Stable and pH-Sensitive Nanogels Prepared by Self-Assembly of Chitosan and Ovalbumin

Shaoying Yu, Jinhua Hu, Xiaoyun Pan, Ping Yao,* and Ming Jiang

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

Received November 22, 2005. In Final Form: January 11, 2006

Two natural macromolecules, chitosan and ovalbumin, were used to produce nanoparticles by a new, green, and convenient method. Chitosan and ovalbumin solutions were mixed; the pH of the resulting solution was adjusted; and the solution was successively stirred and heated. After that, ovalbumin gelled forming nanospheres. The chitosan chains are supposed to be partly trapped in the nanogel core upon heating because of the electrostatic attractions between chitosan and ovalbumin, while the rest of the chitosan chains should form the shell of the nanogels. The nanogels did not change the size distribution after long-time storage and did not dissociate in the pH range of 2−10.5. The dispersibility, size, and hydrophobic/hydrophilicity of the nanogels are pH-dependent. The nanogels are good candidates for cosmetic and pharmaceutical applications.

Introduction

The amount of the world chitin production ranks second in terms of natural polysaccharides, but its insolubility in common solvents limits its utilization.1 Chitosan is obtained from deacetylation of chitin and is a cationic polysaccharide composed of β-[1→4]-2-amino-2-deoxy-D-glucopyranose and some N-acetylglucosamine units depending upon its degree of deacetylation (DD). Differently from chitin, chitosan is soluble in acidic solutions (pH < 6.4) as a result of the quaternization of the amino groups on the D-glucosamine residues (pK0 ≈ 6.3).2 Because of its numerous appealing biomedical properties, such as safety, biocompatibility, biodegradability, bioadhesivity, and anticancer, hemostatic, and bacteriostatic effects,3,4 chitosan has been extensively used in the pharmaceutical and biomedical area.5−7 In the drug-delivery field, the vehicles based on chitosan can be used for transdermal,8 nasal,9 ocular,10 oral,11 and parenteral12 administration and other applications.2 In the drug-delivery field, chitosan can be used in the form of nanoparticles, microparticles, hydrogels, tablets, films, and fibers. It is understandable that nanoparticles have particular advantages because they not only protect the associated drugs but also facilitate the drugs to go across critical and specific biological barriers and hit specific targets.13−16 Various methods have been developed to fabricate chitosan nanoparticulate systems. These methods, in principle, can be divided into noncovalent ionotropic gelation methods and covalent cross-linking methods.6 For example, Alonso and co-workers reported poly(ethylene oxide) (PEO) or PEO−poly(phenylene oxide) (PPO)-coated chitosan nanoparticles that were ionically cross-linked by tripolyphosphate (TPP) as carriers for proteins and vaccines.19,20 Ohya et al. reported the preparation of chitosan nanospheres for the anticancer drug 5-fluorouracil by employing a water-in-oil emulsion method and then glutaraldehyde cross-linking.21 Jiang and co-workers employed chitosan and acrylic acid to produce hollow chitosan nanoparticles for the anticancer doxorubicin by forming a polymer−monomer complex, which was sequentially polymerized with K2S2O8 as the initiator and cross-linked with glutaraldehyde.22 Recently, Zhang et al. used a layer-by-layer method to prepare chitosan hydrogel microcapsules with a single component by selectively cross-linking the chitosan chains with glutaraldehyde and removing the second polymer, poly(acrylic acid), that was built up in the capsule walls.23 However, in most of these methods, a long-time reaction and a separation procedure are needed.24

Gelation of food proteins is a property with important applications in food science and technology and has been well-studied.16 The gelation process follows three steps after the initial heat denaturation of the food protein.25 The first is the formation of aggregates via hydrophobic interactions; the second is the stiffening of the aggregates through the sulfhydryl−disulfide

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

reaction; and the third is a large increase in elasticity, resulting from the formation of many hydrogen bonds upon cooling. Proteins are polypeptides, and their charges and hydrophobicity/hydrophilicity are pH-dependent. Protein hydrogel is a network stabilized by hydrophobic interactions, disulfide bonds, and hydrogen bonds. It is reasonable to think that food protein hydrogels with nanoscale dimensions are ideal candidates for loading and releasing drugs because the pH-dependent properties offer reversible sites to bind and release drugs. The low density and network organization of the gel offer sufficient space and binding sites to load drugs. The cross-linking can suppress dissociation upon dilution. The nano- or micro-size can respond to environmental stimulation immediately.

Food protein—polysaccharide interactions have been studied by many scientists and have been well-covered in the reviews. It was reported that micro precipitates and micro particles with the dimensions of micrometer could be obtained through protein—polysaccharide interactions by manipulating environmental conditions, such as pH, ionic strength, temperature, and concentrations of protein and polysaccharide. Delben and Stefancich have reported the interaction of ovalbumin with glutamate concentrations of protein and polysaccharide. Delben and Stefancich have reported the interaction of ovalbumin with glutamate concentrations of protein and polysaccharide, which are a derivative of chitosan. They found that glutamate glucan could hinder the thermally induced aggregation of ovalbumin. In this paper, we tried to combine the merits of chitosan nanoparticles and food protein nanogels together. We developed a novel, green, and convenient method to produce chitosan–ovalbumin nanogels, which may be used for cosmetic and pharmaceutical applications. Ovalbumin is the most abundant protein in the egg white. It is a single-chain phosphoglycoprotein consisting of 385 amino acid residues with a molecular weight of 47 000 Da and an isoelectric point (pI) of 4.8. The ovalbumin molecule has an ellipsoidal shape with dimensions of 7 × 4.5 × 5 nm, one internal disulfide bond, and four free sulphydryl groups (PDB 1OVA). Heating ovalbumin aqueous solution produces a transparent solution, turbid suspension, transparent gel, translucent gel, or opaque gel, depending upon the concentration of the protein and the pH and ionic strength of the solution. The transparent solution or transparent gel is formed if the pH of the solution is far from the pI and the ionic strength is low; turbidity appears when the pH is near the pI and/or the ionic strength is high. With increasing temperature, ovalbumin undergoes a cooperative conformational transformation from α-helix to β sheet and coil structure, in which the globular form is maintained. Heat-induced ovalbumin aggregates or gels are built from the partially unfolded molecules with a significant amount of secondary structure. In our chitosan–ovalbumin nanogels, which have been characterized by ζ-potential, dynamic light scattering, and transmission electron microscopy, we suggest that the chitosan chains are cross-linked by ovalbumin, which gelates forming the core of the nanogels. In our model, some of chitosan chains are trapped in the core and others form the shell of the nanogels.

**Experimental Section**

**Materials.** Hen egg white ovalbumin from Sigma (grade IV) was used without further purification. Chitosan (DD of about 65%, ash content of 0.43%, and moisture content 7.7%) originated from crabs was purchased from Dalian Chitin Co. Ltd. (Liaoning, China) and was further deacetylated and characterized according to the literature. The final DD was 95%, and the molecular weight was about 300 kDa. Pyrene was purchased from Aldrich (98%) and was recrystallized twice from benzene. All of the other reagents were of analytical grade, were purchased commercially, and were used as received. All samples were prepared using water that was deionized with a Vaponic pure system to a resistance of 17–18 MΩ.

**Nanogel Preparation.** Chitosan acetic acid solution (0.75% chitosan in 0.75% acetic acid, w/v) was titrated into 0.02% ovalbumin aqueous solution under agitation until the weight ratio (WR) of chitosan/ovalbumin of 0.2 was reached, and the mixture solution was stirred slowly at room temperature for 2 h. After that, the pH of the solution (about 4.3) was adjusted to 5.4. Heating the mixture at 80 °C for 20 min produced homogeneously dispersed nanogels. This nanogel preparation method was applied throughout the present research. Other conditions used are indicated specially.

**ζ-Potential Measurement.** ζ-Potential measurements were performed on chitosan solution, ovalbumin solution, a solution containing both chitosan and ovalbumin with a WR of 0.2, and solutions in the presence of nanogels with a WR of 0.2 or 1.3. The measurements were carried out at 25 °C on ZetaSizer Nano ZS90 (Malvern Instrument, U.K.) equipped with MPT-2 Autotitrator and 4 mW He—Ne Laser (λ0 = 633 nm) using the technique of Laser Doppler Electrophoresis. The electrophoresis mobility, U0, was measured, and the ζ-potential, ζ, was calculated by the Henry equation, ζ = 2ε(3/2π)(kT/ε)(ζ)(f(kT/ε)), where c, η, f(kT/ε) were the dielectric permittivity of the solvent, viscosity of the solution, and Henry’s function, respectively. f(kT/ε) here was determined to be 1.5, according to the Smoluchowski approximation.

**Ultraviolet and Visible (UV—Vis) Absorption Measurement.** The changes of transparency of chitosan—ovalbumin solutions with a WR of 0.2 were recorded at 500 nm on a spectrophotometer (Lamda 35, Perkin—Elmer) at 60, 70, and 80 °C with a time interval of 1 s.

**Dynamic Light Scattering (DLS) Measurement.** A commercial laser light scattering (Malvern Autosizer 4700, Malvern Instrument) equipped with a multi-r digital time correlator (Malvern PCS7132) and a diode-pumped, solid-state continuous wave laser (Compass 315M-100, Coherent, Inc.; output power ≈ 100 mW, λ = 532 nm) as a light source was used to measure the nanogel size. DLS measurements were performed at 25 °C at a fixed scattering angle of 90°. The relative error was less than 2%. The measured time correlation functions were analyzed by an automatic program equipped with the correlator, and the apparent z average hydrodynamic radius, R0, and polydispersity index (PDI, [ξf(Γ/2)]2) were obtained by a CONTIN mode analysis. After it was verified that the

---

DLS results were the same for the samples prepared with and without filtering, the DLS measurements were carried out without any dust removal.

Transmission Electron Microscopy (TEM) Measurement. TEM observations were conducted on a Philips CM 120 electron microscope at an accelerating voltage of 80 kV. The samples were prepared by dropping nanogel solutions onto copper grids successively coated with thin films of Formvar and carbon. The samples were dried naturally and frozen-dry under vacuum.

Fluorescence Measurement. The fluorescence emission spectra were recorded at 25 °C on a fluorescence spectrophotometer FLS-920 (Edinburgh Instruments). Recrystallized pyrene was dissolved in acetone to prepare a concentration of 2 × 10⁻⁷ g/mL stock solution. Nanogel solutions were first adjusted to different pH values with HCl or NaOH; then a pyrene acetone solution was added; and the resultant solutions were equilibrated at 4 °C for 48 h. An alternative method is that pyrene was first added into nanogel solutions at acidic pH, after 48 h of equilibrium at 4 °C, the solutions were adjusted to the desired pH and then were left at 4 °C overnight before performing the analysis. The final concentration of pyrene in nanogel solutions was 2 × 10⁻⁷ g/mL. The emission spectra were recorded with an excitation wavelength of 335 nm. The spectral resolution was 1 nm for both excitation and emission, and six scans were accumulated for each run.

Results and Discussion

Self-Assembly Conditions of Chitosan–Ovalbumin Nanogels. Chitosan is a polycation with a pKₐ of about 6.3, and ovalbumin is a polyanalyphosphate with a pI of 4.8. Electrostatic attraction happens when chitosan and ovalbumin carry opposite charges. If the inhomogeneous charge distribution of ovalbumin is irrespective, ζ-potential is a good index of electrostatic interactions between chitosan and ovalbumin because ζ-potential is directly related to the net charges on the surface of the macromolecules and particles. Figure 1 shows the ζ-potentials of both chitosan and ovalbumin at different pH values and their products from pH 4.9 to 7.9, a pH range where they have opposite potentials and hence electrostatic attraction should occur. The product reaches a minimum at pH 5.9, indicating that the strongest electrostatic attraction between these two polyelectrolytes should happen at pH 5.9.

However, the electrostatic attractions of chitosan and ovalbumin cannot induce effective self-assembly in the pH range of 4.9–7.9 as observed by the DLS measurement. Analogous with the pH-induced coacervation in complexes of bovine serum albumin with the cationic polyelectrolyte poly(dimethylallylammonium chloride), the chitosan–ovalbumin complexes can occur because of their net opposite charges. There are no particles detected by the DLS measurement, indicating that the attractive forces between chitosan and ovalbumin are insufficient to form clusters in our explored conditions of pH 4.9–7.9 and a WR of 0.1–3.75. Considering the gelation property of ovalbumin, a heat treatment was also performed at a temperature higher than that of denaturation to induce intermolecular hydrophobic association and the formation of disulfide bonds, which may promote the association of chitosan and ovalbumin. The effects of heat treatment at a different temperature and time were monitored by the turbidity change that rose as a result of phase separation occurring in the process (Figure 2). When a mixture of chitosan and ovalbumin with a WR of 0.2 was heated at 60 °C for 1 h, the solution almost appeared transparent, indicating that no significant aggregation happened. However, as the heating temperature was increased to 70 °C, the mixture solution gradually became turbid. Heating at 80 °C led to a jump of turbidity in the initial 5 min and then to a slow increase of turbidity. This is in agreement with the observation that the denaturation process of ovalbumin spans the temperature range from 58 to 85 °C and shows a peak at 76.2 °C, as measured by differential scanning calorimetry (unreported). The DLS measurement indicated that the turbid system was a homogeneous nanogel dispersion, which cannot be obtained by heating the solutions of chitosan and ovalbumin alone at any pH and concentration.

To further explore the suitable pH condition for chitosan–ovalbumin nanogel preparation, DLS was used to study the hydrodynamic radius (R_h) and the scattering light intensity of the nanogel suspension obtained by heating the chitosan and ovalbumin mixture (WR of 0.2, 80 °C for 20 min) at various pH. Figure 3 shows that the scattering light intensity is very weak in the pH range of 4.3–4.8 but increases rapidly when pH changes from 4.8 to 5.3 and increases slowly in the pH range of 5.3–5.8.

The $R_h$ is almost constant in the pH range of 4.3–5.0, increases in the pH range of 5.0–5.3, and strongly increases in the pH range of 5.3–5.8. When pH exceeds 5.8, precipitates occur immediately after the heat treatment. Figure 1 indicates that the electrostatic attractions between chitosan and ovalbumin increase from pH 4.9 to 5.8; therefore, it is reasonable that both $R_h$ and the intensity increase in this pH range. After pH 5.9, the charges and the solubility of chitosan decrease; thus, we cannot obtain dispersible nanogels and precipitates occur. At pH 5.4, the $R_h$ is close to its minimum, while the intensity is close to its maximum, suggesting that the number of the nanogels is the largest. Therefore, pH 5.4 was chosen in this work to produce stable nanogels with these two polyelectrolytes.

Heating ovalbumin alone at the concentration adopted here only produced coagula in the pH range of 4.3–5.8, where pH is close to the pI of ovalbumin, in agreement with the results of the literature. However, in the presence of chitosan, nanogels were obtained in the same conditions. This indicates that the highly protonated chitosan chains prevent ovalbumin from forming coagula. This result is somewhat similar to the interaction of ovalbumin with glutamate glucan mentioned above, in that glutamate glucan hinders the thermal-induced aggregation of ovalbumin studied by rheology experiments. It is interesting to observe that, in the pH range of 4.3–4.8, no coagula were observed by heating the chitosan and ovalbumin mixture, indicating that there are interactions between chitosan and ovalbumin although their $\zeta$-potentials are both positive. It has been proven by experiments and theories that the protein heterogeneous distribution of charges favors complexation with a polyanion at the pH above the protein isoelectric point, where both the protein and the polyanion carry net negative charges. Possibly, the phase separation of ovalbumin is hindered in the pH range of 4.3–4.8 by the interaction of the local negative charges of ovalbumin with chitosan. On the other hand, the interaction between chitosan and ovalbumin is weak in the pH range of 4.3–4.8 because of their net positive charges; therefore, the $R_h$ and the scattering light intensity are very small after the heat treatment of the mixture of chitosan and ovalbumin.

The interactions between chitosan and ovalbumin depend not only upon the pH of the solution but also upon their ratio. As investigated by DLS, $R_h$ rapidly decreases with the WR and then increases quickly after the minimum at a WR of 1.1 (Figure 4). The same behavior is exhibited by the scattering light intensity, which decreases quickly at low WR values and then shows a slow increase after the WR reaches 1.1. Noticeably, in the WR range of 0.02–1.5, stable nanogels were produced. Therefore, the ratio WR of 0.2 was chosen for studying the nanogels of chitosan and ovalbumin in most cases. When the WR was 0.2, the PDI was 0.38 and a single peak appeared in the size distribution. Besides, a WR of 1.3 was also chosen as the reference.

The nanogel formation was controlled by the electrostatic attraction between chitosan and ovalbumin, electrostatic repulsion among chitosan molecules, hydrophobic association, and disulfide bonds among ovalbumin molecules after ovalbumin denaturation. The nanogels produced with the method described in the Experimental Section are very stable. Figure 5 shows that the nanogel suspension does not change its size distribution. Besides, after long-time (more than 90 days) storage in the absence of antiseptics, the nanogels did not go bad, suggesting that chitosan still kept its bacteriostatic and fungistatic functions.

The larger aggregates shown in the TEM image are apparently composed of the smaller particles. Perhaps, the secondary aggregation happened during the drying process. On the other hand, the size of the single ovalbumin molecule is about 10 nm wide.

in the TEM image (data not shown); thus, the smaller particles shown in Figure 6, whose size is about 120 nm in diameter, are not individual ovalbumin molecules, and the aggregates are not strings of beads formed by individual ovalbumin molecules, as described by Doi and Kitabatake.\textsuperscript{35,36} The average size of the smaller particles in Figure 6 is about 120 nm, which is much smaller than the hydrodynamic diameter obtained by DLS at the same pH, i.e., about 320 nm. This difference could be ascribed to the large amount of water contained in the nanogels and the shrinkage of the nanogels after water evaporation. Enlargement of a nanogel in the inset of Figure 6 reveals a difference of the core and the shell in density, which is compatible with the existence of a core–shell structure in the nanogels.

Figure 7 shows the ζ-potential curves of a WR of 0.2 nanogels, a WR of 1.3 nanogels, and a WR of 0.2 mixture before heating. The ζ-potentials of individual chitosan and ovalbumin, which have been shown in Figure 1, are also reported as the references. A WR of 0.2 mixture shows an amphoteric ζ-potential curve, and its zero potential appears at pH 6.6, which is about the average of those of chitosan and ovalbumin. However, a WR of 0.2 nanogels shows the zero potential at pH 7.2, which is close to the zero potential of chitosan, indicating that the surface of the nanogels is enriched with chitosan chains and ovalbumin molecules are enriched in the core of the nanogels, compared with the corresponding mixture. When the WR of chitosan/ovalbumin in nanogels increases to 1.3, the ζ-potential curve becomes very similar to that of chitosan. In particular, their pH values at zero potential are identical, suggesting that the surface of the nanogels is totally occupied by chitosan chains.

The solubility/dispersibility of chitosan, ovalbumin, and their nanogels is useful in exploring the structure of the nanogels. Ovalbumin alone is soluble at room temperature, and coagula occur after heat treatment in the pH range studied. However, chitosan can suppress the phenomenon discussed above. Chitosan alone is insoluble when pH is above 6.4, as indicated in the literature\textsuperscript{2} and verified in our experiment. A WR of 1.3 nanogels form secondary aggregates when pH is above 6.7. The similarity of a WR of 1.3 nanogels with chitosan in both ζ-potential and dispersibility/solubility indicates that in these conditions the surface of the nanogels is totally occupied by chitosan chains. From this picture, the gelating mechanism of ovalbumin, and the electrostatic attractions between chitosan and ovalbumin, the structure of a MR of 1.3 nanogels can be suggested: ovalbumin gelates after heat treatment, forming the core of the nanogels; chitosan chains are partly trapped in the nanogel core after heating because of the electrostatic attractions between chitosan and ovalbumin, which exist before and after the heat treatment; the rest of the chitosan chains form the shell of the nanogels; the electrostatic repulsions of chitosan chains in the shell hinder any further aggregation of ovalbumin. For a WR of 0.2 nanogels, precipitates occur only in the pH range of 6.9–8.1. The hypothesis of the core–shell structure of a WR of 1.3 nanogels can also be used to interpret the results obtained with a WR of 0.2 nanogels. Chitosan is only 16.7 wt % in these nanogels, and its chains possibly are insufficient to occupy the whole surface of the nanogels. When pH is lower than 6.9, the protonated chitosan chains screen the charges of ovalbumin on the surface of the nanogel core; therefore, a WR of 0.2 nanogels shows positive potentials and is dispersible. When pH is higher than 8.1, the about zero-charged chitosan shell shrinks and the charges of ovalbumin on the core surface exhibit their effect; thus, a WR of 0.2 nanogels shows 17–29 mV negative potentials, and the nanogels can form a dispersion. In the pH range between 6.9 and 8.1, the net charges on the nanogel surface are too few to stabilize the nanogels; therefore, precipitation happens. The shells of the nanogels with a WR of 1.3 are much richer in chitosan. As a consequence, the negative charges of the ovalbumin on the core surface cannot significantly influence the ability of forming a dispersion and the ζ-potential of the nanogels. The proposed core–shell structure is also compatible with the TEM image (Figure 6), which indicates that the density of the core is higher than that of the shell for the WR of 0.2 nanogels.

The nanogels do not disaggregate in the pH range explored, even at pH 2.0, where between chitosan and ovalbumin strong electrostatic repulsions exist. This results from hydrophobic interactions, disulfide bonds, hydrogen bonds among ovalbumin molecules after the heat treatment,\textsuperscript{26} and many hydrogen bonds between the denatured ovalbumin and the chitosan trapped in the core of the nanogels because of the presence of many proton donor and acceptor groups on both chitosan and ovalbumin.

**pH Dependence of the Nanogels.** Although the nanogels do not dissociate in the pH range studied, their size depends upon the pH. After the preparation, the pH of the solution in which the nanogels were dispersed was adjusted to different values to investigate its influence on the nanogel size. The data in Figure 8 show that the $R_g$ values of a WR of 1.3 nanogels are smaller than that of a WR of 0.2 nanogels. As reported previously,\textsuperscript{46,47} for surfactant-free particles, the average surface area stabilized by one hydrophilic moiety should be constant. Our nanogels were produced at pH 5.4, where chitosan is protonated. A larger number of chitosan chains can stabilize a larger surface area,
leading to a smaller size of the nanogels; therefore, the decrease of \( R_h \) of a WR of 1.3 nanogels can be explained.

Both nanogels have a similar dependence upon pH when their pH values are lower than 6.9. In particular, the minima of \( R_h \) occur in the pH range of 6.5–5.5, the \( R_h \) values increase when the pH changes from 5.5 to 3.5 and then decrease when pH is lower than 3.5. This behavior is compatible with the nanogel structure discussed above. When the pH is in the range of 6.5–5.5, the protonated chitosan in the nanogel shell produces a dispersion of the nanogels and the electrostatic attractions between chitosan and ovalbumin result in a shrinkage of the nanogels. In the pH range of 5.5–4.8, chitosan is about fully protonated, while the net negative charges of ovalbumin decrease. As a consequence, the electrostatic attractions within the nanogel core and the core surface decrease and chitosan chains are more extended in the shell, causing an expansion of both the core and the shell. When pH is in the range of 4.8–3.5, ovalbumin also carries net positive charges, so that the intermolecular electrostatic repulsions lead to a further expansion of the nanogels. The \( R_h \) values that decrease below pH 3.5 may be attributable to the electrostatic screen of ions increased in the adjusting pH. The size distributions of the nanogels in the pH range from 6.8 to 2.0 show that the nanogels with a single peak do not increase their PDI when pH was changed. This result excludes the possibility that the size changes are caused by the aggregation or dissociation of the nanogels.

For the pH range between 6.9 and 10.5, the change of the nanogel size is also compatible with the core–shell structure. When pH was adjusted to 6.9, precipitation happened for both nanogels. The precipitates occurring at a WR of 1.3 cannot be redispersed by a further increase of pH. On the opposite side, the precipitates occurring at a WR of 0.2 can be redispersed by increasing pH above 8.1, although multiple peaks appear in the size distributions (data not shown). In the pH range of 6.9–8.1, partly protonated chitosan in the nanogel shell may be neutralized by the negative charges of ovalbumin on the core surface, which leads to the secondary aggregation. When pH is higher than 8.1, chitosan does not carry any charge and the shell shrinks. However, the negative charges of ovalbumin on the core surface play their role in keeping a WR of 0.2 nanogels dispersed, although a partial secondary aggregation cannot be prevented as evidenced by the appearance of a new peak with a larger size in the size distributions. For a WR of 1.3 nanogels, ovalbumin on the core surface should be fully covered by the chitosan-rich shell and, consequently, the nanogels cannot be redispersed when pH is 6.9 or higher. The secondary aggregates formed in the pH range of 6.9–8.1 for a WR of 0.2 nanogels and 6.9–10.5 for a WR of 1.3 nanogels can be redispersed when the pH of the solution is out of this range; i.e., the nanogels exhibit a reversible transition between aggregation and dispersion that is pH-driven. This property may be useful in the practical applications of the nanogels.

The real-time measurement of the nanogel size showed that the nanogels change their size immediately when the pH is changed from neutral to acidic, while the nanogels need about 10 min to reach their equilibrium when the pH is adjusted from acidic to neutral (unreported data).

The hydrophobicity/hydrophilicity of the nanogels was investigated with pyrene fluorescence. Pyrene has a much lower solubility in water (about 10\(^{-7}\) mol/L) than in hydrocarbons (0.075 mol/L). It migrates from the water phase into hydrophobic regions once they are formed in aqueous solution, with remarkable photophysical changes.47–50 The hydrophobicity/hydrophilicity of a WR of 0.2 nanogels at different pH values was investigated by examining the intensity ratio of the first/third band (\( I_1/I_3 \)) in pyrene fluorescence emission spectrum. The samples were prepared by adjusting the pH of the nanogel medium to different values and then adding pyrene. Figure 9 shows that when the nanogels are in the pH range of 2–5, \( I_1/I_3 \) values are about 1.4, which indicates a relatively hydrophobic environment of pyrene. In the pH range of 5.5–6.8, \( I_1/I_3 \) values are about 1.5, suggesting a little increase in hydrophilicity. The \( I_1/I_3 \) value 2.0 at pH 7.0 is the same as that of the pyrene in water, indicating an entirely hydrophilic environment of pyrene. When the pH is further increased, the \( I_1/I_3 \) value rapidly drops to about 1.5. Results similar to the data in Figure 9 were obtained by adding pyrene to the nanogels at low pH values and then increasing the pH to the desired values. This evidence showed that the measurements were performed after the systems had reached the thermodynamic equilibrium and that the nanogels are very hydrophilic at neutral pH, although they form aggregates. At acidic pH, chitosan chains in the shell of the nanogels are protonated; therefore, the shell should be hydrophilic. The \( I_1/I_3 \) values of about 1.4–1.5 indicate that the core of the nanogels is relatively hydrophobic, which indicates that ovalbumin molecules, after heat treatment, expose their hydrophobic residues toward the surface and the net charges of the protein decrease at the pH around its pI. At neutral pH, ovalbumin carries more net charges and chitosan is partly protonated; thus, both the shell and core of the nanogels are very hydrophilic. At alkaline pH, chitosan does not carry charges and the shell may shrink, forming relatively hydrophobic regions, toward which the pyrene migrates.

The pH-dependent hydrophobic/hydrophilic property and the size change of the nanogels may be used for drug delivery. For example, the nanogels can adsorb positively charged drugs at alkaline pH through electrostatic attractions and release them at acidic pH, where nanogels also carry positive charges. The nanogels can adsorb molecules with low polarity at acidic pH, where the core of the nanogels is relatively hydrophobic, and release them at neutral pH, where the nanogels are more hydrophilic. Chitosan–ovalbumin nanogels have the advantages of both chitosan and ovalbumin. The chitosan shell structure gives the nanogels the appealing biomedical properties of chitosan. The chitosan–ovalbumin nanogels have pH-dependent property and stability. Another advantage of these systems is that the production of the nanogels is convenient and green and no separation procedure is needed during the nanogel preparation.

Acknowledgment. Financial support of Unilever Shanghai Co. Ltd. (Unilever Research China) is gratefully acknowledged.

LA053158B