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A Way to Prepare Core-Shell Biocompatible Polymeric Nano-Particles from Gelatin and Acrylic Acid

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Narrowly distributed core-shell nano-particles at relatively high concentration (30 mg/mL) were prepared via in situ polymerization of acrylic acid in an aqueous solution of biocompatible gelatin. These polymeric nano-particles, in aqueous solution, had cores mainly comprised of an insoluble inter-polymer complex of poly (acrylic acid, PAA) and gelatin and shells comprised of soluble gelatin (denoted as gelatin/PAA nano-particles). Dynamic light scattering and electrophoretic light scattering techniques were used to trace the in situ polymerization process. The structure of the gelatin/PAA nano-particle was further locked-in via shell crosslinking; i.e., the reaction between glutaraldehyde and gelatin. Scanning force microscopy (SFM) was used to observe the morphologies of the particles before and after cross-linking. Furthermore, the pH responsive behaviors of the gelatin/PAA nano-particles before and after shell crosslinking were studied.

Keywords nano-particles, interpolymer complex, *in situ* polymerization, pH-response, biocompatible, high-concentration

Introduction

Because of their great potential in both the application and theoretical research fields, core-shell polymeric particles have attracted much attention; they can be used as carriers for catalysts, drugs, molecules with electronic and photonic functions, and bio-macromolecules (1–3). The polymeric particles with core-shell structure can be fabricated via the traditional micellization of block copolymers in selective solvents. In the process, the difference in the solubility between the blocks of a block copolymer is the driving force for the micellization. In the past decades, several new routes to the micellization have been developed (4–7). For example, it was reported that interpolymer complexation due to electrostatic interaction or hydrogen bonding interaction could induce the micellization (6, 7).

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However, micellization is usually conducted at a concentration no more than 5 mg/mL (8); otherwise, irregular aggregates or even precipitates will be produced. The rapid occurrence of micellization at a relatively high concentration results in the soluble block chains having no time to disentangle from each other and to adjust their conformation so as to surround the aggregated insoluble block. Consequently, the micellization method to produce core-shell polymeric particles (micelles) is of low efficiency.

Here, we report our progress in efforts to prepare core-shell polymeric nano-particles at higher concentration. Through *in situ* polymerization of acrylic acid (AA) in the presence of gelatin, which slowed down the micellization induced by the complexation between PAA and gelatin, core-shell biocompatible nano-particles of narrow size distribution at a concentration of 30 mg/mL were prepared successfully. The resulting nano-particles have a core composed of inter-polymer complex of PAA and gelatin and a shell of gelatin, and their structures were further locked by crosslinking the gelatin of the particles with glutaraldehyde.

Experimental

Materials

Gelatin (gelatin B, obtained by the basic hydrolysis method) from Nanxiang Chemical Co. Ltd. was of chemical grade. AA monomer of analytical grade from Shanghai Chemical Co. Ltd. was distilled under vacuum before use to remove the inhibitor. Ammonium persulfate (APS) was of analytical grade from Shanghai Qianjin Chemical Plant. N,N,N',N'-tetramethylethylenediamine (TEMED) was of analytical grade from Merck Co. Ltd. Glutaraldehyde(GA, 25% aqueous solution) employed as a cross-linking reagent for gelatin, was from Shanghai Chemical Co. Ltd. Deionized water was used in all the experiments.

Preparation of Gelatin/PAA Nano-particles

Gelatin was first dissolved in an aqueous solution and the pH of the solution was adjusted to around 2.5 by adding HCl aqueous solution followed by filtration to remove insoluble impurities. Next, AA (20 mg/mL) was added dropwise into 60 mL of the purified gelatin solution (10 mg/mL) under stirring in a nitrogen atmosphere. After the reaction temperature was raised to 40°C, the initiator APS and accelerator TEMED were added to initiate the polymerization. The reaction was allowed to proceed for 180 min. GA was then added to the reaction system and the crosslinking occurred for 1.5 h.

Measurements

Using a MALVERN DTS1060, the average dynamic diameter and zeta-potential of the uncrosslinked and crosslinked gelatin/PAA nano-particles at 25°C were determined by dynamic light scattering (DLS) and electrophoretic light scattering, respectively. DI-NSIV Scanning force microscope (Digital Instruments) was used to observe the morphology of the resultant particles. The samples were prepared by placing 5 ml of the suspension on freshly cleaved mica and allowing them to dry in the air. The topographies were taken in the tapping mode to reduce any damage of the sample caused by tip contact.

Results and Discussion

Formation of Core-Shell Gelatin/PAA Nanoparticles

Gelatin is a biological polymer derived from a partial collagen denaturation with good biocompatibility. Gelatin B, produced by a basic hydrolysis method, with an isoelectric point of 4.8 was used here. When the pH of the gelatin solution is adjusted below 4.8, gelatin macromolecules are positive charged due to protonation of the amino groups; while they are negative charged due to ionization of the carboxy groups when pH is above 4.8. Therefore, when adding poly(acrylic acid) solution dropwise into a gelatin solution with pH around 2.5 under stirring, we can expect that micellization driven by complexation interactions between gelatin and PAA will take place. As only a small part of the carboxyl groups of PAA are ionized in the acid medium, it is reasonable to believe that besides electrostatic interactions between the ionized carboxyl groups of PAA and the protonated amino groups of gelatin, hydrogen bonding interactions between the un-ionized carboxyl groups of PAA and the carbonyl groups of gelatin also contribute to the formation of an inter-polymer complex between PAA and gelatin. In order to prepare gelatin/PAA nano-particles at high concentration, we slowed down the micellization by in-situ polymerization of AA in the gelatin solution.

To monitor the change of the micellization during the polymerization process, the size and zeta-potential of the complex particles were traced by dynamic light scattering and electrophoretic light scattering, respectively (Figure1). Several facts can be drawn from Figure 1. First, the average hydrodynamic diameter ($\langle D_h \rangle$) of the initial gelatin/AA micelles was around 590 nm. As polymerization proceeded, the micelle size contracted greatly in the first 20 min to about 270 nm, and then the size contraction slowed down and contracted to around 240 nm at the end of the polymerization. Second, the change of zeta-potential of the micelles with polymerization time was similar to that of micelle size, that is, in the first 20 min, it decreased rapidly from an initial value of 14.7 mv to

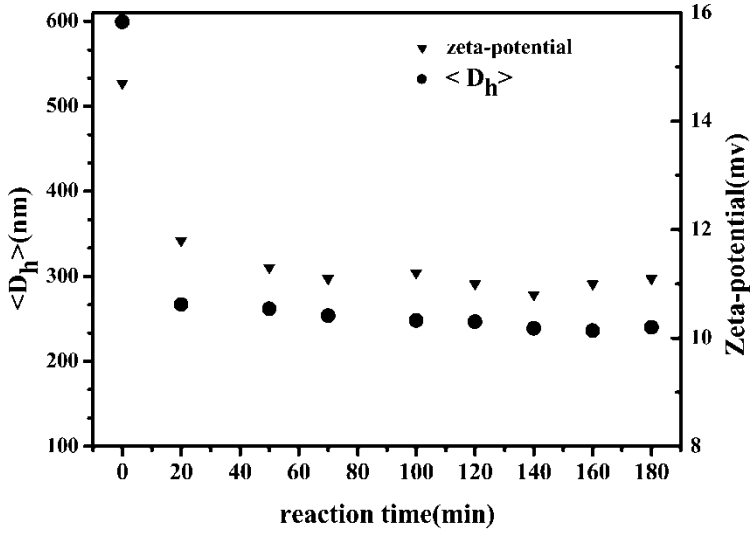

Figure 1. Change in the size and zeta-potential of gelatin/AA (PAA) micelles with polymerization time.

around 12.0 mv, and then the decrease slowed down and it was around 11mv at the end of the polymerization. As polymerization proceeded, the molecular weight of PAA increased greatly, which is accompanied by a great increase in the interactions (including electrostatic and hydrogen bonding interactions) between gelatin and PAA, thus causing a rapid contraction in the complex core and subsequently a drastic decrease in the micelle size. The decrease in the micelle zeta-potential indicated the enhancement in the electrostatic interactions between gelatin and PAA. Finally, the zeta-potential of gelatin/AA (PAA) micelles through the whole polymerization remained positive, which suggested that gelatin molecules with protonated amino groups were in the outermost layer of the particles. Therefore, it was reasonable to propose that the shells of the particles were comprised of positively charged gelatin chains, while the cores of the particles were comprised mainly of the complex of PAA and gelatin.

Figure 2 shows the hydrodynamic diameter distribution curve of the gelatin/PAA nano-particles. The resultant nano-particles are narrowly distributed around 240 nm with a P.D.I. of 0.08, proving that the *in situ* polymerization of AA on the template of gelatin did slow down the micellization process and led to the formation of well-defined core-shell nanoparticles at high concentration. The obtained gelatin/PAA nano-particles were stable during storage in the refrigerator for four weeks (Figure 3). However, when the storing time was longer, an obvious decrease in the particles size was observed, which may be caused by degradation of gelatin in the fairly strong acid medium (pH 2.5).

As the pH value affected the ionization of both gelatin and PAA and subsequently, the complexation between them, it can be expected that the change of pH value would have an important influence on the size of gelatin/PAA nano-particles. Figure 4 shows the size and the zeta-potential of gelatin/PAA particles solution at different pH value. The particles were stable only in the pH range of 2.5–3.0. When the pH was increased above 3.0, aggregation between particles occurred, caused by the decrease in the surface charge of the gelatin/PAA particles, leading to a drastic increase in the particles' size. When the pH increased further and approached the isoelectric point of gelatin, the gelatin shell of the particles became insoluble in the water. As a result, precipitates appeared. Upon further

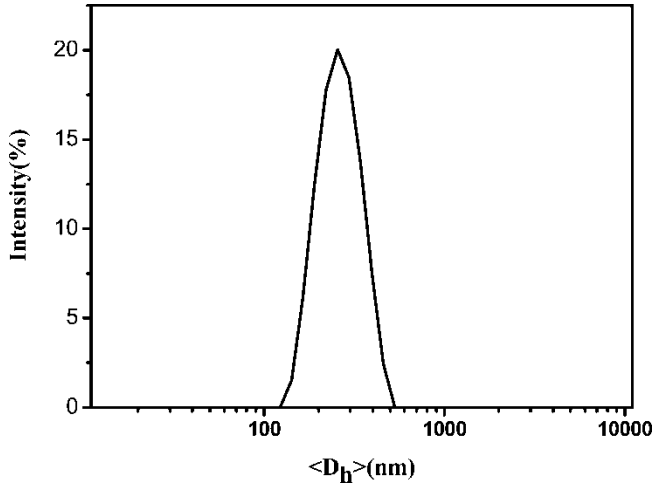

Figure 2. Hydrodynamic diameter distribution curve of core-shell gelatin/PAA nano-particles.

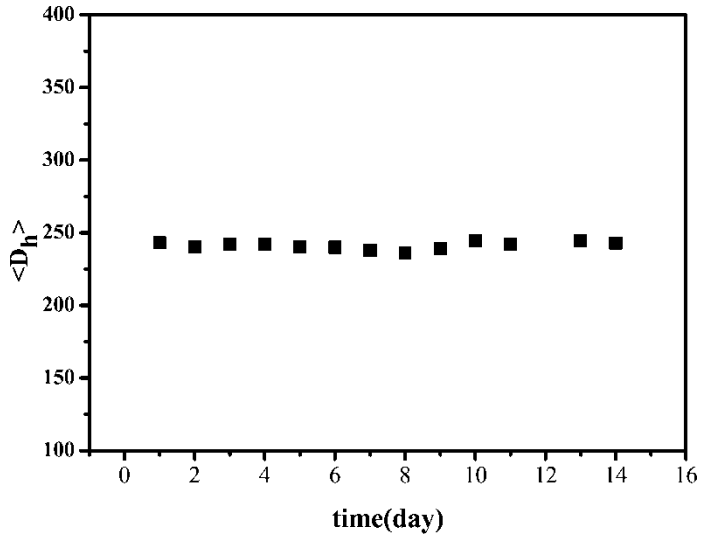

Figure 3. Change in hydrodynamic diameter of gelatin/PAA particles with storage time.

increasing the pH, there were no gelatin/PAA particles in the solution due to the decomplexation between PAA and gelatin.

Crosslinking of Gelatin/PAA Nano-Particle with GA

To improve their stability, we further locked the structure of the particles by shell crosslinking. GA, which has extensively been used as a cross-linking reagent for chitosan, cellulose, and starch (9, 10), was chosen to selectively crosslink gelatin

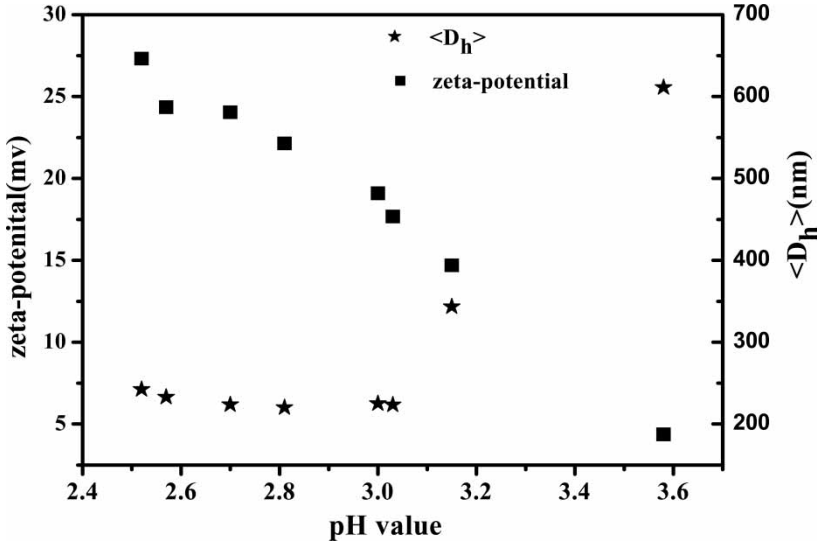

Figure 4. Size and zeta-potential of gelatin/PAA particles at different pH.

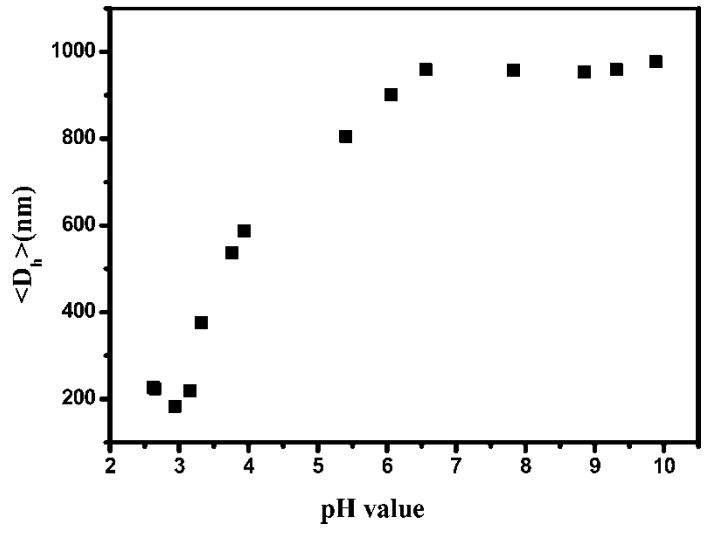

Figure 5. Size of crosslinked gelatine/PAA particles at different pH.

through the reaction of the hydroxyl groups or amino groups of gelatin molecules with aldehyde groups of GA.

After shell crosslinking, the size of the nano-particles decreased slightly, to around 226 nm. The pH-size dependence of crosslinked gelatin/PAA particles was also

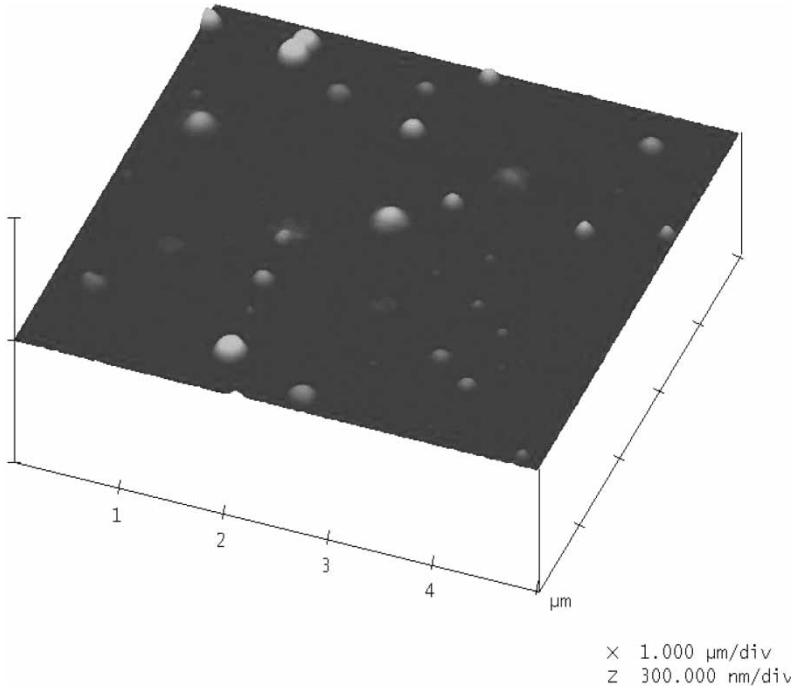

Figure 6. SFM micrograph of the gelatin/PAA particles before crosslinking.

studied; the results were shown in Figure 5. The size change of crosslinked gelatin/PAA particles with pH in the acid range of 2.5–4.0 is similar to that of uncrosslinked ones. It was mentioned above that in the near neutral and basic medium, un-crosslinked gelatin/PAA particles disintegrated, while the crosslinked particles remained stable and the particle size expanded greatly: the size increased from the initial value of 226 nm at pH 2.5 to around 900 nm at pH around 7. This large expansion in the particle size was caused not only by the decomplexation between PAA and gelatin but also by the repulsion interaction of the ionized carboxyl groups of gelatin and PAA. Obviously, the crosslinked gelatin/PAA particles exhibited good pH-response.

Scanning force microscopy (SFM) was used to observe the morphologies of the gelatin/PAA nanoparticles before and after crosslinking; the results are shown in Figures 6 and 7, respectively. The diameters of the uncrosslinked particles and the crosslinked ones in the SFM micrographs are in the range of 198–442 nm and 110–316 nm, respectively, which corresponds well with the diameter size range measured by DLS. The height of the uncrosslinked particles and the crosslinked ones are in the range of 33–58 nm and 60–88 nm, respectively, which are much smaller than their corresponding diameters, indicating that both uncrosslinked and crosslinked nanoparticles collapsed on the mica substrate during water evaporation in the sample preparation. In addition, by comparing Figure 6 with Figure 7, the particles collapsed less after shell crosslinking, indicating that shell crosslinking can improve the mechanical stability of the nanoparticles.

Conclusions

Gelatin/PAA nano-particles with gelatin as the shell and an inter-polymer complex based on PAA and gelatin as the core were prepared at high concentration via *in situ*

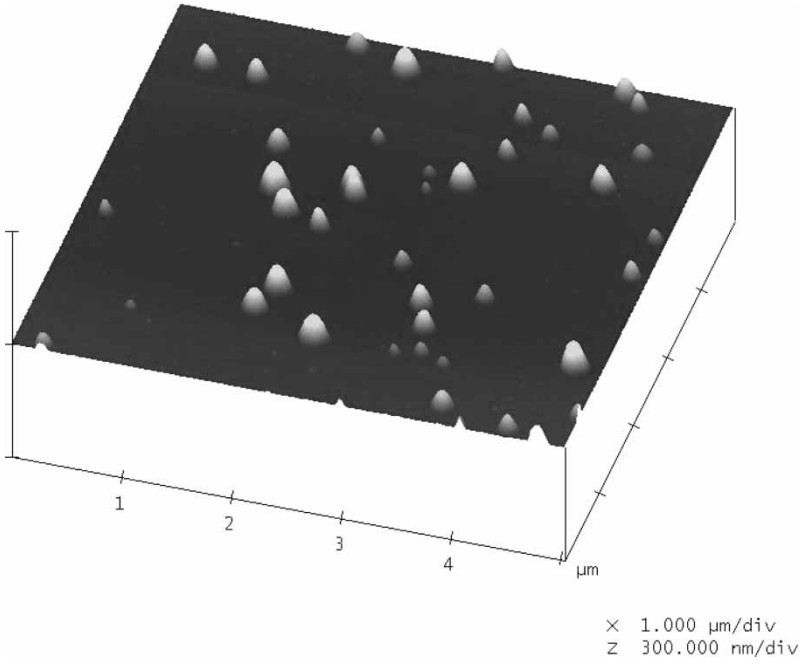

Figure 7. SFM micrograph of the gelatin/PAA particles after crosslinking.

polymerization of AA monomer in the presence of gelatin. GA was added as a crosslinking reagent to lock the structure of the particles. The resulting crosslinked gelatin/PAA particles were pH-responsive: when the pH of the solution changed from 2.5 to about 7.0, the volume of the particles expanded more than 60 times. This pH-sensitivity and the biocompatible gelatin shell will surely make the resultant particles an attractive candidate for applications in the biomedical field.

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