Self-Assembly of Ibuprofen and Bovine Serum Albumin–Dextran Conjugates Leading to Effective Loading of the Drug

Juan Li and Ping Yao*

Key Laboratory of Molecular Engineering of Polymer and Department of Macromolecular Science, Fudan University, Shanghai 200433, China

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A simple and green process of simultaneous formation of albumin nanoparticles and encapsulation of hydrophobic drugs in aqueous solution was developed. Bovine serum albumin (BSA)—dextran conjugates were prepared through the Maillard reaction. Ibuprofen was used as a drug model. The solubility of protonated ibuprofen decreases, and then precipitation occurs when the pH of saturated ibuprofen solution is changed from alkali to acidic value. In the presence of the conjugates, a binding of ibuprofen with BSA through hydrophobic and electrostatic interactions can suppress the precipitation of ibuprofen. After a heat treatment, the gelation of BSA results in the formation of nanoparticles and fixing of the ibuprofen in the core. The nanoparticles were characterized with dynamic and static light scattering, ζ-potential, and transmission electron microscopy. The nanoparticles are of spherical shape having a hydrodynamic diameter of about 70 nm. As much as 0.7 unit weight of ibuprofen can be loaded into one unit weight of the conjugates. The dextran conjugated to BSA stabilizes the nanoparticles in aqueous solution.

Introduction

Albumin has a long history in pharmaceutical applications.† Because of the good biocompatible, biodegradable, and nonantigenic properties of albumin, there is a growing interest in fabricating albumin nanoparticles as drug carriers.‡–§ Albumin nanoparticles can be prepared by desolvation or water-in-oil (w/o) emulsion followed by cross-linking with glutaraldehyde or nanoparticles in aqueous solution, i.e., it is possible to develop a possible that hydrophobic drug and albumin self-assemble to port in the body and release at the cell surface.¶–‖ Therefore, it is natural carrier of hydrophobic molecules, such as vitamins, paclitaxel approved by the Food and Drug Administration (FDA) in 2008,¶¶ which is prepared by high-pressure homogeneration of paclitaxel in the presence of human serum albumin.† Albam is a natural carrier of hydrophobic molecules, such as vitamins, hormones, and other plasma constituents. The binding of hydrophobic substances to albumin is reversible and allows for transport in the body and release at the cell surface.¶¶¶ Therefore, it is possible that hydrophobic drug and albumin self-assemble to nanoparticles in aqueous solution, i.e., it is possible to develop a simple process of simultaneous formation of albumin nanoparticles and encapsulation of hydrophobic drugs in aqueous solution.

Bovine serum albumin (BSA) is a globular protein having a molecular weight of 66 kDa, 580 amino acid residues, 17 intra-chain disulfide bonds, and 1 free thiol group. BSA contains three domains specified for metal-ion binding, lipid binding, and nucleotide binding.¶¶¶¶ The gelation property and mechanism of BSA on heating have been reported by Ferry et al.¶¶¶¶ The thermally induced gelation of BSA involves heat-induced unfolding followed by protein—protein interactions. The interprotein interactions include hydrogen bonding, electrostatic and hydrophobic interactions, and disulfide—sulfhydryl interchange reactions.

It is known that a “stealth” property is necessary for a nanoparticle shell for the purpose of avoiding reticuloendothelial recognition and subsequent elimination of the nanoparticles, so that the circulation time in the bloodstream is prolonged.¶¶¶¶ It has been reported that polysaccharides, such as dextran, can provide nanoparticles with a stealth property and decrease the adsorption of plasma protein.¶¶¶¶¶ Water-soluble dextran is a family of 1−6-α-d-glucans, which has been used as a plasma expander.¶¶¶¶¶ BSA—dextran conjugates prepared through the Maillard reaction have been reported.¶¶¶¶¶¶ Maillard reaction is a natural and nontoxic process that conjugates polysaccharide and protein by linking the reducing end carbonyl group in the former to the amino group in the latter.¶¶¶¶ BSA—dextran conjugates can form stable nanogels having a structure of BSA core and dextran shell after heating the conjugate aqueous solution at pH around the isoelectric point (pI) of BSA.¶¶¶¶¶ Iupbrofen (Scheme 1) is a nonsteroidal drug and widely used to treat inflammation. It has a low solubility in acidic aqueous

*To whom correspondence should be addressed. E-mail: yaoping@fudan.edu.cn. Fax: 86-21-65640293.


solution. Giacomelli et al. reported a 100% encapsulation of ibuprofen using poly(ethylene oxide)-b-poly(2-(disopropylamino) ethyl methacrylate) through electrostatic and hydrophobic interactions with indirect dissolution or cosolvent method.\(^{21}\) Jiang et al. reported ibuprofen nanoparticles prepared by a process including a precipitation of ibuprofen in a supersaturated solution and a deposition of diethylaminoethyl (DEAE) dextran onto the precipitated ibuprofen particles through electrostatic interaction.\(^{23}\) In the present report, ibuprofen was used as a drug model to prepare ibuprofen–BSA–dextran nanoparticles based on the hydrophobic and electrostatic interactions between ibuprofen and BSA as well as the gelation of BSA. By a simple process of mixing ibuprofen with the conjugate solution, pH adjusting, and a heat treatment in succession, ibuprofen can be effectively loaded in the core of the nanoparticles.

### Materials and Methods

**Materials.** BSA (grade IV) was from Sigma. Dextran with molecular weight of 62 kDa was from Amersham Pharmacia Biotech. Ibuprofen (>98%) was supplied by Shanghai Shunqiang Biotechnology Co., Ltd. Glutaraldehyde (50%) was from Sino Pharm Chemical Reagent (Shanghai) Co., Ltd. All solutions were prepared using deionized water.

Pretreatment of BSA–Dextran Conjugates. BSA and dextran with molar ratio of 1:1 were dissolved together in water. The pH of the mixture was adjusted to 8.0 using 0.1 mol/L NaOH, and the solution was lyophilized. The lyophilized powder was reacted at 60 °C under 79% relative humidity in a desiccator containing saturated KBr solution for 24 h.\(^{17,23}\) The conjugation degree of the resultant Maillard reaction products was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and gray scale analysis method (Supporting Information). The Maillard reaction products were denoted as BSA–dextran conjugates and were used without separation.

Preparation of Ibuprofen–BSA–Dextran Nanoparticles. As mentioned above, the molar ratio of BSA to dextran was 1:1 in the conjugate preparation. For simplifying the description, we only denote the concentration of BSA in BSA–dextran conjugates. If there is no special indication in this report, the preparation process of the nanoparticles is as follows. The conjugates were dissolved in water and then ibuprofen was added. The final concentration of ibuprofen was 10 mg/mL, and BSA was 10 mg/mL too (weight ratio of ibuprofen to BSA (WR) 1:1). The pH of the mixture was adjusted to 5.2 with 1 mol/L HCl. The solution was heated at 80 °C for 60 min to produce ibuprofen–BSA–dextran nanoparticles. The resultant nanoparticle solution was kept at 4 °C before use.

Preparation of Glutaraldehyde-Cross-Linked Ibuprofen–BSA–Dextran Nanoparticles. The nanoparticles were further cross-linked using glutaraldehyde. Typically, 10 μL of 50% glutaraldehyde solution was added in 1 mL of the nanoparticle solution. The cross-linking was performed under stirring at room temperature for 24 h.

Ibuprofen Loading Capacity. The unloaded ibuprofen was separated from the nanoparticles by ultrafiltration (cutoff molecular weight of 30 kDa; MicroconYM-30, Millipore). The loaded ibuprofen was calculated by subtracting the free ibuprofen in the ultrafiltrate from the initial ibuprofen in feed. Ibuprofen concentration was determined by its absorbance at 222 nm (Lambda 35, Perkin-Elmer) according to the working curve measured using standard ibuprofen solutions.\(^{24}\) At least two batches of loading samples were analyzed, and average data were reported.

**Ibuprofen Release.** Ibuprofen–BSA–dextran nanoparticles solution of 0.5 mL was dialyzed (cutoff molecular weight of 14 kDa) against 49.5 mL of release buffer (pH 7.4 phosphate buffer) at 37 °C. Periodically, 3 mL of the release buffer was taken out, and the same volume of fresh buffer was added. The release experiments were repeated two times, and average data were reported.

Dynamic Laser Scattering (DLS) Measurements. DLS measurements were carried out on a Malvern Autosizer 4700 (Malvern Instruments) equipped with a multi-r digital time correlator (Malvern PCS712) and a solid-state laser (Compass 315M-100, Coherent, Inc.; output power: 100 mW at λ 532 nm). In DLS measurements, the line width distribution G(τ) was calculated from the Laplace inversion of the measured intensity–time correlation function G(τ) can be converted to the translational diffusion coefficient distribution G(D) and to the hydrodynamic diameter distribution f(Dn) by the Stokes–Einstein equation.\(^{25}\) The measurements were performed at 25 °C and a fixed scattering angle of 90°. The measured time correlation functions were analyzed by Automatic Program equipment. The apparent z-average hydrodynamic diameter (Dn) and polydispersity index (PDI, Dn/(Dn)) were obtained by CONTIN mode analysis.\(^{26}\) The nanoparticle samples were diluted 10 times with water for DLS measurements.

\[ R_g/R_b \text{ Ratio.} \]  
\[ R_g \text{ (z-average root-mean-square radius of gyration)} \]  
\[ R_h \text{ (z-average hydrodynamic radius)} \]  
were measured on an ALV/CGS-8F laser light scattering spectrometer equipped with a multi-r digital correlator (ALV/LSE-5004) and a solid-state laser (IEC60825-1, ADLAS; output power 35 mW at λ 633 nm). In static light scattering (SLS) measurements, \( R_h \) was determined from the angular dependence of the Rayleigh ratio \( R_0(q) \).

\[ \zeta \text{-Potentials.} \]  
\[ \zeta \text{-Potentials were measured at 25 °C on a ZetaSizer Nano ZS90 (Malvern Instruments) equipped with a 4 mW He–Ne laser (λ 633 nm) using the technique of laser doppler electrophoresis.} \]

Transmission Electron Microscopy (TEM) Measurements. TEM observations were conducted on a Philips CM 120 electron microscope at an accelerating voltage of 80 kV. Samples were prepared by depositing solution onto a carbon-coated copper grid, followed by removal of excess solution by blotting the grid with filter paper. The samples were dried for 72 h at room temperature in a desiccator containing dried silica gel.

**Results and Discussion**

Ibuprofen–BSA Complexes. As the \( pK_a \) of ibuprofen is 5.2–5.6, the solubility of ibuprofen in aqueous solution is pH dependent.\(^{22}\) At pH 5.0 and 6.0, the solubility is 0.096 and 0.52 mg/mL, respectively. The solubility increases to 14.8 mg/
mL at pH 9.2. When changing its saturated alkali solution to acidic pH, ibuprofen precipitates. Our study (Table 1) shows that the precipitation does not occur after adjusting the solution pH to 5.2 in the condition of BSA concentration of 10 mg/mL and WR of 0.4 or less. The pl of BSA is about 4.8. When the alkali solution of ibuprofen and BSA mixture was adjusted to pH 5.2, protonated ibuprofen molecules increased and the net negative charges of BSA decreased. Therefore, at pH 5.2, the hydrophobic interactions between protonated ibuprofen and BSA increase; the electrostatic repulsion between unprotonated ibuprofen and BSA decreases, whereas electrostatic attraction occurs because of the asymmetric charge distribution of protein. Therefore, the precipitation of ibuprofen at pH 5.2 can be suppressed by binding with BSA through hydrophobic and electrostatic interactions. Precipitation occurs when WR is larger than 0.4. This result suggests that the hydrophilicity of BSA is not enough at pH 5.2 to stabilize ibuprofen–BSA complexes and/or that the binding sites of BSA are saturated by ibuprofen. In order to increase the hydrophilicity of BSA, we used BSA–dextran conjugates to interact with ibuprofen. The data in Table 1 show that precipitation does not occur in the mixtures of ibuprofen and the conjugates at pH 5.2 until WR reaches 1.6. These results verify that the dextran covalently conjugated to BSA stabilizes the ibuprofen–BSA complexes when WR is less than 1.6 at pH 5.2. In such conditions, the binding sites of BSA and the free ibuprofen in the mixtures do not reach saturation. The physical mixture of BSA and dextran cannot increase the hydrophilicity of BSA, and therefore the behavior of the mixture containing ibuprofen, BSA, and dextran is the same as the behavior of the mixture of ibuprofen and BSA.

**Preparation of Ibuprofen–BSA–Dextran Nanoparticles.** However, the mixtures of ibuprofen and the conjugates precipitate after long-term storage, especially for the samples with higher ibuprofen ratio. We found that heating the mixture of ibuprofen and the conjugates can produce long-term stable nanoparticle dispersion because of the gelation of BSA after the heat treatment. In order to optimize the nanoparticle fabrication, we investigated the factors that influence the nanoparticle formation as follows.

**Effects of Heating Temperature and Heating Time.** The denaturation temperature of individual BSA is 59–63 °C. Laura et al. reported that BSA–dextran conjugates have higher solubility and thermal stability than the native protein at the pH around its pl. We used DLS to investigate the effects of heating time and temperature on the nanoparticles prepared from the mixture with WR 1:1 and BSA concentration of 10 mg/mL at pH 5.2. The data in Table 2 show that heating at 80 and 90 °C can produce narrowly dispersed nanoparticles. The Dₙₐ and intensity increase with the increases of heating time and temperature. In order to avoid the decomposition of ibuprofen after long-time heating at higher temperature, we adopted a heat treatment at 80 °C for 60 min to prepare ibuprofen–BSA–dextran nanoparticles in the following study. After the heat treatment, high-performance liquid chromatography (HPLC) analysis verified that the decomposition of the ibuprofen did not happen during the heating process (data not shown). The resultant nanoparticles have a size of about 67 nm and a narrow size distribution.

**Effect of the Conjugation Degree of BSA–Dextran Conjugates.** Table 1 shows that at pH 5.2, BSA–dextran conjugates can stabilize ibuprofen–BSA complexes in a WR range of 0.4–1.6, but the physical mixture of BSA and dextran cannot. The conjugation degree of the conjugates used in this study is about 41% (Supporting Information). This result means that about 41% of the BSA molecules are conjugated with dextran, and there are about 59% free BSA and 59% free dextran in the conjugates after the Maillard reaction. In order to investigate the influence of different conjugation degrees on the resultant nanoparticles, we mixed different weight percent of BSA–dextran Maillard reaction product with a physical mixture of BSA and dextran. The resultant conjugation degree was calculated and shown in Table 3. Then, we used these mixtures to prepare ibuprofen–BSA–dextran nanoparticles. The data in Table 3 show that the nanoparticles form when the mixtures have 10% and higher conjugation degree. The particle size decreases with the increase of the conjugation degree. This phenomenon can be explained by the fact that more hydrophilic dextran conjugated to BSA can stabilize a larger surface area, which leads to smaller nanoparticles. The Dₙₐ values are almost the same when increasing the conjugation degree from 31% to 41%, but the scattering light intensity decreases significantly. This result indicates that the efficiency of the nanoparticle formation decreases. It is possible that the steric hindrance of the dextran conjugated to BSA somewhat restricts the gelation of BSA when the conjugation degree is 41%. Considering the much narrower PDI, we chose 100% Maillard reaction product, i.e., the conjugates with a conjugation degree of 41%, to prepare the nanoparticles in this report.

**Effect of heating pH.** Table 4 shows the DLS result of the particles prepared by heating the mixture of ibuprofen and the conjugates at different pH values. Nanoparticles form in the pH range of 3.5–6.0. At pH 6.0, the nanoparticles have relatively larger Dₙₐ and smaller intensity, indicating that some of the...
molecules do not take part in the particles. This is ascribed to the fact that both ibuprofen and BSA carry negative charges at pH 6.0, electrostatic repulsion exists, and hydrophobic interactions are not strong enough. At pH 2.0, large aggregates appear in the solution. The reason may be that pH 2.0 is away from the pI of BSA (4.8), and therefore BSA carries more positive charges that weaken the gelation ability of BSA and also decrease the hydrophobic interactions between BSA and protonated ibuprofen. In this paper, we chose pH 5.2 to prepare the nanoparticles.

Effect of WR. Figure 1 shows the $D_h$ and PDI of the nanoparticles prepared with different WR values at pH 5.2. As mentioned above, the molar ratio of BSA to dextran is 1:1 in the conjugates. For simplifying the description, we only denote the ratios of ibuprofen to BSA in this report. The individual conjugates can form nanoparticles with $D_h$ of about 200 nm. In the WR range of 0.05–0.15, soluble complexes form, as shown in Table 1, but precipitation occurs after the heat treatment. Dispersible nanoparticles form when increasing WR to 0.2. The $D_h$ values of the nanoparticles are about 70 nm when WR is in the range of 0.3–1.6. Further increasing WR to 1.8 or 2.0, precipitation occurs even before the heat treatment because the binding sites of BSA and the free ibuprofen in the solution have reached saturation as discussed above. After the heat treatment, the supernatant solution shows $D_h$ values of around 100 nm. If there is no special indication, the nanoparticles were prepared with WR 1:1 in this report.

**Ibuprofen–BSA–Dextran Nanoparticles Cross-Linked through Glutaraldehyde.** For the purpose of characterization, we fixed the structure of the nanoparticles by cross-linking with glutaraldehyde. Although glutaraldehyde may react with amines, thiol, phenols, and imidazoles of protein, the cross-linking effect of glutaraldehyde is dominated by the reactions with ε-amino groups of lysine residues. Table 5 exhibits the DLS results of the nanoparticles after glutaraldehyde cross-linking. Compared with the nanoparticles without cross-linking, the intensity, $D_h$, and PDI of the nanoparticles are almost the same after adding 5 or 10 μL of 50% glutaraldehyde solution into 1 mL of the nanoparticle solution and reacting at room temperature for 24 h. This result suggests that the structure of the nanoparticles does not change significantly. The intensity increases significantly when the glutaraldehyde was increased to 20 μL, indicating a significant increase of the mass of the nanoparticles. In this report, we added 10 μL of glutaraldehyde into 1 mL of nanoparticle solution to prepare the cross-linked nanoparticles, in which the ratio of total aldehyde groups to total free amino groups was about 13. The glutaraldehyde-cross-linked nanoparticles are denoted specially in this report.

**Characterization of Ibuprofen–BSA–Dextran Nanoparticles.** *Stability.* The nanoparticles prepared in the WR range of 0.4–1.6 are stable. No precipitates were observed after long-term storage. Figure 2 shows the size distributions of the nanoparticles after lyophilization and then rehydration. The nanoparticles can disperse well in water after lyophilization, and the size distribution does not change significantly compared with the sample freshly prepared. These are valuable properties for practical use.

**Ibuprofen Loading.** The free ibuprofen in the nanoparticle dispersions was separated by ultrafiltration. The loading efficiency and loading amount of ibuprofen were calculated according to the following equations:

\[
\text{Loading efficiency} (\%) = \frac{\text{ibuprofen in feed} - \text{free ibuprofen}}{\text{ibuprofen in feed}} \times 100\%
\]

\[
\text{Loading amount} (\%) = \frac{\text{free ibuprofen}}{\text{conjugates in feed}} \times 100\%
\]

Figure 3 shows that the loading efficiency and loading amount increase with the increase of WR. At WR 1.6, the loading efficiency and loading amount reach to 91% and 72.8%.
However, the ibuprofen molecules can leave the particles, and nanoparticles with glutaraldehyde cross-linking cannot dissociate; therefore, the nanoparticles dissociate. The nanostructure becomes more compact, i.e., more ibuprofen molecules from 1.00 to 0.84. The decrease of \( R_h \) values measured at 90°, 60°, and 30° are almost same for all of the samples. These nonangular dependent \( R_h \) values indicate that the particles are spherical, whereas the nanoparticles without cross-linking exhibit larger absolute values of \( \zeta \)-potential at pH away from 4.2, which are ascribed to the dissociation of the nanoparticles at these pH conditions verified by the DLS results (Table 6).

**Morphology.** The morphology of the nanoparticles was observed by TEM. Figure 4 shows that both kinds of nanoparticles (with and without glutaraldehyde cross-linking) are spherical. The nanoparticles were prepared with and without glutaraldehyde cross-linking, which is consistent with the DLS results shown in Table 5. The nanoparticles in TEM images exhibit a narrow size distribution that also consists with the result of DLS. The diameters of the nanoparticles in TEM images are in the range of 30–40 nm, which is smaller than the 60–70 nm of \( D_h \) determined by DLS. As we know, DLS provides the data for the particles swollen in solution, whereas TEM shows the images of dried particles. Figure 4c shows the image of the cross-linked nanoparticles after ibuprofen release. The particles exhibit a structure of hollow sphere after the release. This image reveals that the ibuprofen molecules form the core of the nanoparticles.

\[ 5 \quad \text{na} \quad \text{decrease, and the electrostatic repulsion increases inside the neutral and alkali pH values.} \]

The nanoparticles were prepared with and without glutaraldehyde cross-linking.

**ζ-Potential.** \( \zeta \)-Potential is directly related to the net charges on the surface of the macromolecules and particles. Figure 5 shows \( \zeta \)-potentials of the nanoparticles as a function of pH. Both of the nanoparticles with and without cross-linking exhibit a zero \( \zeta \)-potential at pH of about 4.2, which is close to the pl of BSA. The absolute \( \zeta \)-potential values of the cross-linked nanoparticles are close to zero in the pH range of 2–11. This result indicates that the charges of the BSA are screened, and the dextran on the surface stabilizes the nanoparticles in the solution, whereas the nanoparticles without cross-linking exhibit larger absolute values of \( \zeta \)-potential at pH away from 4.2, which are ascribed to the dissociation of the nanoparticles at these pH conditions verified by the DLS results (Table 6).

**Structure.** The structure of the nanoparticles was further characterized with DLS and SLS. The data in Table 7 show that the \( R_h \) values measured at 90°, 60°, and 30° are almost same for all of the samples. These nonangular dependent \( R_h \) values indicate that the particles are spherical, whereas the nanoparticles without cross-linking exhibit larger absolute values of \( \zeta \)-potential at pH away from 4.2, which are ascribed to the dissociation of the nanoparticles at these pH conditions verified by the DLS results (Table 6).

\[ \begin{array}{c|c|c|c|c|c|c} \hline \text{pH} & \text{intensity (kcps)} & \text{nanoparticles} & \text{glutaraldehyde-cross-linked nanoparticles} & \text{intensity (kcps)} & \text{D}_{h} (\text{nm}) & \text{PDI} \\ \hline 5 & 39 & 66 & 0.10 & 40 & 66 & 0.05 \\ 7 & 2 & 47 & 0.44 & 20 & 65 & 0.02 \\ 9 & 1 & 34 & 0.30 & 22 & 67 & 0.06 \\ 11 & 0.5 & 40 & 0.33 & 21 & 68 & 0.09 \\ \hline \end{array} \]

\( \text{a} \) The nanoparticles were prepared with and without glutaraldehyde cross-linking.

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<th>Table 6. DLS Results of Ibuprofen–BSA–Dextran Nanoparticles at Different pH</th>
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\( \text{a} \) The nanoparticles were prepared with and without glutaraldehyde cross-linking.

**Brownian motion.** Brownian motion is the random thermal motion of particles. The nanoparticles were observed under TEM images. The ratios of \( R_{g}/R_{h} \) are listed in Table 7. It has been reported that \( R_{g}/R_{h} \) changes from 1.8 to 0.774 when the structure of polymer changes from a random coil to a uniform hard sphere. For a polymeric vesicle or a hollow sphere, the ratio \( R_{g}/R_{h} \) may be less or larger than 1.0, depending on the thickness and the density of the wall. Table 7 shows that increasing ibuprofen ratio from 1.00 to 1.93 that the nanoparticles dissociate after the ibuprofen release. For the nanoparticles cross-linked with


glutaraldehyde, the $R_g/R_h$ value of 0.78 suggests a uniform hard sphere structure. The $R_g/R_h$ value increases to 0.90 after ibuprofen was released. As the TEM image (Figure 4c) shows a structure of hollow spheres, the value of 0.90 indicates a compact BSA wall structure.

**Ibuprofen Release.** Figure 6 shows the release of ibuprofen from the nanoparticles in different media by means of dialysis. The nanoparticles exhibit an almost sustained release in water; whereas the individual ibuprofen releases about 85% in 2 h. DLS (Table 6) and $\zeta$-potential (Figure 5) results demonstrate that the nanoparticles dissociate at pH 7.0. The sustained release in water reveals that the ibuprofen molecules are bound with BSA even at neutral pH. Increasing the ionic strength of the release buffer can effectively increase the release rate. In 0.1 mol/L pH 7.4 phosphate buffer, a burst release of the nanoparticles was observed. The ionic strength-dependent rate of the release indicates that ions can disrupt the interactions between ibuprofen and BSA at pH 7.4. For glutaraldehyde-cross-linked nanoparticles, the release does not change very much. In 0.1 mol/L pH 7.4 phosphate buffer, about 50% and 80% of the ibuprofen was released after 1 and 3 h, respectively. This result implies that the cross-linking does not change the interactions between ibuprofen and BSA but decreases the diffusion rate of the ibuprofen from the core of the nanoparticles to the solution. The release of the ibuprofen in 0.1 mol/L HCl solution (pH about 1.2, stomach condition) from the nanoparticles prepared with WR 0.2 was also measured. Compared with the release in 0.1 mol/L pH 7.4 phosphate buffer, the release in 0.1 mol/L HCl was slower: about 63% and 96% of the ibuprofen was released in the initial 2 and 4 h, respectively (data not shown).

**Mechanism of the Nanoparticle Formation.** From the study above, the mechanism of the nanoparticle formation can be understood. The solubility of protonated ibuprofen decreases, and the ibuprofen aggregates. When changing the pH of ibuprofen and conjugate mixture from alkali to acidic value, the hydrophobic interactions and electrostatic attraction between ibuprofen and BSA increase, which suppresses the precipitation of ibuprofen. After the heat treatment, the gelation of BSA leads ibuprofen to be fixed in the core of the nanoparticles. The dextran
conjugated to BSA stabilizes the nanoparticles in the solution, and also effectively prevents the glutaraldehyde cross-linking between the nanoparticles. Scheme 2 illustrates the formation of the nanoparticles.

Conclusion

A simple and green process was developed in this study to fabricate albumin nanoparticles and load hydrophobic drug simultaneously. BSA–dextran conjugates were prepared through the Maillard reaction. Ibuprofen was used as a drug model. After mixing ibuprofen with the conjugate solution, adjusting pH, and heating in succession, ibuprofen can be effectively loaded in the core of the nanoparticles through hydrophobic and electrostatic interactions as well as BSA gelation. The knowledge gained in this study may be applied to load other drugs in albumin nanoparticles.

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Supporting Information Available: The analysis of conjugation degree of the Maillard reaction product (BSA–dextran conjugates). This material is available free of charge via the Internet at http://pubs.acs.org.