Gelatin/Tannin Complex Nanospheres via Molecular Assembly

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ABSTRACT: Nanospheres were produced by molecular assembly between tannin and gelatin because of the synergistic interaction of the hydrophobic effect and hydrogen bonding. The factors that influenced the production of nanospheres, such as sample concentration, mass ratio between tannin and gelatin, reaction temperature, pH, and reaction time, were studied. Moreover, the nanospheres were analyzed and characterized by a particle size analyzer, UV–vis spectrophotometer, and TEM. It was concluded that the critical point was important for the assembled nanospheres. The tannin/protein mass ratio should be lower than the critical point. The concentration of tannin should be confined to a relatively low level. The proper range of the reaction temperatures was usually between 10°C and 50°C. It was steady for nanospheres assembled when the pH value of the gelatin solution was within ±1 IEP of the gelatin. After the reaction had gone on for more than 48 h, the assembled nanospheres became stable. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 3125–3130, 2006

Key words: tannin; gelatin; molecular assembly; nanospheres

INTRODUCTION

Polymeric assembly is defined as a system in which molecular entities, through noncovalent interaction, spontaneously order into structures composed of many molecules.[1] These structures must exhibit a long-range order distinguishing them from simple molecular aggregates. Nanospheres with controlled spatial arrangement and shapes are fabricated by polymeric assembly with the aid of van der Waals bonding, hydrogen bonding, and electrostatic interactions into superstructures or nanostructures with well-defined spherical configurations on the molecular level.[2]

Tannins are polyphenolic substances that are widely distributed in almost all plant tissues.[3] Tannin plays an important role in plant physiology,[4,5] tanning hides,[6] removal of proteins,[7,8] medicine,[9] and so on. Polyphenolic structures endow tannins with a series of unique chemical characteristics. Their ability to interact with, and precipitate, proteins are their most important property. In 1803, Davy discovered reversible binding between tannins and proteins. In the 1980s, when separable and analyzable technology developed, the interactions related to their structures and activities began to be sketched out and suggestions about their molecular bases and interpretations were presented. This mechanism has been of interest for a long time, and hydrogen bonding[10–12] and hydrophobic interaction[13,14] have been reported to be major binding modes. Interactions between tannins and proteins are complicated. A two-stage mechanism for their coprecipitation has been reported.[15] Initially, making use of a weak interaction, tannin molecules assemble on the surface of a single protein molecule to form complexes; subsequently, complexes are crosslinked by more tannins until precipitation occurs.

Nanospheres prepared by polymeric carriers have been applied in many fields, including medicine,[16,17] tissue engineering, and cell culturing.[18] On the basis of previous research, we wanted to find a new way to prepare nanospheres by using interactions between tannin and gelatin.[19]

In our research, we tried to find a critical point in the two-stage mechanism at which interactions between tannin and gelatin could be controlled. In the second stage, before complexes are crosslinked by more tannin molecules to form precipitate, tannin was able to assemble nanospheres with gelatin. The mass ratio between tannin and gelatin played an important role in the fabrication of nanospheres. When the ratio reached a certain point, the system began to show turbidity. This point was termed the critical point at which gelatin interacted with tannin to assemble nanospheres. The influential factors in the preparation

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of nanospheres, such as the concentrations of systems, the mass ratio between tannin and gelatin, the pH, the reaction temperature, and the reaction time also are discussed in this article.

EXPERIMENTAL

Materials

Gelatin A was purchased from the Tianjin Chemical Factory (Tianjin, China). The number-average molecular weight of the gelatin (2.15×10^4) was measured by GPC (model 410, Waters Company; with PEO used as a standard sample and $NaNO_3$ as the flowing phase). The number-average molecular weight of the hydrolyzable tannins, provided by the Tianjin Jiangtian Chemical Company, was 1.52×10^3. All other agents used in the experiments were of analytical grade. All aqueous solutions were prepared from distilled water.

Preparation of nanospheres

The experimental procedure is as follows. First, 2 mL of a 0.5 mg/mL tannin solution was added into 10 mL of a 0.5 mg/mL gelatin solution to react at 20°C for 48 h. Factors influencing preparation of the nanospheres such as the concentration, composition, and temperature of the system were changed to meet the purposes of different experiments in this study. The nanospheres were then centrifuged at 12,000 rpm (model MR 18.22, Jouan Company), and finally, they were lyophilized in a freeze-dryer for 24 h (model Alpha 2-4, Chaist Company).

Particle size and distribution

The particle size and distribution of the nanospheres were analyzed by a laser particle size analyzer (model BI-90 Plus, Brookhaven Instruments).

Transmission electron microscope studies

The morphology of the nanospheres was observed with a transmission electron microscope (model 100LX, Phillips Company).

UV–vis spectrophotometer

The interaction between the gelatin and the tannin was tested with a UV–vis spectrophotometer (model U-1800, Hitachi Company).

RESULTS AND DISCUSSIONS

Hydrophobic interaction was the driving power in the reaction between tannin and gelatin. With a strong

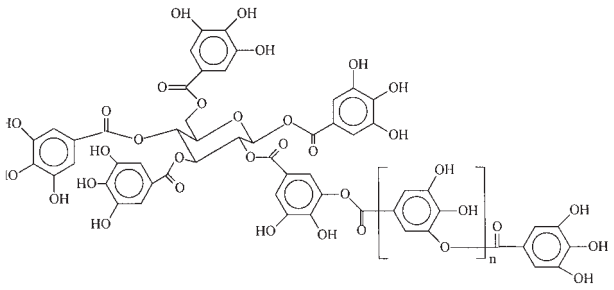

Figure 1 Chemical structure of tannin.

hydrophobic interaction, the phenolic hydroxyl groups of the tannin molecules were able to enter the hydrophobic areas of the gelatin. The polyphenolic structure of tannin (shown in Fig. 1) enabled it to interact by hydrogen bonding with the polar groups of the gelatin, such as peptide, carbonyl, and guanidine groups. Then hydrophobic areas were formed by tannin combining with many sites of the protein molecules by hydrogen bonding: complexes of tannin and gelatin began to precipitate. The interaction mechanism between tannin and gelatin belonged to the "hand–glove" model form. This kind of model required that donors and receptors have flexibility, which enabled a system to form multiple stable combinations of tannin and gelatin.

The interactions between tannin and gelatin should conform to a matching principle, such as the complementary matching of hydrogen bonding and hydrophobic interaction.

Effect of the mass ratio of tannin and gelatin on nanospheres

In 20°C, 10 mL of gelatin at a concentration of 0.5 mg/mL interacted with different amounts of tannin at the same concentration. After 48 h, nanosphere particle size was analyzed.

Figure 2 shows that the ability of tannin to precipitate gelatin increased with an increased amount of tannin. When the mass ratio reached a critical point prior to precipitation, the system began to show turbidity. In this system, the critical point was about 0.8. When the mass ratio was less than 0.8, tannin and gelatin were able to assemble nanospheres (see Fig. 3) whose diameters were in the range of 150 to 250 nm.

In the gelatin structure, the number of binding sites with tannin were constant at a specific concentration. As the amount of tannin increased in the system, more phenolic hydroxyl groups interacted with the gelatin molecules, so networks between tannin and gelatin could be formed. When the amount of gelatin far exceeded that of tannin, monotannin molecule binding with two or more gelatin molecules could form bipolymers or tripolymers. Because of an insufficient

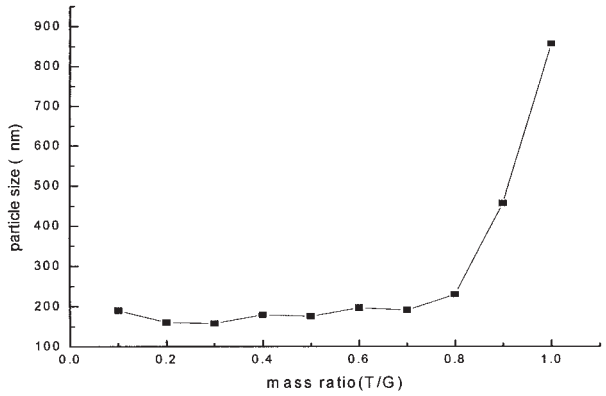

Figure 2 Relationship between mass ratio (tannin/gelatin) and diameters of the nanospheres. In the reaction, which occurred at 20°C for 48 h, 10 mL of gelatin at 0.5 mg/mL interacted with tannin at 0.5 mg/mL from 1 to 10 mL.

number of tannin molecules to combine with the complexes, the solution had many assembled nanospheres. With an increased amount of tannin, the number of phenolic hydroxyl groups in tannin was about equal to the number of gelatin binding sites, leading to precipitation (see Fig. 4). In sum, to avoid precipitation, the mass ratio between tannin and gelatin should below the critical point.

Effect of system concentration on assembled nanospheres

Gelatin was interacted with tannin at the same concentration, which varied from 0.05 to 0.7 mg/mL. With different concentrations, different amounts of tannin were added to the gelatin solution. In the same conditions as mentioned above, nanospheres were

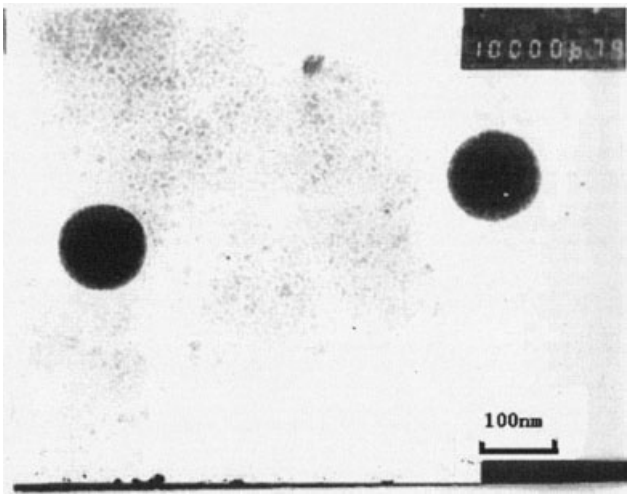

Figure 3 TEM photograph of the nanospheres (5 mL of tannin at a concentration of 0.5 mg/mL was added to 10 mL of gelatin at the same concentration at 20°C for 48 h).

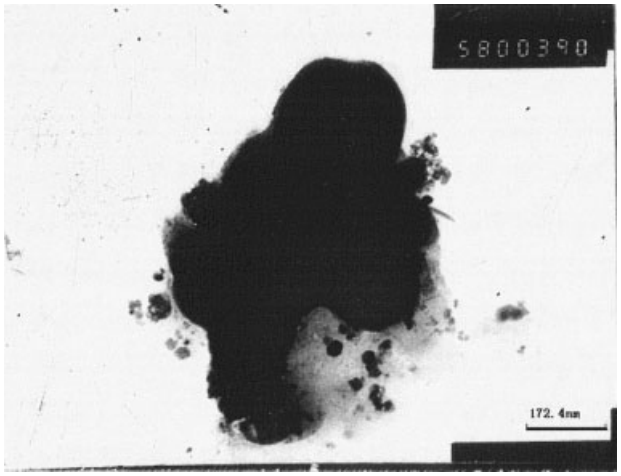

Figure 4 TEM photograph of crosslinked nanospheres (10 mL of tannin at a concentration of 0.5 mg/mL was added to 10 mL of gelatin at the same concentration at 20°C for 48 h; the Tannin/protein mass ratio was higher than the critical point).

produced. Figure 5 shows the relationship between concentration and tannin/gelatin mass ratio. We were able to determine different critical points at different concentrations (see Table I).

With an increased concentration of tannin, the ability of tannin crosslinking complexes increased. To obtain stable nanospheres whose diameters were 150–250 nm, tannin concentrations needed to be relatively low.

Effect of reaction temperature on assembled nanospheres

Gelatin solution at a concentration of 0.5 mg/mL interacted with various amounts of tannin at the same

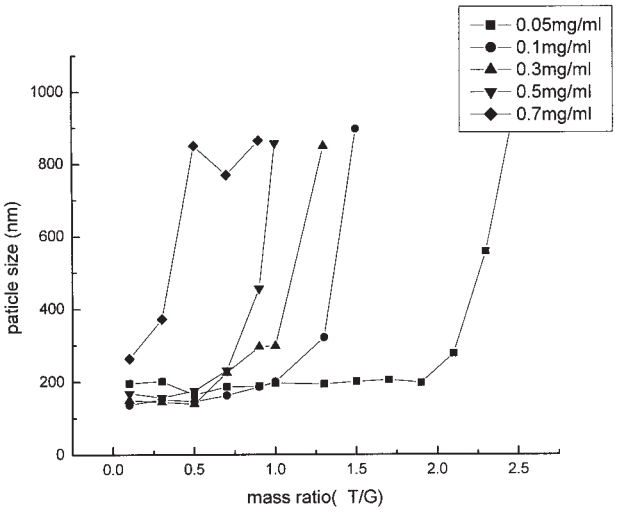

Figure 5 Relationship between mass ratio (tannin/gelatin) and the diameters of the nanospheres at different concentrations. Gelatin interacted with tannin at the same concentrations from 0.05 to 0.7 mg/mL. The preparation conditions were as described in Figure 2.

TABLE I
Various Critical Points at Different Concentrations

Concentration (T+G)	Critical point (T/G)
0.7 + 0.7 mg/mL	0.4
0.5 + 0.5 mg/mL	0.8
0.3 + 0.3 mg/mL	1
0.1 + 0.1 mg/mL	1.2
0.05 + 0.05 mg/mL	2.3

T, tannin, G, gelatin.
Critical point indicates T/P mass ratio.

concentration at different temperatures ranging from 5°C to 65°C.

Figure 6 shows the effect of temperature on the assembled nanospheres. The amount of tannin interacting with gelatin was different at different concentrations at the same temperature. A high tannin concentration provided more binding sites between tannin and gelatin to form larger aggregates, so the particle size of the nanospheres increased. When the temperature was below 40°C, the influence of temperature was weak because of the modest reaction rate. At about 45°C, the diameter of particles suddenly enlarged with an increase in the reaction rate, so it was easy to observe turbidity in the system.

With an increase in the reaction temperature, the structure of the gelatin became looser. Hydrophobic groups would be exposed enough to interact easily with tannin. When the temperature was above 40°C, the interactions between tannin and gelatin were drastic, and complexes were easy to combine with tannin molecules. As the temperature increased from 40°C to 60°C, the ability of tannin to precipitate gelatin became stronger, and then a network structure was formed with precipitation. When the temperature was higher than 65°C, the system became unstable because gelatin was uncomplexed easily. If the temperature was below 10°C, interactions would be slow.

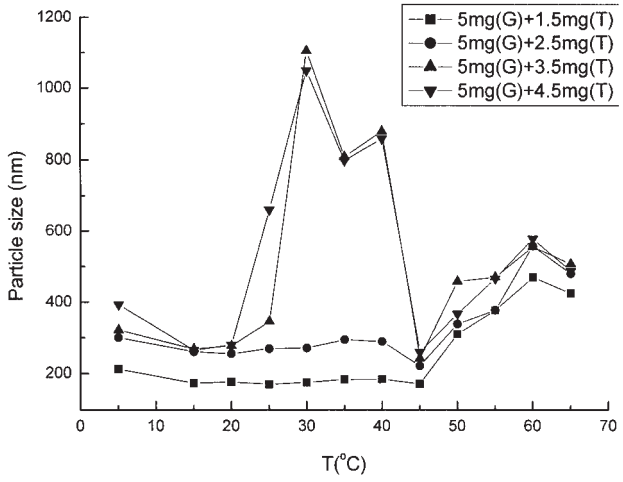

Figure 6 Effect of temperature on the nanospheres (10 mL of gelatin at a concentration of 0.5 mg/mL interacted with tannin at a concentration of 0.5 mg/mL from 3 to 9 mL; the reaction time was 48 h at different temperatures).

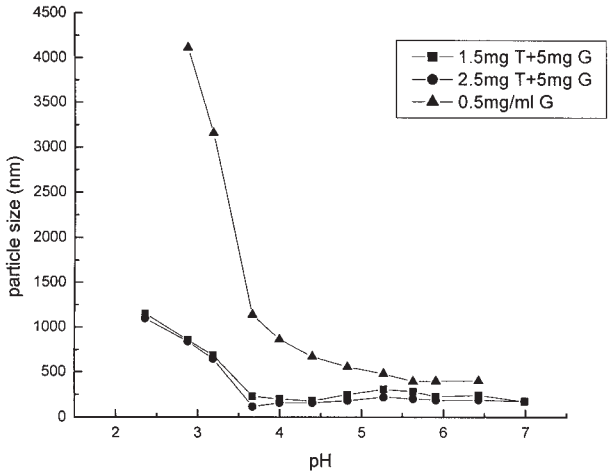

Figure 7 Effect of pH value of gelatin solutions on the nanospheres (3 mL of tannin and 5 mL of tannin at a concentration of 0.5 mg/mL were added to 10 mL of gelatin at a concentration of 0.5 mg/mL with different pH values; the preparation conditions were as described in Fig. 2).

As discussed above, we were able to conclud that a temperature between 10°C and 40°C was suitable for the assembled nanospheres.

Effect of pH on assembled nanospheres

Buffer solutions were used to adjust the pH of the gelatin solution from 2.5 to 7, and then tannin solution was added to gelatin solution at different pHs at 20°C for 48 h.

The relationship between the pH and diameters of the particles is shown in Figure 7. When the pH varied from 5 to 7, the system was stable and the diameters of the nanospheres were distributed evenly. When the pH was below 4, the solution appeared turbid and began to show flocculent deposits.

It was obvious that pH played an important role in these tannin–gelatin systems. The pH could affect the electrical charge of gelatin solution, leading to changes in some characteristics such as solubility and conformation. Whether the interaction between the tannin and gelatin was stronger than the electrostatic repulsion among the gelatin molecules was important. When interaction occurred near the isoelectric point (IEP) of the gelatin, electrostatic repulsion was at its least and the interaction of gelatin and tannin was strongest, producing more stable assembled nanospheres. In this work, the isoelectric point (IEP) of gelatin was 6.8.

When the pH was above 8, tannin would be subject to oxidative hydrolysis. If the pH was low enough, the

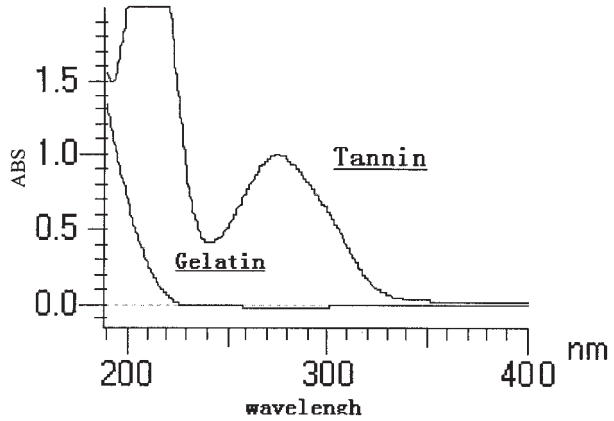

Figure 8 ABS of tannin and gelatin.

tannin was easy to condense, leading to an increased molecular weight of the tannin, resulting in a stronger ability of gelatin to be precipitated.

Effect of reaction time

Figure 8 shows that tannin had absorbance (ABS) at 214 and 275 nm, whereas gelatin did not have an ABS at 275 nm. Then 5 mL of tannin (0.5 mg/mL) solution was added to 10 mL of gelatin (0.5 mg/mL), 0.8-mL samples were taken out at hourly intervals. After centrifugation at 12,000 rpm for 10 min, the supernatant was transferred to a fresh tube, and distilled water was added up to 5 mL. A UV–vis spectrophotometer analyzed the samples at 275 nm (see Fig. 9). After interactions between gelatin and tannin, the ABS of 275 nm decreased.

In the first 2 h, the reaction proceeded rapidly, and then the systemic interaction became slower. It seemed that the ABS of tannin got smaller and changed slowly, but there may have been a local interaction of tannin and protein in the system. After 24 h of reaction, we found that nanoparticles in the system and that the reaction became stable. After about 48 h, the reaction reached a fully stabilized state (see Fig. 10).

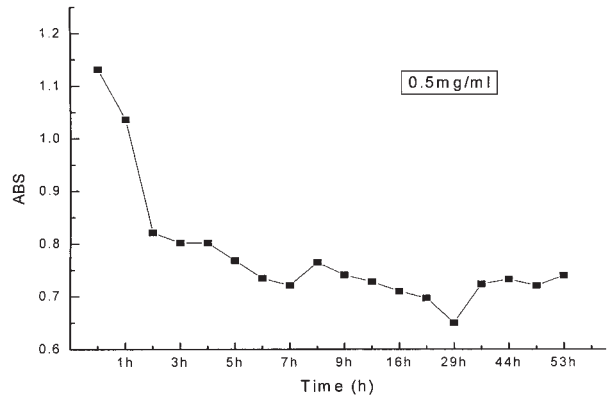

Figure 9 Relationship between reaction time and ABS of tannin.

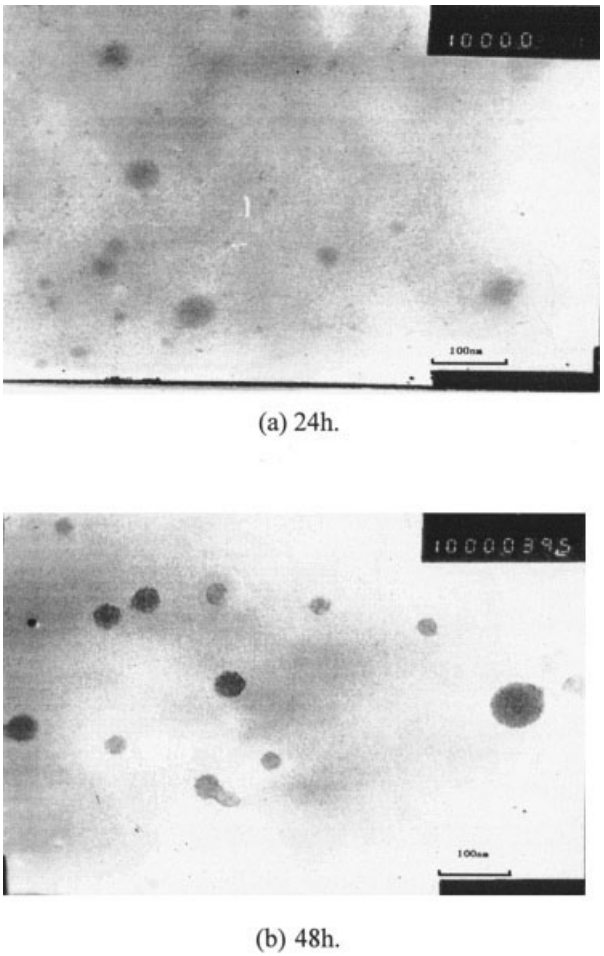

Figure 10 TEM photographs of nanospheres at different reaction times: (a) 24 h, (b) 48 h.

CONCLUSIONS

In this work, we made use of hydrogen bonding and hydrophobic interaction between tannin and gelatin to assemble gelatin/tannin nanospheres. Based on a two-stage mechanism, we found a critical point for assembling gelatin–tannin complex nanospheres before precipitation. The effects of mass ration, concentration of the system, pH, reaction temperature, and reaction time on the assembled nanospheres were discussed. In sum, each concentrations had its own critical point of precipitation for the reaction between tannin and gelatin. When the tannin/gelatin mass ratio was higher than this point, precipitation began. Hence, when nanospheres were to be assembled, it was very important to find the critical point with a given concentration. In addition, the concentrations of the system also were important. Tannin had a strong ability to precipitate at a higher concentration, so nanospheres could

not be assembled easily. Usually 0.5 mg/mL was close to the proper concentration needed to produce stable assembled nanospheres. With an increasing temperature, it was easy to observe system precipitation, but the reaction became slow at low temperatures. The proper range of reaction temperature was usually between 10°C and 50°C. Moreover, the assembled nanospheres were stable when the pH of the gelatin solution was within ±1 IEP of gelatin. We could see the shape of the nanoparticles about 24 h after the reaction, but the reaction was still ongoing. After more than 48 h, the reaction became more complete as the ABS of tannin remained at a steady value.

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