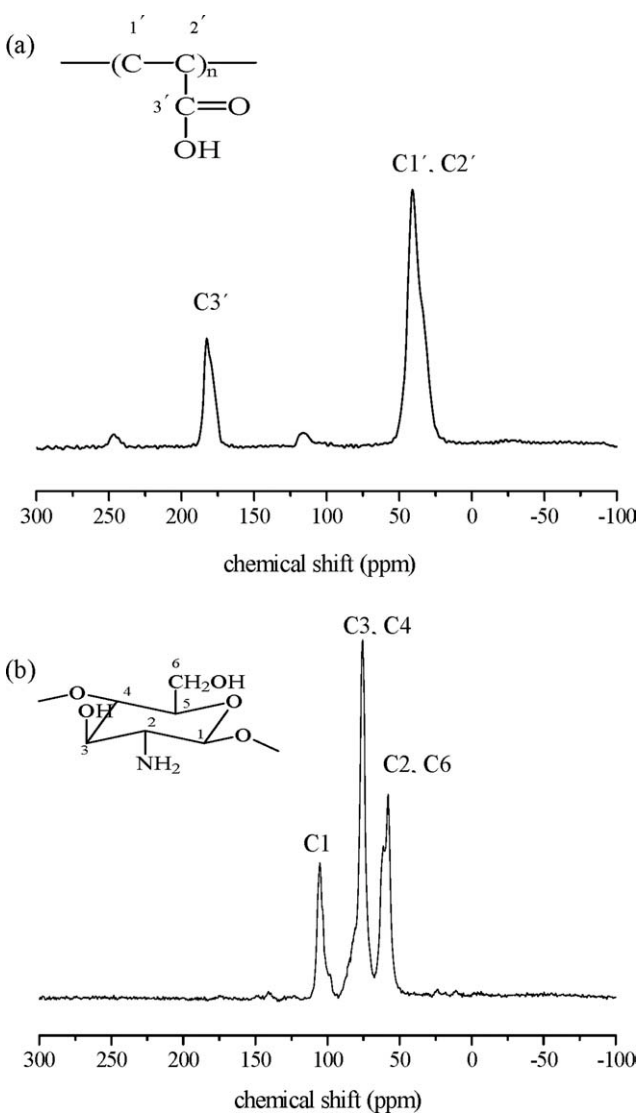

Figure 3 ^{13}C-NMR spectra of pure PAA (a) and pure CS (b).

TABLE II
^{13}C-NMR Chemical Shift of Various Carbons in the CS, PAA, CS/PAA Blend and CS-PAA Complex Particles (CS/AA = 1.0/1.1)

Sample	Carbon position	Chemical shift (ppm)
CS	C1	105.38
	C2, C6	57.95
	C3, C4	75.66
PAA	C1′, C2′	40.84
	C3′	176.77
CS/PAA blend	C1	99.98
	C2, C6	57.77
	C3, C4	75.11
	C1′, C2′	40.92
	C3′	177.84
CS-PAA complex particles	C1	98.92
	C2, C6	62.06
	C3, C4	75.89
	C1′, C2′	46.11, 44.34, 36.89, 33.00
	C3′	177.96

CS and PAA, and the chemical shift values of various carbons are listed in Table II. Figure 4 displays the spectra of a polyelectrolyte complex formed by directly blending CS and PAA (CS/PAA blend) and the synthesized complex particles of CS/AA = 1.0/1.1. Compared with the spectra of pure CS and PAA, the resonance absorption peaks of the CS/PAA blend in Figure 4(a) are exactly the addition of the respective peaks in the two individuals. It is because that there is no chemical bond formation between the CS and PAA in the blend. The formation of polyelectrolyte complex between CS and PAA still can not cause the change of chemical shift. Yet, in the spectrum of CS/AA = 1.0/1.1, as shown in Figure 4(b), slight changes in chemical shift occur in the C1′ and C2′ absorption peaks of PAA. The absorption peaks become broad and split into several peaks at 33, 36, 44, and 46 ppm. This strongly suggests that the electron densities around C1′ and C2′ in some AA repeating units are different from the others. The chemical bonding of AA unit on the degraded CS chain radical during polymerization is therefore believed to be the reason for the findings in the NMR spectra, in agreement with the previous studies.[27,28] In other words, CS-*co*-PAA copolymer and PAA homopolymer were both produced during the reaction, if KPS was used as the initiator.

Morphology observation of CS-PAA complex particles

Figure 5 shows TEM micrographs of CS-PAA complex particles in the system of CS/AA = 1.0/1.1 developed at different reaction times. It is found that the micelle-like structure was already formed before polymerization as shown in Figure 5(a), which is believed due to the electrostatic interaction between protonated amino groups (NH_3^+) in CS and anionic acrylate (AA^-). At this stage, the pH value of the solution and zeta potential for the CS-AA complex micelle were measured as 4.28 and 45.9 mV, respectively. The positive surface charge indicates that there were still some free protonated amino groups left in CS and resided on the outer surface of micelles. After polymerization, both AA and AA^- constituted the PAA polymer and the morphology of micelles gradually changed. At 30 min of polymerization, the droplets tended to become spherical in shape and appeared in a smaller size as shown in Figure 5(b). After 2 h of polymerization, CS-PAA complex particles with a diameter of 95 ± 12 nm were produced as shown in Figure 5(c), and their zeta potential was found to be 26.2 ± 0.8 mV. The reason for the decrease of particle diameter and zeta

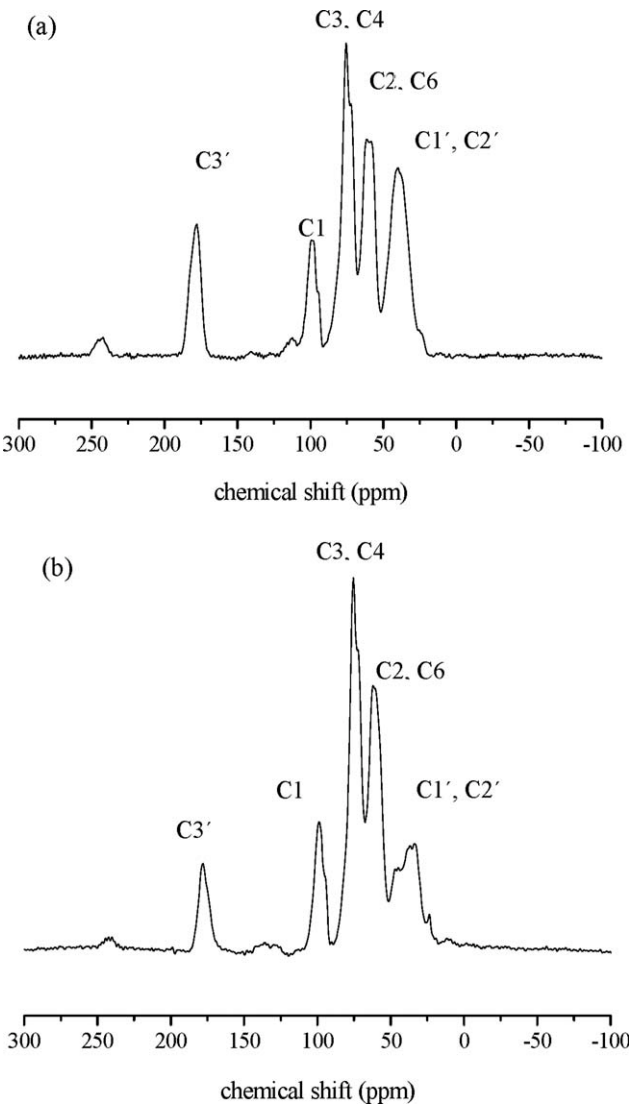

Figure 4 ^{13}C-NMR spectra of (a) CS/PAA blend and (b) synthesized CS-PAA complex particles, CS/AA = 1.0/1.1.

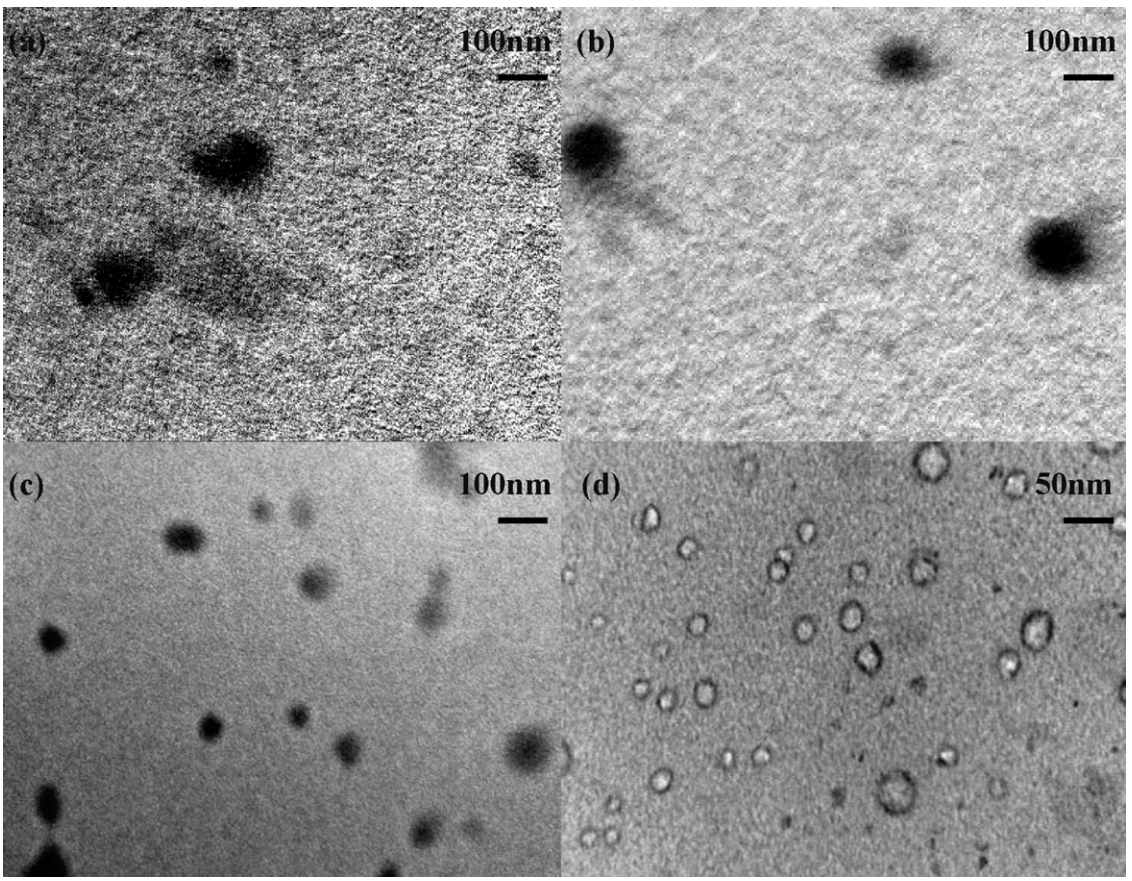

Figure 5 Morphology of the CS/AA = 1.0/1.1 complex particles at different polymerization times (a) before the addition of KPS initiator, (b) 30 min, (c) 120 min, (d) slice section of complex particles obtained at 120 min of reaction.

potential is that more and more PAA chains were produced as polymerization proceeded. These PAA chains with negative charges could form more polyelectrolyte complex with cationic CS chains and thus decreasing the magnitude of positive charge. Because the polyelectrolyte complex is more rigid and hydrophobic, its formation thus could cause the decrease of particle diameter.

To examine the interior structure of CS-PAA complex particles, ultra-thin section of about 90 nm was prepared by microtoming an acrylic resin embedded with CS-PAA complex particles. A TEM photograph of the CS/AA = 1.0/1.1, Figure 5(d), shows many dark rings present in the picture, suggesting these complex particles have a hollow structure. The formation of a hollow structure originated from the initial CS-AA micelle.[4] Since there were nearly equivalent amounts of glucosamine and AA in the feed, most anionic AA^- would interact with protonated amino groups in CS to form CS-AA micelles. After the addition of KPS initiator, AA and anionic AA^- polymerized to form anionic PAA chains, which were then attracted to the cationic CS chains on micelle's surface to form polyelectrolyte complex. The polyelectrolyte complex thus constituted the shell of nanoparticles. After freeze-drying, a hollow structure in these CS-PAA complex particles was thus observed.

Moreover, it is found that the formation extent of polyelectrolyte complex and the resulting morphology of CS-PAA depend on the concentrations of CS and AA, CS/AA molar ratio in the feed, and the pH value of the reaction medium. In some cases, polyelectrolyte complex precipitated out from the solution if the molar ratio of CS to AA was not controlled. In other cases, if the concentration of AA monomer was too high, the reaction solution became gel. The effect of feeding molar ratio of CS to AA on the morphology of complex particles was also studied by TEM. Figure 6 shows TEM photographs of two CS-PAA systems prepared from two different CS/AA molar ratios other than 1.0/1.1. When CS was less than AA in the feeding amount (CS/AA = 0.2/1.1), not only CS-PAA complex particles but also PAA homopolymer particles were observed, as shown in Figure 6(a). This indicates that in addition to the self-assembled CS/AA micelles, the excess AA monomer would be freely dissolved in the solution. Therefore, as KPS initiator was added into the solution, the excess free AA monomer could undergo polymerization to form PAA homopolymer. Yet, by increasing the feeding amount of CS, more and more AA monomer would

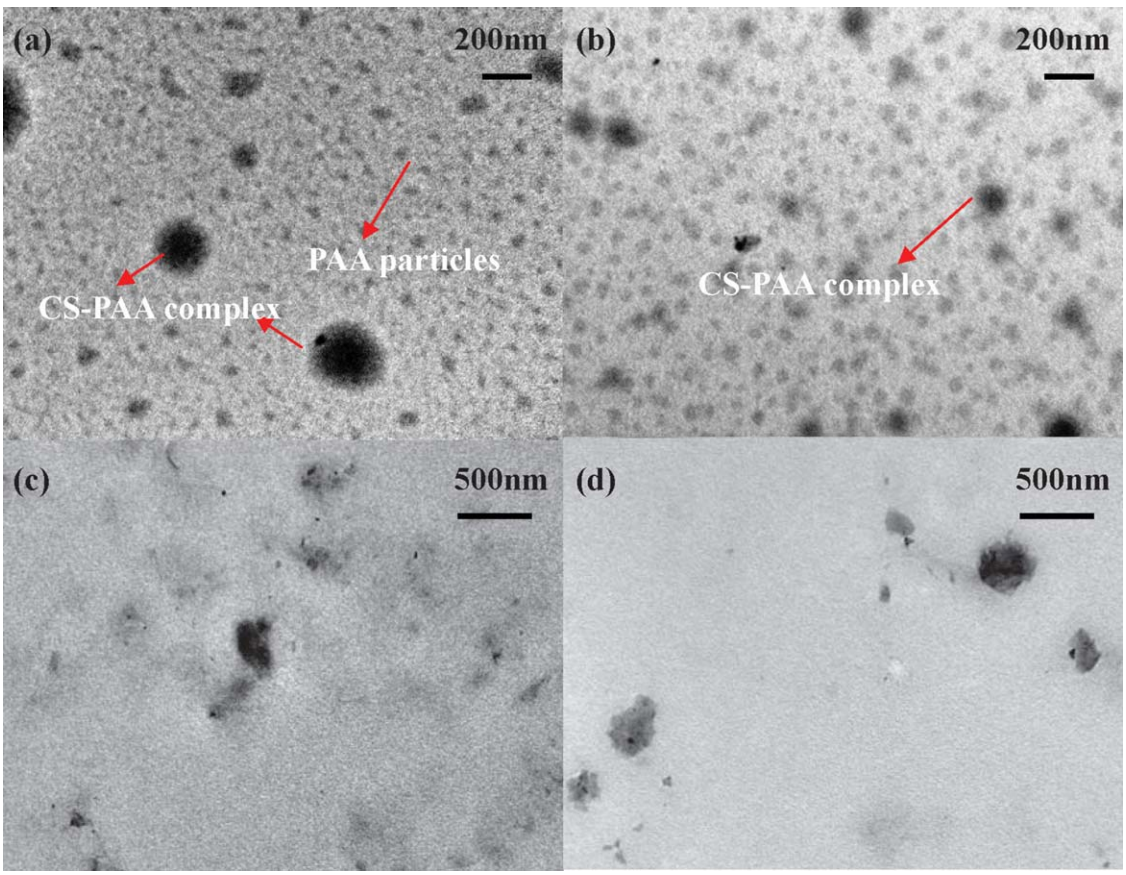

Figure 6 TEM images of synthesized CS-PAA complex particles with different CS/AA molar ratios. (a) CS/AA = 0.2/1.1, (b) CS/AA = 2.0/1.1, (c) slice section of complex particles of CS/AA = 0.2/1.1, (d) slice section of complex particles of CS/AA = 2.0/1.1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

diffuse to assemble with CS, thus decreasing the amount of free AA in the solution. The increasing amount of CS-AA micelle could be indirectly proved by the observation of an increase in the pH value of medium from 2.69 for the CS/AA = 0.2/1.1 solution to 4.28 for the CS/AA = 1.0/1.1 solution and to an even higher value of 5.10 for the CS/AA = 2.0/1.1 solution. Furthermore, when CS was fed in excess as in the system of CS/AA = 2.0/1.1, Figure 6(b), coagulation would occur among particles in the dispersion solution after 3 days of storage, indicating an insufficient stability in this dispersion solution.

TEM photographs of microtomed sections of CS-PAA complex particles are shown in Figure 6(c,d) for the CS/AA = 0.2/1.1 and CS/AA = 2.0/1.1, respectively. In contrast to the sample CS/AA = 1.0/1.1 shown in Figure 5(d), the hollow structure is no longer observed. To examine the composition of complex particles, TEM-EDS was used to analyze the interior composition of CS/AA = 0.2/1.1 complex particles, and the results are displayed in Figure 7. Only carbon and oxygen atoms were found in the center of complex particles, where nitrogen atom could not be traced. On the contrary, nitrogen atom was detected on the edge of complex particles, proving that CS was on the surface shell of complex particles. It is thus reasonable to deduce that the main component in the interior of CS/AA = 0.2/1.1 complex particles is PAA homopolymer. The results therefore suggest that only a certain value of CS/AA molar ratio would result in the formation of hollow particles. When the molar ratio of CS/AA is low, there is a plenty of AA monomer which can polymerize to form both CS-PAA polyelectrolyte complex and PAA homopolymer. The PAA homopolymer can be formed not only inside but also outside the complex particles; therefore, complex particles of a solid structure (instead of a hollow structure) and smaller PAA homopolymer particles are found. When the CS/AA molar ratio is high, there would not be enough AA monomer to supply protons for the amino groups in CS. Therefore, positive charge along CS chains would not be sufficient to maintain the hollow structure during the formation of CS-PAA complex particles.

Particle size and zeta potential of CS-PAA complex particles

Table III shows the particle size and surface charge of CS-PAA complex particles measured by a DLS

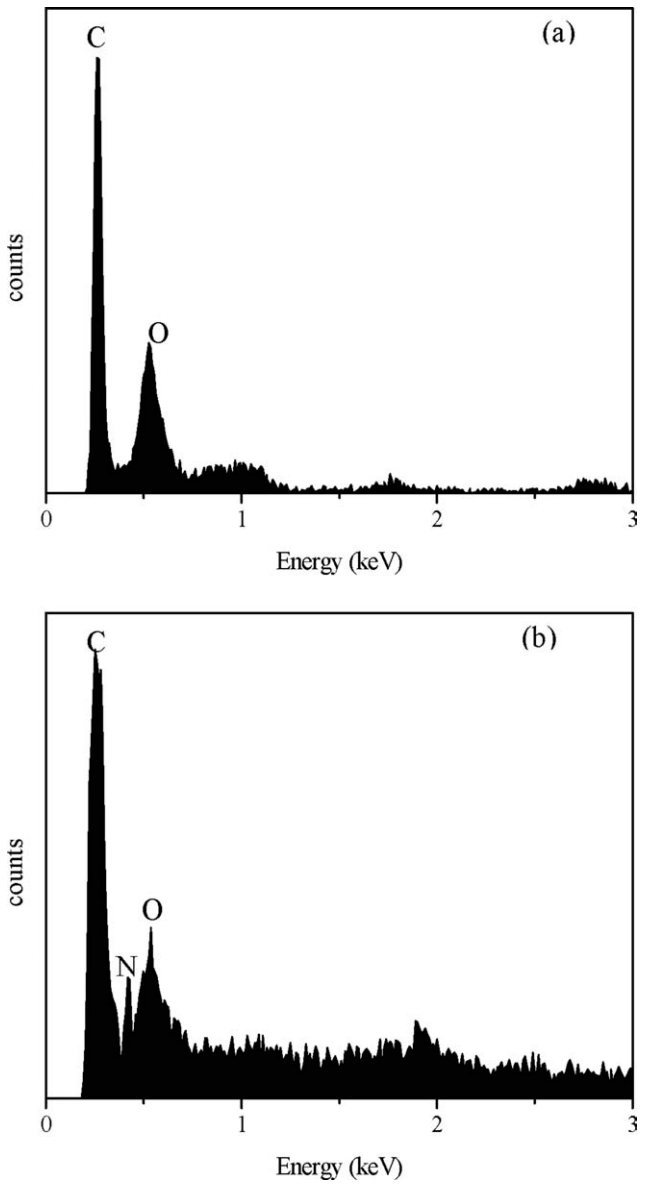

Figure 7 TEM-EDS analysis of CS-PAA complex particles of CS/AA = 0.2/1.1. (a) Center of complex particles, (b) edge of complex particles.

and electrophoretic LS, respectively. For the present system, all complex particles have particle size smaller than 200 nm and the smallest one is found for the CS/AA = 1.0/1.1 system. This is explained by the extent of formation of polyelectrolyte complex. In the system of CS/AA = 0.2/1.1, CS was less than AA, and the pH value of reaction medium was the lowest. Therefore, CS chains would be in the most extended state among three systems. Moreover, it is possible that some PAA chains could be absorbed to the CS-PAA complex particles due to the electrostatic attraction. As a result, the particle size of CS/AA = 0.2/1.1 system would be the largest. This has the same trend as already observed in TEM pictures (Fig. 6). In the system of CS/AA = 1.0/1.1, the molar amount of glucosamine unit in CS was close to that of carboxylic acid group in AA. After polymerization, this system thus would have the highest formation extent of polyelectrolyte complex, resulting in the tightest structure and the smallest particle size. In the system of CS/AA = 2.0/1.1, AA was less than CS, and thus only a limiting amount of proton could be supplied for the protonation of amino group in CS. Therefore, positive charge in CS chains was smaller that would decrease the formation extent of polyelectrolyte complex. The data in Table III show that this system had the larger particle size than CS/AA = 1.0/1.1.

The results of zeta potential are also listed in Table III. It is found that all the CS-PAA complex particles have positive surface charge of about 21–26 mV. The positive surface charge is generally observed for most CS particles prepared in the acidic environment as reported by other authors, because of the cationic characteristic of CS in the acidic environment.[4,34,35] However, the zeta potential of CS-PAA complex particles would vary with the feeding molar ratio of CS to AA. In the system of CS/AA = 0.2/1.1, AA was in excess. Therefore, there would be the fewest CS chains for each complex particle in this reaction system, though most amino groups in CS would be protonated. In addition, since AA was more than five times amount of glucosamine unit of CS, there would be enough PAA to form polyelectrolyte complex with CS, thus decreasing the positive charge of CS. As a result, the zeta potential of CS/AA = 0.2/1.1 complex particles was the smallest. In the system of CS/AA = 1.0/1.1, which had the nearly equivalent amounts of glucosamine unit and carboxylic acid group, most amino groups would also be protonated as in the system of CS/AA = 0.2/1.1, but the amount of CS chains on each complex particle in the former was higher than that in the latter. In addition, it is known that by decreasing the particle size, the surface charge density would be increased. As shown in Table III, the particle size of CS/AA = 1.0/1.1 was the smallest among three systems. Therefore, complex particles of CS/AA = 1.0/1.1 had the highest zeta potential. On the other hand, in the system of CS/AA = 2.0/1.1, CS was in excess so that the degree of protonation of

TABLE III
Mean Diameter and Zeta Potential of CS-PAA Complex Particles

Sample	Mean diameter[a] (nm)	Zeta potential (mV)
CS/AA = 0.2/1.1	191 ± 28	21.0 ± 0.1
CS/AA = 1.0/1.1	146 ± 16	26.2 ± 0.8
CS/AA = 2.0/1.1	179 ± 14	24.6 ± 0.3

[a] Base on intensity.

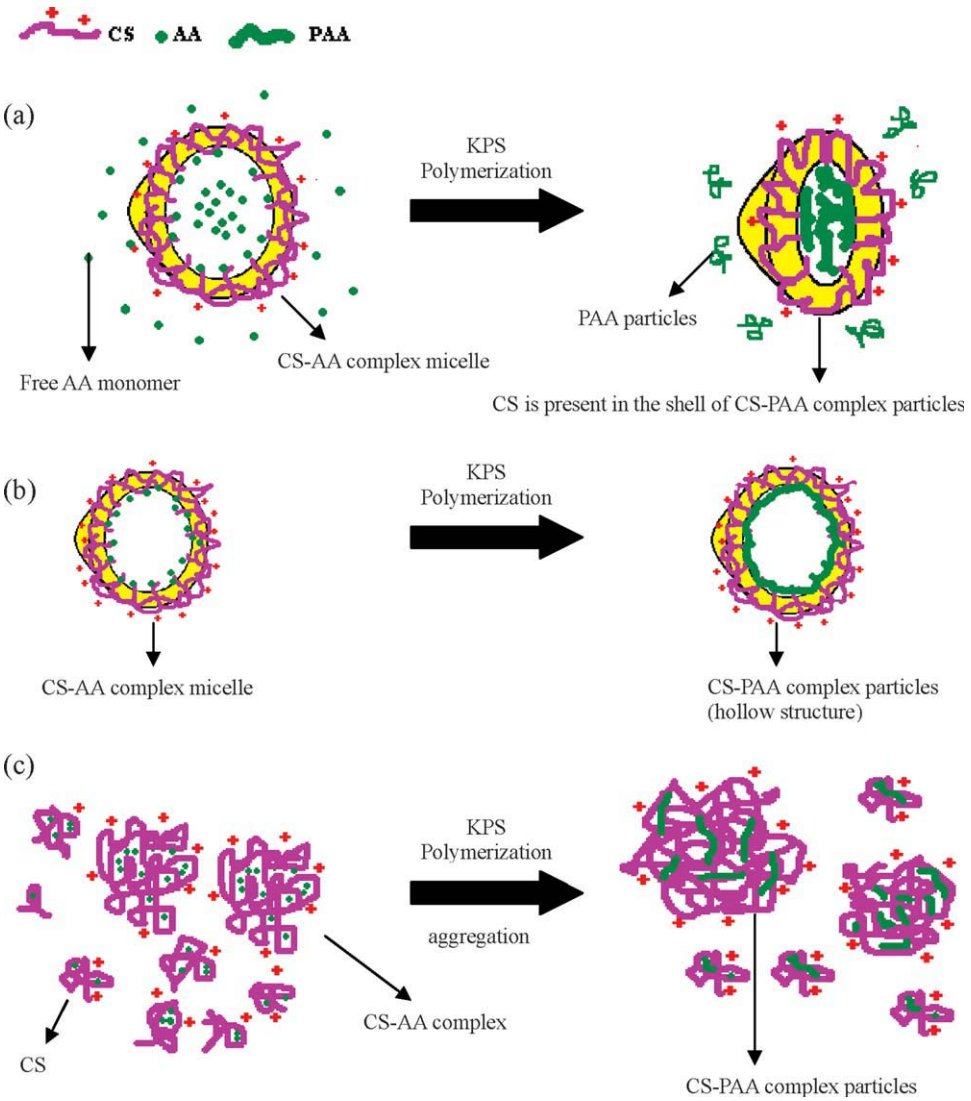

Figure 8 Formation mechanisms of CS-PAA complex particles with different feeding molar ratios of CS/AA. (a) CS/AA = 0.2/1.1, (b) CS/AA = 1.0/1.1, (c) CS/AA = 2.0/1.1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

amino group in CS would be the least. Although, there should be the highest amount of CS chains on each complex particle compared to the other two systems, the zeta potential still decreased. In conclusion, complex particles of CS/AA = 1.0/1.1 had the smallest particle size and the highest zeta potential.

Formation mechanism of CS-PAA complex particles

Figure 8 shows possible formation mechanisms of CS-PAA complex particles with different feeding molar ratios of CS/AA. When the feeding molar ratio of CS/AA is low as in the system of CS/AA = 0.2/1.1, in addition to the formation of CS-AA complex micelle, the excess AA monomer would be freely dissolved in the solution or be trapped inside the micelle, as depicted in Figure 8(a). When KPS initiator is added to initiate the polymerization, both PAA homopolymer and CS-PAA complex particles are thus produced. The PAA homopolymer synthesized within the micelle would fill in the interior space of CS-PAA complex particles, so that solid CS-PAA particles are observed after drying. When the feeding amounts of CS and AA are nearly equivalent as in the system of CS/AA = 1.0/1.1, AA can provide a sufficient amount of H^+ to protonate amino groups in CS, as shown in Figure 8(b). As a result, most CS chains would assemble with AA to form CS-AA complex micelles. The formation of CS-AA complex micelles would decrease the original hydrophilicity of AA monomer; therefore, the majority of AA is present closer to the inner surface of micelle. Upon polymerization, CS-PAA complex particles, which are polyelectrolyte complex in nature, are thus formed. The polyelectrolyte complex structure

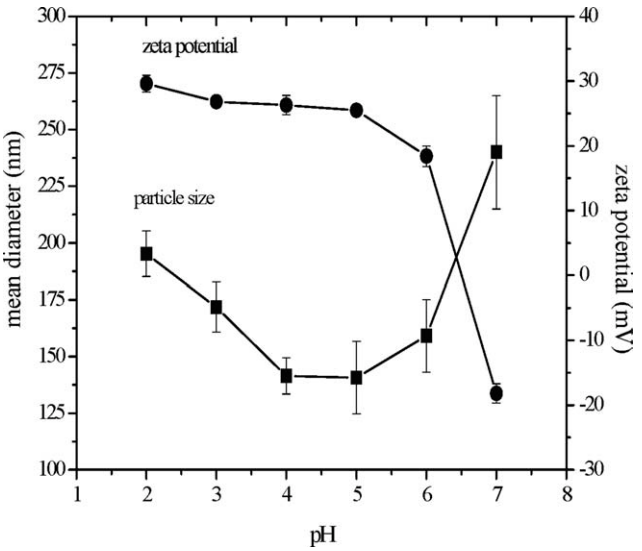

Figure 9 The variation of mean diameter and zeta potential of CS-PAA complex particles in CS/AA = 1.0/1.1 with the pH value of buffer.

enhances as the polymerization proceeds. Therefore, CS-AA complex micelles with a loose structure are gradually changing to compact CS-PAA complex particles. Actually, there are two kinds of force: an electrostatic attractive force between CS and PAA drawing particles to shrink, and an electrostatic repulsive force from positive charges (NH_3^+) along CS chains causing particles to expand. When these two opposing forces reach a balance, a hollow structure is formed and the shell is composed of CS-PAA polyelectrolyte complex.[4] When the feeding molar ratio of CS to AA is high as in the system of CS/AA = 2.0/1.1, Figure 8(c) shows that not all of CS molecules can assemble with AA to form CS-AA complex micelles. Under this condition, the protonation degree of CS is low due to the insufficient amount of AA. After the addition of KPS initiator, the CS-AA micelles go to polymerization to form CS-PAA complex particles. However, the surface charge of CS-PAA complex particles may not be enough to stabilize themselves, resulting in the coagulation and precipitation of particles.

pH response of CS-PAA complex particles

As we have described above, the polyelectrolyte complex plays an important role in the formation of CS-PAA particles. It is expected that changing the pH value of medium may cause the changes in particle size and surface charge of complex particles. Figure 9 shows the variation of mean diameter and zeta potential of CS-PAA complex particles in CS/AA = 1.0/1.1 with the pH value of medium. The mean diameter of complex particles had the minimum in the pH range of 4.0–5.0 and increased significantly as the buffer became more acidic or basic. This is because the ionization degrees of both CS and PAA change with the pH value, thus causing the change of the polyelectrolyte complex structure and therefore its mean diameter. At a low pH environment such as pH 2, nearly all the amino groups in CS are completely ionized. On the contrary, most carboxylic acid groups in AA remain neutral. Therefore, the electrostatic attraction between CS and PAA is weak. On the other hand, strong electrostatic repulsion exists among NH_3^+ groups along CS chain. Therefore, a larger particle size of CS-PAA complex particles is observed. At pH 7, PAA becomes highly ionized while CS has much less ionization. Thus, the electrostatic attraction between CS and PAA is also weak, but the electrostatic repulsion among COO^- groups of PAA chain becomes strong. Therefore, the expansion of complex particles is expected. When the pH value of medium is in the range of 4.0–5.0, both CS and PAA are partially ionized to some extent, and a compact polyelectrolyte complex is formed by the strong electrostatic interaction between CS and PAA. Therefore a minimum in mean diameter of complex particles is observed. Meanwhile, the zeta potential of complex particles shows a slow decrease yet still maintains a positive value as the pH increases from 2.0 to 6.0. This is because that the degree of protonation of CS is higher than the degree of ionization of PAA in this pH range, resulting in a positive zeta potential of complex particles. Increasing the pH value of medium would decrease the degree of protonation of CS and yet increase the degree of ionization of PAA. When the pH value reaches 7.0, a negative zeta potential is observed. At this pH value, the degree of ionization in PAA becomes much higher than the degree of protonation in CS, as it is known that the pKa of CS is about 6.3.[36] As a result, the zeta potential goes through a sharp decrease and changes from a positive value to a negative one.

Both individual CS and PAA are pH-responsive substances which can release or consume proton according to the environmental pH value. In an acidic medium, the amino groups of CS are protonated to form NH_3^+, and yet carboxylic acid groups remain in PAA. In a basic medium, CS has most neutral NH_2 groups, while the carboxylic acid groups of PAA react with hydroxyl ions to become carboxylate groups. Therefore, it is possible that CS-PAA complex particles can also be applied as a pH-buffering material. Figure 10 shows the temporal response of the sample CS/AA = 1.0/1.1 in pH buffering by changing the initial pH value of the test solution. It shows that the complex particles of CS/AA = 1.0/1.1 are most effective in buffering the basic medium. The possible reason is that the CS/AA = 1.0/1.1 system has the highest formation extent of

polyelectrolyte complex in a medium with a pH value ranged from 4 to 5 as proved in Figure 9. Therefore, these complex particles do not have much pH buffering effect in the acidic medium. Only in a very acidic medium such as pH 3, some free amino groups in CS could consume protons, thus increasing the pH value, yet only to 4. However, in the basic medium, the polyelectrolyte complex is disrupted in a way that most of the protonated amino groups in CS react with hydroxyl ions to become neutral amino groups ($-NH_3^+ + OH^- \rightarrow -NH_2 + H_2O$). As a result, the pH value is decreased and the medium approaches to the neutral state.

Release of doxycycline hyclate from drug-loaded CS-PAA complex particles

Doxycycline hyclate, which is a water soluble antibiotic and widely used in clinical therapy, was used as the model drug to carry out the drug-release test. Figure 11 shows the *in vitro* release profile of doxycycline hyclate from drug-loaded CS-PAA complex particles of CS/AA = 1.0/1.1 in a normal saline solution at 37°C. The encapsulation efficiency of doxycycline hyclate is 44% and the drug loading content is 1.6%. From Figure 11, an initial burst was observed and about 25% doxycycline hyclate was released in the first 8 h. After the initial burst, there was a continuous release of the encapsulated doxycycline hyclate up to 8 days. Finally, about 51% of encapsulated doxycycline hyclate was released from the drug-loaded complex particles. This result suggests that the CS-PAA complex particles have a sustained release behavior for doxycycline hyclate. A possible reason is that the amide groups of doxycycline hyclate can interact with some free carboxylic acid groups of PAA in the CS-PAA complex particles. This interaction could retard the release of doxycycline hyclate from the complex particles, resulting in a low release percentage of doxycycline hyclate.

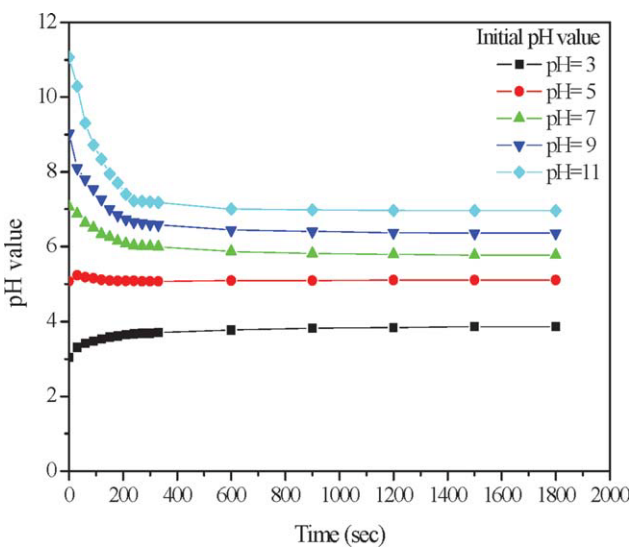

Figure 10 The pH temporal response of CS-PAA complex particles sample, CS/AA = 1.0/1.1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

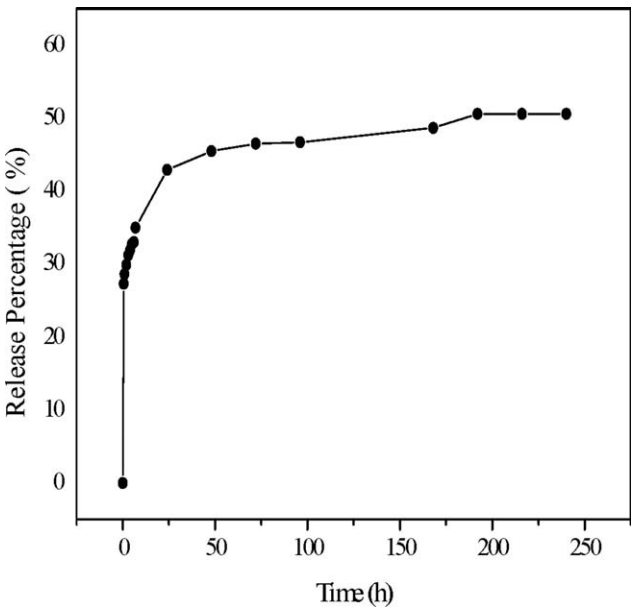

Figure 11 *In vitro* release behavior of drug-loaded CS-PAA complex particles in CS/AA = 1.0/1.1 in a normal saline at 37°C.

CONCLUSIONS

In this study, CS-PAA polyelectrolyte complex particles were successfully prepared by a free radical polymerization of AA monomer in the presence of CS using KPS as the initiator. Because of the proton transfer from AA to CS and thereby the electrostatic interaction between cationic CS and anionic AA^-, CS-AA complex micelles were formed during the dissolution of CS in the AA solution. After polymerization, these micelles turned into CS-PAA complex particles due to the formation of polyelectrolyte complex. When the polymerization was carried out with a feeding molar ratio of CS/AA = 1.0/1.1, the prepared complex particles had the smallest mean diameter and the highest zeta potential compared to the other two systems, i.e., CS/AA = 0.2/1.1 and CS/AA = 2.0/1.1. From TEM observation, a hollow structure in the complex particles of CS/AA = 1.0/1.1 was developed after polymerization. Moreover, the synthesized complex particles were pH-responsive. The mean diameter of complex particles can be manipulated by changing the pH value of medium. In the *in vitro* drug release test, a continuous release of the encapsulated doxycycline hyclate up to 8 days was observed. Therefore, the CS-PAA complex

particles have potential to be applied as the controlled release vehicle in hydrophilic drug delivery.

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